RAT MIDDLE CEREBRAL ARTERY VASOREACTIVITY IN CONTROLS, SHAM OPERATIVE CONTROLS, AND FOLLOWING ACUTE SUBARACHNOID HAEMORRHAGE BY ARTERIAL RUPTURE WITHOUT CRANIOTOMY

LAURENCE A.G. MARSHMAN MB ChB, FRCS

THESIS FOR THE DEGREE OF M.D
UNIVERSITY OF GLASGOW
JANUARY 2003

Departments of Neurosurgery and Experimental Medicine
Royal Hallamshire Hospital
Sheffield
S10 2JF

ABSTRACT

Acute subarachnoid haemorrhage (SAH) is a devastating event prevalent mostly in adult life. The vast majority occur from structural defects on major arteries within the basis cerebri called berry aneurysms. Apart from rare exceptions, there are no definite conditions—inherited or acquired—that predispose to these. Sudden death may accompany the impact, and up to a half of patients do not reach neurosurgical units alive. Of those that do, technical improvements in definitive surgery and interventional radiology have largely obviated the risk from secondary haemorrhage. However, a third of patients surviving intact to convalescence instead succumb to another process which mysteriously develops with some delay. In spite of considerable efforts, both clinical and experimental, there continues to remain no wholly satisfactory account for either the mechanism, or the treatment, of the stroke that frequently results. The current consensus, however, is that the basic mechanism involves an increasingly severe local arterial spasm ('vasospasm', VSM) that causes increasing focal cerebral ischaemia ('delayed cerebral ischaemia', DCI); and that VSM is caused purely as a result of subarachnoid clot lysis.

Although vessel-surface clot can, experimentally, simulate many of the appearances of VSM, such studies (i.e. 'clot-VSM' models) rarely elicit DCI. Moreover, none of the results of 'clot-VSM' studies have led to the development of efficacious treatment strategies (apart, that is, from the controversial exception of the calcium antagonist nimodipine). Such a failure in extrapolation suggests that either DCI is primarily a species-specific phenomenon, or that such models are fundamentally flawed (in either design or application). Since most current 'SAH' models are in reality only models of VSM (i.e.'clot-VSM' models), one obvious potential design flaw is their implicit assumption that 'VSM = DCI'. The fact that, clinically, VSM occurs with twice the frequency of DCI might suggest to many that other factors are operative. Such factors could relate to generalised reductions in cerebral perfusion pressures, a generalised dysautoregulation within the cerebrovasculature, or to electrolyte disturbances. However, they could also relate to factors long since forgotten associated with the acute ictus. Such factors could conceivably include the central physiological disturbance (i.e. systemic and intracranial pressor effects) associated with the acute ictus, as well as the properties of the ruptured vessel per se. Perhaps related to both of these would also be the conjectured occurrence of

acute VSM as distinct from delayed VSM. The potential gravity of the central physiological disturbance is reflected by the fact that sudden death occurs in up to a half of SAH patients; whilst many of the remainder are rendered comatose. By ignoring such potentially key 'early' factors—and, in consequence, by potentially over-emphasizing the importance of later 'clot-VSM'—the failure of most current 'SAH' models in adequately accounting for DCI might therefore be potentially explained.

In particular, and as implied by proponents such as Nagai, it is possible that the acute ictus 'primes' the larger basal arteries within the subarachnoid space such that they might be more—*or even less*—susceptible to the later effects of chronic clot lysis. If this is so, then an analysis of cerebrovascular reactivity in the acute phase following a physiologically representative SAH—**an area that has been overwhelmingly under-explored**—might yield important clues: particularly in the form of subtle differences between post-SAH vessels and controls. Such differences, if found, might therefore be a factor in explaining why current 'clot-VSM' SAH models fail to elicit clinically useful results: because, in such latter studies, subarachnoid clot is chronically applied to vessels that are, in fact, indistinguishable from controls. In reality, however, it could be that 'clot lytic' effects ought to be superimposed upon cerebral vessels *that have already been rendered significantly different from controls* by 'acute ictal' effects.

The principle hypothesis of the current study is that events associated with the acute ictus of SAH elicit changes in cerebrovascular physio-pharmacology that are independent of any subsequent effects of chronic clot lysis. As a result, such changes might interact with the effects of chronic clot lysis in a way that may, in consequence, ultimately explain why currently favoured 'clot-VSM' fail to adequately explain delayed VSM and DCI. **The principle aim of the current thesis was to formally categorize cerebrovascular physio-pharmacological properties in sham and non-operative controls, and to compare these with those obtained in the acute period after SAH in a model more representative of the true clinical scenario.** Clearly, the most ideal SAH model would be one in which arterial rupture is created inside a closed skull, since only here will the acute intracranial pressure changes be faithfully recreated. Ironically, the earliest SAH models came close to such an ideal: nevertheless, all utilized a prior craniotomy. More recently, endovascular filament (EF) models have instead gained

credence. Because, in such models, intracranial arterial rupture is created in small animals by EF advancement through the intracranial internal carotid artery from an extracranial source in the neck, the potential disadvantages associated with a prior craniotomy are avoided. **However, to date no EF-SAH model has yet been used to study cerebrovascular reactivity**: this thesis is the first to do so.

Hypothetically, however, two sources of 'side bias' potentially thwart the application of EF-SAH cerebrovascular study. One source relates to the manipulation and temporary ischaemia suffered during EF insertion (in particular, with prolonged carotid clamping). Another relates to relative uncertainty regarding the precise site of intracranial rupture. **A secondary aim of the thesis, then, was to formally assess the hypothesis that EF-SAH introduce procedural 'side bias' by comparing cerebrovascular responses ipsilateral and contralateral to EF insertion using a range of constrictory and dilatory agonists**—conceivably all relevant to SAH. The successful exclusion of 'side bias' would be important to any future study of delayed (i.e. chronic) VSM because, should any side differences be subsequently found in such delayed studies, then these could be attributable to focal factors—such as thickened clot lateralization—with somewhat greater confidence.

Another potential flaw with current 'clot-VSM' models, however, resides not in their design but in their application. Thus, many do not use as substrate for cerebrovascular study vessels from the anterior cerebral circulation—where both VSM and DCI are most frequently apparent. Instead, most choose vessels from the posterior circulation: in particular, the basilar artery. Furthermore, many do not appear to restrict study to particular segments of specific arteries, and thus potentially thwart 'like-with-like' comparisons. Most studies also do not quote vessel diameters in relation to sub-groups and their responses: in this regard, most assume that randomisation for vessel side would make up for any 'size effects' on vessel responses. And finally, most ignore a principle implied by Brandt, that variation in vasoreactivity in individual arteries might be discontinuous—and not continuous—and so therefore not amenable to routine statistical analysis. It was therefore considered critical in this thesis to assess and limit such potentially confounding factors by fully reviewing what is already known about cerebral vessels before and after SAH, by critically reviewing all experimental models used to

derive such data, and by assiduously analyzing normal vessel responses in a standard small vessel study model *before* progressing to their ultimate study post-SAH. To this end, **middle cerebral arteries (MCA) medial to the olfactory tract were analyzed by 'wire myography'**.

Whilst the ultimate aim of all DCI research must clearly be in the effects of delayed VSM on a post-ictal brain, it is a scientific duty to delineate cerebrovascular reactivity at all stages prior to this. Unfortunately, it is not possible to apply the methodology of this current thesis to assess all of the 192 hours that pass between the acute ictus and the typically delayed start of VSM: instead, restriction of study to a shorter time interval would only be possible in one thesis. As a result, **the current thesis was restricted to an analysis of the first three hours of EF-SAH** for the following reasons:

1. To assess the principle hypothesis that events associated with acute SAH elicit changes in MCA physio-pharmacology that are independent of any subsequent effects of chronic clot lysis
2. To assess the secondary hypothesis that EF-SAH models introduce a procedural 'side bias' by comparing constrictory and dilatory MCA responses ipsilateral and contralateral to EF insertion

CONTENTS

Part 5 : Results

Part 6 : Discussion

LIST OF PLATES

ACKNOWLEDGEMENTS

I would like to thank the following who helped me throughout this work:

Prof C Welch	Postgraduate Dean, University of Sheffield. Professor of Vascular Surgery, Royal Hallamshire Hospital, Sheffield
Dr Brian Howard	Vetinary physician, Field Laboratories, RHH
Dr Alyn Morice	Consultant Respiratory physician, Dept Experimental Medicine, RHH
Dr J Sarah Thompson	Postgraduate student, Dept Experimental Medicine, RHH
Mr Ken Allen	Head of Medical Physics, King's College Hospital, Denmark Hill, London SE5 9RS
Dr Christine Smith, Dr Walter Timperely	Consultant Neuropathologists, RHH
Mr Toni Buxton	Senior technician, Dept Neuropathology, RHH
Mr Gyorgy Seifert, Mr Otto Major	Fellow Neurosurgical and Research Registrars, Dept Neurosurgery, RHH
Dr Nicky Brown	Lecturer, Dept Experimental Surgery
Mr William Pease	Harcourt Brace printing Ltd.
Dr David Grey	Lecturer, Dept of Statistics, University of Sheffield
Ms Sophie Kirk	Parker-Stratton Design

DECLARATION

All of the experiments—both in vivo and in vitro—were performed by the author. The only extraneous material herein contained relates to the distension-diameter study of n = 13 pulmonary arteries (Fig 13) performed by Dr J. Sarah Thompson; and the 'bandwidth contraction' experiments on the multi-channel pen recorder (Fig 7) by Mr Ken Allen.

THIS WORK IS DEDICATED TO

Veronica
John & Edna
Charlie, Sam, Freddie & Tilly

ABBREVIATIONS

AC	Adenylate Cyclase
ACommA	Anterior communicating artery
ARDS	Adult respiratory distress syndrome
ATP	Adenosine triphosphate
AT	Agonist-induced Tone
BBB	Blood-Brain Barrier
Ca	Calcium
CA	Catecholamines
cAMP	Cyclic Adenosine-3', 5'-Monophosphate
CBF	Cerebral blood flow; *Regional* CBF; and *Local* CBF
CCA	Common Carotid Artery
cGMP	Cyclic Guanosine-3', 5'-Monophosphate
CHR	Cushing Hypertensive Response
CM	Cisterna magna
$CMRO_2$	Cerebral Metabolic rate for Oxygen uptake
CoW	Circle of Willis
CO	Cardiac output
CPP	Cerebral Perfusion Pressure (i.e. [MAP - ICP])
C-R curve	Concentration-response curve
CSF	Cerebrospinal Fluid
DCI	Delayed Cerebral Ischaemia
DID	Delayed Ischaemic Deficit
DSS	Denervation Super-Sensitivity
EC_{50}	Mean Effective concentration producing 50% maximal response (i.e. response potency or affinity)
*e*Ca	*Extracellular* Calcium
ECA	External Carotid Artery
EDCF	Endothelial-derived constricting factor
EDRF	Endothelial-derived relaxing factor
EEL	External Elastic Lamina
EF	Endovascular filament
E_{max}	Efficacy of agonist response (i.e. mean maximal response)
GC	Guanylate Cyclase
HA	Histamine
Hb	Haemoglobin
5HT	5-Hydroxy Tryptamine (Serotonin)

*i*Ca	*Intracellular* Calcium
ICA	Internal Carotid Artery
ICP.	Intracranial Pressure.
ΔICP	ICP_2-ICP_1 where ICP_2>ICP_1 (hence ΔICP is always positive—i.e. an elevation)
IEL	Internal Elastic Lamina
*i*NOS	*Inducible* Nitric Oxide Synthase
K	Potassium
L-ARG	L-Arginine
L-NAME	Nω-Nitro-L-Arginine Methyl Ester
MAP	Mean Arterial Pressure
ΔMAP	MAP_2-MAP_1. MAP_2>MAP_1 (+ΔMAP) = hypertension, MAP_2<MAP_1 (−ΔMAP) = hypotension, MAP_2=MAP_1 (zero) = invariant
MCA	Middle Cerebral artery
MT	Myogenic Tone
NE	Norepinephrine (Noradrenaline)
NO	Nitric Oxide
PGF2α	Prostaglandin F2α
PKC	Protein Kinase C
PPV	Papaverine
PSC	Potential specific channel
RSP	Reserpine
SAH	Subarachnoid Haemorrhage
SAS	Subarachnoid Space
SIADH	Syndrome of inappropriate anti-diuretic hormone secretion
SIT	Subintimal thickening
SNP	Sodium nitroprusside
SNS	Sympathetic Nervous System
UTP	Uridine-3', 5'-triphosphate
V_E	Volume of blood extravasated (i.e. extent of SAH)
VSM	Vasospasm
VSMC	Vascular Smooth Muscle Cell

NB

1. The notation **[substance]$_{CSF}$** or **[substance]$_{plasma}$** will be used to denote the **concentration of a substance** in either the **CSF** or **plasma** respectively.

2. All values for continuous data are represented as mean±SE (standard error of the mean) except functional vessel **diameters** which are represented as **mean±SD** (standard deviation of the mean).

3. Maximal agonist responses will generally be denoted as [substance symbol] E_{max}, e.g. KCl E_{max}. This is because the maximal response is the **efficacy (E)**. However, sometimes the notation will be shortened to [substance symbol]$_{max}$, e.g. K_{max}. Since there is no agonist used with a symbol E, no undue problems are envisaged.

PART 1

ACUTE SUBARACHNOID HAEMORRHAGE

Chapters 1-3

Chapter 3 Treatment policies for delayed cerebral ischaemia

Chapter 1
Historical background. Current theoretical and therapeutic perspectives

1.1 Introduction

Subarachnoid haemorrhage (SAH) is, in large part, a consequence of the unusual arrangement of the cerebral vasculature. The usual angiology in an organ involves the major arterial supply entering at the hilum, with successive ramification then occurring within the organ. The only 'exposed' region of the supply is therefore at the hilum, although even here extra support is still often afforded. For example, the splenic artery gains further protection within the lienorenal ligament; the hepatic artery within the lesser omentum; and the pulmonary artery within the pulmonary ligament. The cerebral vasculature, however, contrasts in both aspects. Its major supply, from two sources bilaterally, **ramifies** over the **surface** of the brain **prior** to entering it; whilst the major divisions are **poorly protected** by little—if any—connective tissue. These somewhat exposed cerebral vessels thus pulsate freely within the clear CSF of the subarachnoid space (SAS)—the latter providing ambient pressures for them of around 12 mmHg[659]: their major support, in fact.

To make an already precarious situation somewhat worse, the major cerebral arteries are frequently **defective** in their most structurally important layers: the **internal elastic lamina**[385, 499], and the **smooth muscle** of the **tunica media**[186, 253, 385]. In fact, the cerebral vasculature abounds in all sorts of anomalies[87, 396, 592], but it is such defects—characteristically occurring at points of **bifurcation**—that are related to the precipitation of acute SAH. Nevertheless, it is not until early adult life (especially over 30 years[253]) that problems usually arise: eventually bulges form at such points. Since velocity of flow must necessarily slow as the lumen suddenly widens, kinetic energy must also fall. Potential energy must therefore increase, in order to compensate: the total energy must be conserved. Increased potential energy is absorbed by **further increases in wall tension**, at sites already structurally weakened (Part 2, 1.4.3). Once started, a vicious cycle may thus ensue, the bulging wall becoming forever larger. These bulgings are referred to as **berry aneurysms**. Some attempt at compromise is reached by successive clot formation and calcification, the result of slower and turbulent sac flow. But eventually the equilibrium may fail. Blood at systemic arterial pressure then bursts out into the SAS of the craniospinal axis—characteristically following a surge in physical activity[87, 315, 396].

Thus is the case with the single most important cause of SAH: a ruptured berry aneurysm (at least 75% cases[396]). However, numerous other causes also abound, and are listed for

convenience in Table 1. Some of these, especially arterio-venous malformations (AVMs), may strike in adolescence or childhood: but SAH is essentially an affliction of adult life. This may possibly relate to increasing levels of MAP[87, 132]. Furthermore, SAH frequently follows heavy lifting, bending, coughing, straining at stool, head injury, and orgasm at coitus[315, 396]. The common denominator in all of these are **acute surges** in **systolic pressure** cranially.

Table 1. The causes of SAH

Common	Berry Aneurysm
	Arterio-venous malformation
	Unknown
Rare	Non-saccular aneurysms
	Atherosclerotic
	Mycotic
	Oncotic
	Dissecting
	Traumatic
	Blood dyscrasias
	Coagulopathy
	Disseminated intravascular coagulopathy
	Anticoagulants
	Myeloproliferative disorders
	Drugs
	Vasculopathies
	Connective tissue disorder
	Henöch Schönlein purpura
	Rheumatic fever
	Drugs
	Amphetamines
	? Anabolic steroids
	Eclampsia

SAH is a serious condition, affecting young and old adults alike, in at least 15 per 100 000 population[477]. With a demonstrable berry aneurysm, rupture will occur in 0.05-0.5% per annum[657]. Although Quincke introduced lumbar puncture in 1891, the diagnosis of SAH was probably rarely made in the patient's own lifetime until only the latter half of this century[556]. SAH is still, in fact, a notable cause of **occult sudden death** (Part 6, Section III). Thus, it was responsible for 5% of sudden deaths in one study[130]: this rising to 18% in a group of young soldiers undergoing strenuous physical activity[374]. Because SAH may be misdiagnosed failing an autopsy, decreased autposy-rates following sudden death necessarily under-estimate its true incidence.

1.1.1 Immediate effects of subarachnoid haemorrhage

Blood at systemic MAP suddenly gushes into the subarachnoid space, where the pressure is usually less than 15 mmHg. There is, naturally, a limit to the amount of extra fluid that can be suddenly accommodated here—particularly within a rigid cranium. With the brain parenchyma itself virtually incompressible, the ICP must suddenly rise. This is probably the cause of **severe headache**: the worst, in fact, of that person's lifetime[396]. However, because extravasation occurs into the subarachnoid space, the ΔICP is conducted pari passu over the entire parenchymal surface and major cerebral vessels. Venous and capillary beds are soon shut down[115, 212], and transmural vessel pressures (and functional diameters) are effectively reduced. In consequence, the CBF becomes drastically reduced (Part 2, 2.4). Brain homeostasis is thus soon compromised, this resulting in an abrupt **loss of consciousness**. Unless the CBF can be quickly restored, brain death will soon follow.

Although the initial SAH can rarely—if ever—be monitored clinically, the effects of a secondary haemorrhage have frequently been recorded. Such studies have confirmed the above reasoning. Thus, the ΔICP occurs rapidly over one or two minutes such that $ICP_{max} \sim MAP$[257, 453, 454, 632]. However, the volume extravasated (V_E) often bears little relationship to the ICP_{max}[221, 256, 453, 561]. Thereafter, the ICP soon falls[453] to a new plateau within minutes, usually only slightly above that of the baseline value[454, 632]. The bleeding is probably stopped by a combination of tamponade and acute spasm in the ruptured artery. The CBF falls acutely to critically low levels, before slowly rising to regain previous values[18]. This, however, is sometimes delayed, even where ICP values have normalized[18]. (Persistently low values, of course, invariably result in brain death[18]). Widespread perfusion defects are prevalent in these latter cases (no-reflow phenomena: NRP[18]), and resemble those seen following cardiac arrest[360]. In others CBF recovery is rapid, with even a temporary overshoot ("hyperaemic overshoot")[360, 632]. This may prove detrimental, serving to disrupt the BBB as it may also do post cardiac arrest[115, 116, 285, 360].

Yet other findings appear less explicable. For example, the cerebral blood volume (CBV) is frequently *increased*[222-224] after SAH; whilst cardiac arrhythmias abound in up to 91% of cases within 48 hours of SAH[12]. Similarly, a persistent state of cardiac dysfunction may pertain, the co-development of ARDS often serving as a harbinger of imminent demise[189].

1.1.2 Natural history of subarachnoid haemorrhage

Acute SAH therefore clearly represents a profound pathophysiological insult, causing both gross central and systemic derangement. It is all the more surprising, therefore, that most current experimental studies choose to ignore this insult (Part 6, Section III), concentrating instead on the effects of the blood clot (*see below*). Although most statistics naturally pertain to survivors, anything up to 40-50%[477] may never reach hospital at all, these

suffering sudden death. Even with recent cases benefiting from efficient emergency services, probably less than 60% of those reaching neurosurgical units alive eventually return to normal life[267].

The natural course in those not operated on is necessarily based upon older studies where operation was not available. Most agree that there is a high risk of re-rupture over the ensuing six weeks: however, this is highest early on. It is especially high in the first 24-48 hours following initial rupture, and thereafter falls over the first one or two weeks. It becomes less likely over the following four weeks. After this crucial period (i.e. the first six weeks), the risk falls dramatically such that it soon approaches 0.5% per annum[657]. However, there is a progressive increase in mortality with each successive re-rupture[369].

Perhaps the majority of patients, then, that survive make at least some degree of recovery. They thus enter the critical period of the first six weeks. Their major complaints at this time centre around a profound headache and photophobia. The latter, coupled with varying degrees of nuchal rigidity, provide evidence of meningeal irritation (**meningism**). The **MAP** is often **elevated**, along with a central **elevation** in core **temperature**[163, 518]. In some, a mixed peripheral **leucocytosis** occurs[445]. Many also appear **drowsy**[163, 396]. All of these signs may correlate with eventual outcome[396], but most neurosurgeons especially rank the latter[87]. As a result, strict bed rest is frequently encouraged: but this and opiate-based analgesia encourage constipation—a potent precipitant of re-rupture in many cases (*see below*).

1.2 Delayed cerebral ischaemia

With improved safety in definitive surgery, the excess morbidity and mortality (it was hoped) would be largely eradicated. Nevertheless, those surviving both primary SAH and definitive surgery may still instead succumb to yet another process. In fact, the **major factor** now **influencing outcome** is attributable to this rather nebulous condition that characteristically appears in the post-acute recovery phase[316]. Only recognised as a distinct entity since the 1950s, it classically evolves several days after SAH, and is thus referred to as **Delayed Cerebral Ischaemia (DCI)**. It results in the formation of **Delayed Ischaemic Deficits (DID)**. Like secondary haemorrhage, it often strikes in a patient having initially made a complete recovery from the primary episode. Although most prevalent synchronous with maximal risk from re-haemorrhage, it contrasts markedly from the latter both in being more gradual in evolution; and by the absence of fresh bleeding on a CT scan. Drowsiness becomes increasingly heightened, and cerebral infarcts eventually arise in the same territory in which rupture had occurred (*see below*). Such infarcts predominantly occur within the internal carotid artery (ICA) territory: posterior circulation infarcts appear especially rare[267].

It is difficult to put a precise figure on the proportion succumbing to this syndrome, there being large variations reported (from 5% to 90%!)[134] amongst different series. But in a recent overview of 10,445 cases amongst 297 studies[134], DCI occurred in **33%**. Its timing varied from 2-4 days to over 14 days, but the mean time suggested an onset at around **8 days after SAH**. Thereafter, DCI steadily attained a zenith over 1 - 4 days; following which, should the patient have survived, some degree of recovery was the rule.

Once established, DCI appears to possess the same natural history as that associated with any other stroke. Thus, **death** occurs in **30%**, whilst a **permanent neurological deficit** occurs in a further **34%**. Ultimately, only 36% achieve a good outcome[134]. In consequence, DCI often serves to thwart the effort and progress made with early aneurysm surgery, blighting otherwise promising recoveries. It is hardly surprising, then, that most current research in SAH has focussed on DCI.

1.3 Delayed vasospasm

In spite of considerable efforts, the precise cause of DCI continues to remain elusive. This is reflected in the relative failure of treatment strategies over the past 20 years[20, 134]. Any proposed mechanism must adequately explain the temporal delay in onset of neurological deficit. Certain associations have accrued. For example, there is frequently a de novo **reduction** in the **CBF** prior to the onset of DCI, both globally and regionally[222-224, 413, 632]. There is also a frequent **reduction** in cerebral metabolism (reduced $\mathbf{CMRO_2}$)[222, 224, 632], as well as **reduced** arterio-venous oxygen differences ($\mathbf{AVDO_2}$)[632] across the brain. The **ICP**, where monitored, often shows **secondary elevation**[454, 632]; and the **CSF** classically reveals the presence of a **lactic acidosis**[195, 487, 632].

But without doubt, the most vivid association—and certainly the most tangible—is that found on cerebral angiography. In a landmark paper in 1951, Ecker and Riemenschneider documented the angiographic occurrence of **severe arterial narrowing** occurring in association with recently-ruptured cerebral aneurysms[146]. Since that time, this phenomenon—forever after referred to as cerebral vasospasm (**VSM**)—has become increasingly established as playing a major, if not indispensable, role in the development of both DCI and DIDs. There are, in fact, several reasons for arriving at this conclusion:

- DIDs suggest that a poor local blood supply (**ischaemia**) has accrued.
- The **severity** of the angiographic **narrowing** often appears **proportionate** to both the **clinical grade** and the **extent** of resultant **DIDs**[632].
- The timing of **angiospasm** frequently appears **concurrent with** both **clinical** and **radiological** onset of **DIDs**. (In a recent overview, Dorsch and King found that across 12 series and 481 cases, the mean onset was around **7-8 days**[134]).

- The location of **VSM** is usually close to the aneurysm and normally **within** the **vascular territory** of **infarcted area**[146].
- An **increased velocity of flow** is characteristically registered on **Doppler studies** concurrent with the onset of DCI (at 7-12 days[538]) in 30% of individuals[538], this being assumed reflective of an acute narrowing.
- **SAH-CSF** is **vasoconstrictory** (*see below*)
- **SAH-CSF** produces equivalent **angiospasm** and **vessel wall pathological change**

Thus, the development of VSM appeared inextricably linked with DCI; coinciding both in time and space. This is so much the case that, until quite recently, possibly the majority of neurosurgeons universally diagnosed "DCI" interchangeably with "VSM"[632]. This process appeared, teleologically, to reflect an extended post-haemorrhagic vascular spasm, that served to reduce CBF in an area likely to re-rupture[146]. It thus appeared—at least to its original discoverers—to reflect a fundamentally **protective phenomenon**[146]. However, even at this stage the authors acknowledged that "unfavourable effects" could accrue when VSM was either severe or extensive[146].

The concept of blood vessels going into spasm and causing distal ischaemic necrosis is a common one both physiologically and pathologically. For example, a purely physiological form occurs in women at the time of the **menses**. Here the long spiral arteries of the uterine stratum spongiosum develop a functional spasm cyclically every 28 days—this causing infarction of the inner lining (Part 3, 1.5.3). The latter is then shed through the vagina as the next menstrual flow. Another example—occurring both physiologically and pathologically—concerns the arterial sphincters of the dermal glomus bodies during hypothermia. Here, blood is indiscriminately shunted away from the skin in order to prevent unnecessary heat loss during drastic reductions in the ambient temperature. When extreme, however, this can result in dermal necrosis, ulceration and in the acropathological tissue losses of **frostbite**[663].

A purely pathological vasoconstriction and, moreover, one occurring within the cerebrovasculature, is that associated with **migraine**. Here, the aura of the attack may be associated with a VSM of larger cerebral arteries which can be appreciated on angiography. Such VSM, however, is normally short-lived. The resultant throbbing headache possibly reflects gross metabolic dilation (Part 2, 2.5) following the ischaemia necessarily incurred. Whilst probably over-simplistic, the latter mechanism appears particularly attractive in that, where excessive, local cerebral infarcts can result (**hemiplegic migraine**)[86, 589]. Furthermore, the arteries involved may, in long-standing cases, show precisely the same changes (medial myonecrosis and SIT, *see below*) as those seen with VSM[589]. This,

therefore, hints that fundamentally similar processes may be involved in both pathologies: functionally and structurally. Particularly interesting from the point of view of universality is the increased frequency of migraine with menstruation, since both conditions fundamentally reflect vasoconstrictory phenomena.

VSM following SAH, then, could easily be construed as a 'percentage error' of a physiological mechanism 'designed' to prevent the catastrophe of a secondary haemorrhage. Clearly there was a need to know the determinants of VSM's severity, since certainly no other artery after haemorrhage exhibited such sustained contraction[526, 527]. Clues were initially sought on histological analysis of such vessels harvested from terminal cases, or from patients dying later from some other complication.

1.3.1 Vasospasm pathology

Pathologically, there was no doubt that the **vessels** associated with VSM were clearly **abnormal**. This was perhaps a little surprising since, on first principles, such vessels ought to represent otherwise normal vessels 'held' in a state of sustained spasm[100]. Nevertheless, such abnormal vessels certainly corresponded both to the vascular territory of the resultant DID[111, 275], as well as to the region of VSM seen angiographically[177, 265, 554]. In some studies a positive correlation was demonstrated between the degree of angiospasm and the extent of DCI[107, 362]. Furthermore, such changes were little apparent within the first week of SAH[100, 107]. In all cases, the lumen of vessels with documented angiospasm appeared narrowed at fixation relative to controls[399, 554]. Thus, the first major conclusion in the study of DCI was that vessels in VSM were definitely *not* normal. Changes could, in fact, be present in all three layers (Part 2, 1.2):

1.3.1.1 Vasospasm pathology: *tunica intima*

In truth, frank endothelial changes are uncommonly reported in SAH patients: they are more frequent in experimental models. Intimal changes may be more frequently inferred than observed. For example, the presence of contrast-enhancement in close proximity to the source of haemorrhage on a CT (computerized tomogram) scan[188] suggests empirically that BBB breakdown has occurred. Since the BBB predominantly relates to the endothelial lining, it is assumed ipso facto that there has been a derangement in this layer. Nevertheless, some authors have reported a denuded endothelium in cerebral vessels after SAH[8, 170, 178].

In contrast, there frequently are abnormalities reported in the subintimal layer. In particular, a marked corrugation is frequently reported in the internal elastic lamina (IEL)[355, 465], which resembles the appearance of normal vessels when previously

constricted[398]. Other abnormalities in the IEL—such as fragmentation or, even, duplication—may also be seen in such cases. The major change, however, is that of a subintimal swelling. This has been presumed to be the result of fluid transudation and, therefore, a BBB defect[111, 398, 584, 587]. Since frank structural endothelial change is a rarity, BBB disruption is probably the consequence of a functional abnormality[19]. However, the appearance of intramural red and white blood cells, in addition to a frankly "oedematous" picture, tends to suggest somewhat more than this[275] Later on, **subintimal thickening (SIT)** becomes associated with the apparent migration of cells with staining characteristics of **myofibroblasts**[78, 555]. The latter cells are ordinarily encountered with either skin wounds healing by second intention, or with vascular tears. As they proliferate, myofibroblasts deposit copious amounts of ground substance and collagen of varying maturity.

1.3.1.2 Vasospasm pathology: *tunica media*

Although, early on, a slight thickening in this layer may be apparent[399], the outstanding feature is eventually one of a gradual **decrease** in medial **thickness**, accompanied by scattered foci of frank **myonecrosis**[7, 170, 584, 587]. In many studies, this somewhat devastating picture is largely apparent only in the **outer layers** of the media[669].

1.3.1.3 Vasospasm pathology: *tunica adventitia*

The major abnormality reported is that of the presence of blood elements in the adventitial layer after SAH. It is possible that these could either be in consequence of an inflammatory response, or the result of their being pressure-driven there at the time of rupture[362].

1.3.1.4 Vasospasm pathology: vessel injury

The major changes are therefore SIT, IEL disruption, and medial myonecrosis. This, in principle, is quite similar to that which results from **vessel injury** following a variety of stimuli[399]. It is, however, rather **more extensive following SAH**, and occurs at some distance from the rupture site[100]. In many ways the changes resemble those of atherosclerosis[399], and both VSM and atherosclerosis may therefore represent final common reparative pathways. Other authorities, however, have likened appearances to an endarteritis obliterans similar to that seen with either meningitis or Buerger's disease[275]. Interestingly, experimental models of atheroma have shown that such changes are maximal at around 7 days[399]: this concurs with the timing of SIT observed after endothelial denudation[469]. Such results obviously concur not only with epidemiological estimates in VSM timing (*see above*)[134], but also with the intensity of the meningeal inflammatory reaction eventually seen[232]. The concept of VSM occurring as a consequence of vascular

"injury" thus began to materialize (*see below*)[399]. Some, in fact, have even employed direct vascular injury as an experimental model for VSM[577] (Part 4, 2.4)

1.3.1.5 Vasospasm pathology: vasospasm is reversible

Perhaps surprisingly, all pathological changes eventually ameliorate—as both VSM and DCI ameliorate—with time. Thus, there is eventually a **regression** in the degree of **SIT** and, in consequence, an **increase in the luminal diameter**. Such resolution occurs at any time between 2 weeks[398, 424, 669] and 6 months[398]. However, a generally "fibrotic" histological picture continues to remain, with collagen deposition apparent in all three vascular layers[398]. Remodelling back to a state of normality may take anything up to two years[584]. However, that resolution does eventually pertain underscores that VSM is an essentially transient phenomenon. This implies that with other processes, such as atheroma, it is merely the persistence of the injurious stimulus that distinguishes it from VSM. The fact that C-type natriuretic peptide has been shown to inhibit SIT following vessel injury—and that it is predominantly present within cerebral vessel walls[199]—suggests that CNP could be one possible agent that mediates ultimate resolution[199].

In summary, at a fairly early stage in VSM study, two schools of thought concerning the possible mechanism of VSM began to emerge:

- Functional (physiological) factors: **unopposed vasoconstriction.**
- Structural (anatomical) factors: **proliferative angiopathy.**

Of course, there were also those already proposing that *both* schemes were correct[584].

1.3.2 Functional spasm

Certain changes—intimal corrugation[355, 467] and medial myonecrosis[275]—immediately appeared to confirm an excessive and unopposed vasoconstriction as the underlying cause of VSM. For example, the thickened wall and reduced luminal diameter vividly suggested—to some—a state of "frozen" vasoconstriction. Perhaps, then, the rest of the pathological changes necessarily followed on from this[275]. Studies continue, in fact, to propose a functional spasm as the fundamental mechanism[376].

1.3.2.1 Functional spasm: sympathetic tone

An obvious first choice as mediator in this scheme was the sympathetic nervous system (SNS)[7, 170]. It had been known for some time that cerebral vessels possessed a fine network of such fibres on their adventitiae, sometimes even down to the capillaries[504]. Furthermore, an excessive SNS discharge had long been implicated with other systemic pathophysiological effects of SAH. For example, the **Cushing hypertensive response** is

the consequence of both increased total peripheral resistance[659], and increased cardiac output[213]. Similarly, following Aschenbrenner and Bodechtal's first report of electrocardiographic changes in 1938[21], clear examples of frank **cardiac injury** became a popular subject for case reports following SAH[218]. Furthermore, **catecholamines (CA)** quickly became connected with this[158, 380, 485, 602]. That reserpine (RSP) antidepressive pre-treatment obviated these changes (RSP depletes neuronal CAs producing a functional denervation), substantiated the case further still[255].

It seemed reasonable, therefore, to link such a SNS "storm" with a sustained and, ultimately, harmful spasm of cerebral vessels. Certainly, acute bursts of circulating CAs can follow abrupt ΔICPs[158, 552]; whilst high local CSF CA concentrations have recreated both angiographic and pathological VSM[7, 669]. In this regard, some have demonstrated that NE accumulates within smooth muscle cells of various organs following previous CA overload[74, 462]. The characteristic histological finding of sympathetic terminal boutons apparently depleted of neurotransmitter[190, 483] further corroborates such a SNS "storm".

The problem with the latter concept is that a continuous state of contraction spanning several days from the acute ictus does not appear to be the case[647]. Certainly a 'lucid interval' usually separates any early VSM from the delayed VSM associated with DCI (although some may disagree[632]). Although VSM may represent an attempt to divert blood away from a dangerous area, the concept that it is a *direct* extension of any acute vasospastic process appears somewhat untenable. This is particularly pertinent when one considers that tachyphylaxis, as a consequence of agonist-induced desensitization, is rather a feature of the CAs acting at adrenoceptors (Part 3, 3.2).

In an alternative scheme, Alksne and Greenhoot suggested, in 1974, that the initial intense vasoconstriction might then itself initiate a self-propagating, self-destructive mechanism in adjacent cells, as a chain reaction spanning several weeks[7]. Exactly how this might be achieved, however, could not be elaborated upon. Another possible mechanism instead proposed that VSM resulted from a gradually-developing **Denervation Super-Sensitivity (DSS)** phenomenon[79] (Part 3, 3.3). Thus, following on from the SNS neuronal 'burn-out', such a DSS could accrue several days hence[2, 125, 162, 367, 368, 435] (Part 3, 3.3).

1.3.2.2 Functional spasm: local effects of the blood clot

Attention soon turned, however to blood, and blood products, as possibly containing the appropriate vasoconstrictory stimulus. Blood ectopically located within the usually clear CSF had long been implicated as the cause of the irritation—the '**chemical (aseptic) meningitis**'[164, 232, 288]—associated with 'post SAH meningism'. Similar degrees of

inflammation were also apparent with major vessels after acute bacterial meningitis[106, 489, 593, 670] or head injury[387, 580]. In both cases, a comparable VSM may also be seen. That this can be non-specific is demonstrated by similar appearances following either the addition of talc[440] or vessel trauma[576]. However, intracerebral bleeding— whilst presumably causing comparable ICP-loading—*rarely* results in either VSM or DCI[275]. In consequence, blood ectopically located within the subarachnoid space might serve as a non-specific irritant to the exposed vasculature. That blood application could also, however, result in an acute **vasoconstriction** was not shown until 1971, when Echlin demonstrated this angiographically[148]. Numerous factors subsequently supported an aetiological role for clot in VSM:

- The laterality of the VSM: **VSM** often occurs **adjacent** to the **clot**[107, 111, 275].
- The **size** of the **clot** often appears proportional to the extent of both VSM and the DCI induced. In fact, clot size (assessed by CT scan) is often used as a **prognostic indicator** for VSM development[177, 675].

(Both of these points better fit the localized nature of the VSM in a way that a generalized SNS over-response can not).

- Conceivably, a **poor washing action** of the **CSF** in the vicinity of the clot might **allow for sustained agonist effect**[614]. The fact that hydrocephalus invariably complicates SAH[52] might also, as a result of poor CSF drainage, elevate effective agonist concentrations (Part 1, 2.5).
- Owing to the abnormal anatomy of the tunica adventitia in cerebral arteries (Part 2, 1.2), **agonists** have been shown to **penetrate** the wall more easily from the **abluminal border**[310, 468].
- Blood clot contains most of the agents (e.g. **5HT**, **NE**, and **UTP**) that are incriminated in other vasoconstrictory phenomena, such as with Raynaud's disease and migraine.
- Blood clot also contains elements known to be associated with sub-intimal proliferative phenomena such as **platelet-derived growth factor (PDGF)**. Such agents are known to be involved with the formation of similar degrees of SIT in other disease states.
- An abundance of experimental studies has confirmed that the pathological, physiological and angiographic features of VSM can be reproduced by the local application of autologous blood. That these changes were effectively abolished by RSP pre-treatment in the blood-donor implies that the agent required was **platelet-borne** (and therefore possibly 5HT, NE or UTP)[584].
- The apparent **effectiveness of clot removal** in alleviating VSM by irrigation[457], fibrinolysis[173, 540] or by operation[425, 465, 526, 570] implicates the clot as the major offender.

- Conceivably, the application of thick clot to the precarious adventitia may interfere with vessel wall metabolism[484, 548, 676] (Part 2, 1.2).

In summary, an abundance of evidence appeared to correlate VSM directly to the presence of CSF clot. In fact, to many authors, subarachnoid clot continues to represent the *conditio sine qua non* of VSM and DCI[539]. Certainly, the presence of subarachnoid clot satisfies two minimal requirements in causing cerebral vasoconstriction observable angiographically, and in causing a representative vessel morphological change.

It is worth mentioning, however, that VSM can occur in the presence of *clear* CSF[215, 265]. Although rarely reported, such occurrences must be awarded due regard when attempting to delineate the mechanism involved. Furthermore, almost all of the above points have, over the years, suffered some degree of detriment under the scrutiny of closer re-examination. For example, VSM may not always correspond to the site of the clot, and any prognostication based upon its size is often erroneous[530, 583]. It is questionable that a *major* source of nutrition derives from the CSF[468], whilst agonists are frequently more effective *intra*luminally than extra-luminally[629]. Moreover, the vessel wall itself metabolizes many agonists, and a persistent supply could, conceivably, be quite easily metabolized[618]. Following clot removal, both VSM and vessel morphological change can continue on unabated[6]; whilst non-physiological doses of catecholamines *alone* can cause a myonecrosis *and* a widespread meningeal fibrosis[7].

1.3.3 Structural spasm: proliferative angiopathy

By 1974, the importance of the structural changes in the vessel wall had begun to be stressed[7]. Not only important as a possible manifestation of generalized inflammation, the extensive cellular-proliferative and noncellular changes gradually became accepted in their own right as possibly being causal to VSM and DCI[94]. Thus, proliferative change can result in a **non-compliant** fixity of **contracted state**[48, 100, 668]. By the resultant loss of normal elasticity at normal pressures—it was argued—the necessarily the less would be the effective functional diameter[48]. In fact it has been estimated that by the latter mechanism, such a **reduction** in **effective luminal diameter** could amount to anything up to 40% from that of normal[191]. This, of course, bears strikingly close agreement to that seen angiographically[584]. Furthermore, increased rigidity has been shown to be present even upon the total abolition of all myogenic tone from the vessel wall[326]. Thus, it may be that the 'aseptic meningitis' principle is in fact the more important one: with **inflammatory changes** throughout the subarachnoid space **affecting** the **mechanical properties** of the major cerebral vessels[94]. Such reasoning explains several short-comings of the "vasoconstrictor" hypothesis:

- The decreased number of vascular smooth muscle cells (VSMCs) in the tunica media of vessels in VSM suggests that such a vessel would be less able to maintain vasoconstriction: this is directly evidenced by studies that have demonstrated reduced contractile responses[94, 289, 472, 598].
- The migratory cells present in the subintima as part of the process of VSM resemble the **myofibroblasts** seen in wounds healing by second intention. The latter function to **contract** and then **hold together** the margins of the wound, by the deposition of a collagen matrix[78, 554]. The presence of these cells could easily explain VSM.
- Although, without doubt, SAH-CSF samples are definitely vasoconstrictory[57, 64], the vasoconstrictory profile does not correspond temporally to either VSM *or* DCI[527]. However, **SAH-CSF** can promote the **transition of SMCs to myofibroblasts**, at least in tissue culture[668]. This suggests that a proliferative angiopathy might predominate.
- Many studies have demonstrated a distinct **lack of efficacy** of common **vasodilators** to reverse VSM[623, 655].
- The apparent **effectiveness of trans-luminal angioplasty (TLA)** in the treatment of VSM and DCI is a strong argument in favour of the fact that the major pathophysiological change is that of a vessel wall 'fibrosis'[449].
- A **myonecrosis** can occur **without** a persistent **vasoconstriction** having been in evidence[7]. This is so where no intimal corrugations were in evidence following application of blood, 5HT and barium chloride[398].
- There is abundant evidence that has demonstrated **immunological change** with in the **vessel wall**. Thus, there is leucocyte adherence at sites of intimal corrugation[444], endothelial expression of intercellular adhesion molecule 1 (ICAM-1)[235], and class II antigens, and the presence of interleukin 1 (IL-1) within the vessel wall[555]. In particular, myonecrosis has been shown to be associated with an immunopathy[235] for which cytokines have been heavily implicated[204-206]. Furthermore, there is evidence of a **generalized systemic inflammatory reaction**, with systemic activation of complement[439] and a peripheral leucocytosis[445].

Nevertheless, vasodilator therapy—particularly when given locally—can successfully reverse angiospasm in approximately 50% cases[400]. In fact, there has been a recent renaissance in local papaverine therapy-particularly at angiography[99, 102, 319, 400, 608, 622]. Furthermore, an increased rigidity per se does not rule out a functional constriction in aetiology, since the latter can be a feature of other diseases also characterized by persistent constriction (such as Hirschprung's disease of the rectosigmoid—Part 2, 3.4). Moreover, the aforementioned 'loss of elasticity' principle cannot adequately explain reduced diameters in cerebral arteries because the development of myogenic tone to distension

would tend to offset elastic distension (Part 2, 2.2). In consequence, a proliferative angiopathy may not successfully explain every case of VSM or DCI. Instead, a combination of factors may be implied.

1.4 Red blood cell lysis and free radicals

The effects of subarachnoid clot continue to appear integral, in the majority of cases, to the development of VSM. Several constituents of the clot may contribute in this fashion. The contribution of platelets (containing NE, 5HT, UTP and PDGF) have already been alluded to. Most current hypotheses on VSM, however, concentrate upon the effects of red blood cells (RBC) and their contained haemoglobin (Hb). In particular, they centre upon the effects of substances released by RBC lysis over ensuing days.

1.4.1 Haemoglobin and its derivatives: contractile effects

Initially, the **oxyhaemoglobin** (HbO_2) released after SAH becomes desaturated and, within a first few hours, **deoxyhaemoglobin** begins to accumulate. The process is probably complete within about 72 hours, but this clearly depends upon clot size. That both agents can potentially cause cerebral arterial constriction was initially an exciting discovery[148]: unfortunately, contractile effects appear greater in animals than in humans[607]. Hb/HbO_2 can cause vasoconstriction directly by PIP_2 activation and the release of IP_3 (Part 2, 3.5.2): however, this is probably more slower than with other agonists. Nevertheless, the contraction can be a prolonged one, spanning several days[196]. Hb/HbO_2 can also **inhibit the transfer of EDRF**[386] by extracellular scavenging of NO (Part 3, 1.8). Perhaps the major mechanism of Hb/HbO_2 action, however, involves the enhancement (or the de novo release) of other agonists, from the vessel wall. Thus, Hb/HbO_2 can **release endothelial prostanoids**[601] and **stimulate endothelin biosynthesis**[466]. Hb/HbO_2 can also potentiate the activity of other agonists[375]: for example, it directly **potentiates a 5HT constriction**[316, 338, 650] **as well as** that of **NE** and **PGF2α**[316, 338] (although in all three cases only with intact endothelia). Therefore, several of these mechanisms show that Hb/HbO_2 particularly works by the **enhancement of pre-existing vascular tone**.

1.4.2 Free radical formation

With prolonged application of Hb/HbO_2, however, different variables arise. Hb/HbO_2 is eventually decomposed into methaemoglobin (methHb) over several days. Not only is the latter prolonged exposure related to the prolonged production of eicosanoids, it is also related to increased release and activity of free radicals. The problem is that when HbO_2 is converted to methHb **superoxide anion** is formed[423]. This, in turn, either directly (or indirectly via **hydroxyl ion, singlet oxygen**, or **alkoyl radical) initiates free radical reactions**, such as the peroxidation of polyunsaturated fatty acids (PUFAs) within the biomembrane[527]. Furthermore, some degradation products of Hb such as haematin, iron

ions and metHb, in turn powerfully catalyze PUFAs to their peroxide forms (hydro- and endo-peroxides); and nearby leucocytes also release superoxides[527]. Thus, the degradation of Hb is instrumental to the release of many deleterious agents.

1.4.3 Vasospasm as a deficiency syndrome

Normally, however, **superoxide dismutase (SOD)** converts superoxide to H_2O_2, which is then inactivated by either **glutathione peroxidase** or **catalase**[527]. SOD, glutathione peroxidase and catalase are all **anti-oxidants**, and tend to stabilize membranes which have been exposed to free radicals in a fashion similar to that with either **NADPH** or **vitamin E**. They thus function as **free radical scavengers**. Anti-oxidants are normally present in significant concentrations within the serum and parenchyma of body tissues, but for some unknown reason appear particularly sparse centrally[527]. In fact, vitamin E and catalase are undetectable in the CSF, whilst glutathione peroxidase is barely detectable at all[527]. Some, then, have proffered the interesting concept that VSM may be a consequence of such central deficiency of anti-oxidants, the detrimental effects of blood degradation being poorly contained here[527]. Such effects can be appreciated with the cellular damage apparent in hereditary acatalasia[161]. However, CSF anti-oxidant concentrations usually rise after SAH and, indeed, some have demonstrated that the cytokines so liberated by SAH (especially IL-1 and TNF) further induce their expression[395]. In addition, glucose-6-phosphate dehydrogenase (G6PD) is also increased[70], whilst many patients with acatalasia experience no untoward consequences throughout their entire lives[161].

Nevertheless, lowered basal anti-oxidant levels (or interference by iron complexes within the vessel wall) may conceivably allow superoxide or H_2O_2 to produce singlet oxygen or hydroxyl ion via the **Haber-Weiss reaction**[527]. Superoxide also reacts with PUFA-hydroperoxides (impurities) to form the alkoyl radical. All of these free radicals combine with PUFAs to produce organic (free fatty acid) radicals which react with O_2 to produce **lipid peroxides**—thus producing a chain of lipid peroxidation. Lipid peroxides can cause both a potent vasoconstriction and, ultimately, endothelial disruption[527].

Thus, a large number of potentially deleterious agents accumulate several days after Hb degradation. Additionally, some have found significant levels of detergent-like ampiphiles (such as lyosophosphatidylcholine) peaking at around 10 days: these are very effective at damaging membranes (particularly myelinated ones)[143]. Furthermore, arachidonic acid itself also causes **detergent-like effects**[655]. This is important because not only do detergents threaten **endothelial breakdown**, they also cause **vasoconstriction**[96, 320, 321]: this being necessarily delayed. Both vascular damage and vasoconstriction may therefore be explained by the effects of clot lysis over ensuing days.

1.5 Caveat: Vasospasm qua delayed cerebral ischaemia?

The structural-functional debate over the mechanism of VSM has somewhat detracted attention away from questioning the place of VSM *qua* DCI. Indeed, anecdotal clinical evidence has increasingly questioned this association. In a recent overview of 13,490 cases (across 223 studies) by Dorsch and King[134], 43% of patients overall developed VSM. This figure, although quite close to that of DCI (33%), is still in excess of it. However, studies that have additionally focussed angiography into the second week after SAH (2738 cases across 38 studies) have inadvertently increased the proportion to 67%[134]. Thus, although the initial figure for VSM more closely resembled that of DCI, this may have been due to a gross under-reporting in angiographic documentation.

The importance of correct timing in angiography (i.e. into the second week) has previously been stressed[555]. Some have even—somewhat rashly—extrapolated the conclusion that daily angiographic studies would reveal that the proportion developing VSM approaches 100%![653]. Nevertheless, since the majority appear to develop VSM at the beginning of the second week[134], it would seem likely that studies based on a single first-week angiogram have inadvertently lowered the true estimate. Under-reporting could, of course, also be applied to DCI figures tending to restore the initial association. In fact, a whole range of confounding factors could thwart statistical analysis in these matters. These would include the lack of agreed narrowing considered representative of angiospasm; and the frequency of excluding groups from study (especially those of poor clinical grade).

Nevertheless, the best data available tends to support anecdotal clinical impressions that **VSM does not invariably equate with DCI**[170]—VSM appears the more common. Moreover, whilst most experimental models have successfully demonstrated a comparable VSM few, if any, have managed to recreate a corresponding DID. This may just be because only **VSM** of a **certain degree** causes DCI, as Ecker and Reimenschneider originally postulated[146]. The latter is suggested in some studies that have demonstrated a correlation between severity of narrowing and DCI[632]. However, perhaps a more sensible policy is to accept that **other factors** may **contribute** to the effects of VSM to appear collectively responsible for DCI[413]. To emphasize this, there have been repeated appellations in recent times to clearly **dichotomize VSM** and **DCI**, and to cease the hitherto practice of using both terms interchangeably[409].

Chapter 2
Contributory factors to delayed cerebral ischaemia

2.1 Introduction

Several factors may contribute toward DCI development. When considered purely as an ischaemic phenomenon, the most important would be the effective **circulatory delivery** of **oxygen** and **glucose** to the cerebral parenchyma. Assuming adequate lung function, this depends upon the cerebral perfusion pressure, (CPP). This is the mean arterial pressure minus the intracranial pressure:

$$CPP = [MAP] - [ICP]$$

2.2 Gradually decreasing cerebral blood flow

The acute falls in CBF (Part 1, 1.1.1) experienced with the acute ictus usually quickly recover[18, 222-224, 454]. However, secondary falls in CBF may follow in the ensuing days[453]. One cause is certainly cardiogenic dysfunction. The latter seems distinct from that which causes arrhythmias in the immediate post-SAH period[351], and may eventually prove terminal not only for the patient but also for any potential recipient, should transplantation subsequently take place. In fact, **donor heart dysfunction** is a major scourge of cardiac transplantation, often occurring along with ARDS[458-461, 552].

Nevertheless, a gradually decreasing CBF may occur without any overt cardiovascular dysfunction, this being a peculiar feature of SAH. In documenting this fact, Meyer and his colleagues assessed CBF in the post-SAH period by the use of the ^{133}Xe inhalational method[413]. In a now classical study, they demonstrated the astonishing fact that the global CBF gradually **falls in all cases** following acute SAH during the **first two weeks**, this occurring as a secondary phenomenon over that of the acute episode[413]. This fall was particularly **greater** in patients in the **poorer clinical grades** and with **older age**, and tended to reach its **nadir** at around **14 days post ictus**. Thereafter it gradually commenced its rise back up to normal values[413]. Similar findings have been reported by others[327]. Clearly, this mysterious process will only serve to worsen any relative ischaemia already being experienced at local levels (i.e. VSM)[583].

The precise cause of this confounding process may, however, vary between individuals. For example, in addition to possible cardiac causes, hypothyroidism (which may be in evidence in up to 45% patients following SAH[673]) is associated with a definite **decrease in circulating volume**[496]. Since some 23% may also suffer Adreno-corticotrophin hormone depletion after SAH[673] this factor may become ever more important. Furthermore, many

patients in this condition appear decidedly drowsy[163, 396], and slow wave sleep is associated with significant CBF reductions[517, 663]. But it is the disturbances in plasma sodium levels, and their associated syndromes, which appear particularly linked to this process (*see below*).

2.3 Decreased cerebral metabolism

The insidious development of global cerebral hypoperfusion is often accompanied by a diminution in cerebral metabolism[222-224] (Part 1, 1.2). The two processes are, of course, directly linked, this denoting metabolic regulation[411] (Part 2, 2.5). It is uncertain which of the two processes represents the *primary* disturbance. Some studies have shown that the reduction in the $CMRO_2$ after SAH is in excess of the CBF. This indicates global "**luxury perfusion**"—a CBF in excess of apparent requirements. Luxury perfusion is more apparent in the **better clinical grades**[632]. The correlation between a poor $CMRO_2$ and a poor CBF is much closer in the poorer clinical grades[632]. Perhaps, then, the fall in the $CMRO_2$ is the primary disturbance—the fall in CBF merely following on. Certainly the frequent drowsiness seen[396], the frequently retarded electro-encephelogram, the observed reductions in $A\text{-}VDO_2$[632], and a CSF lactic acidosis[195, 487, 632] all perhaps tend to support the view that the **primary disturbance** is with **cerebral metabolism**. Nevertheless, there is ample evidence that the circulating volume is frequently reduced after SAH[559], (*see below*).

2.4 Electrolyte disturbance

Disturbances in plasma sodium levels frequently occur in the post-SAH period. Their importance resides not only in their frequent association with the onset of DCI, but also in the way they are treated. Although the predominant cause of hyponatraemia was formerly held to be the syndrome of inappropriate anti-diuretic hormone (SIADH) secretion, this is probably the rarer cause[283, 448, 646]. Thus, the **major causes** of both hypo- and hyper-natraemia **involve** a relative **loss** of **circulating volume,** therefore linking in with the process of Meyer above[413]. In support of this, more than one study has shown evidence of lowered central venous pressures (CVPs) indicating lower circulating volumes[553], one also showing increased plasma levels of atrial natriuretic factor concurrent with reductions in ADH[283].

2.4.1 Hypernatraemia

This is most often due to a failure of posterior pituitary secretion of ADH. It results in the loss of excess water in the urine (**Diabetes Insipidus**), and a **decrease** in the **circulating volume**. It is therefore associated with an **increased plasma osmolality** and increased viscosity. In the former capacity it may offset cerebral oedema formation, but in the latter capacity it may worsen any ischaemia whilst also promoting intravascular coagulation. A

massive depletion in this agent often accompanies the agonal period of brain death, so much that ADH administration is often required to restore circulatory stability, particularly pending a potential transplantation[287].

Table 2 Contributory factors to the development of DCI

Pulmonary dysfunction

1. Central factors
   - Decreased conscious level
   - Direct brainstem factors
2. Pulmonary factors
   - Pneumonia - decreased conscious level
   - ARDS
   - Cardiac failure

Cardiovascular dysfunction

1. Circulating volume
   - Decreasing CBF
   - Natriuresis
   - Hypothyroidism
   - Ant pituitary dysfunction
   - Diuretics - antihypertensives
   - Fluid overload - SIADH
   - Hypervolaemic hypertensive therapy
2. Cardiac Function.
   - Acute dysfunction - catecholamines
   - Chronic dysfunction - others
   - Hypervolaemic hypertensive therapy
3. Systemic blood pressure
   - Hypervolaemic hypertensive therapy

Cerebrovascular dysfunction

1. Dysautoregulation
2. Diminished CO_2 regulation (and ∴metabolic regulation)
3. Hypervolaemic hypertensive therapy

Hydrocephalus

2.4.2 Hyponatraemia

This is often synonymous with **plasma hypo-osmolality** since $[Na^+]_{plasma}$ is the major determinant of the latter[65]. Hypo-osmolality threatens **cerebral oedema** formation. It is most likely due to the secretion of a natriuretic factor possibly from the CNS itself[133, 646]. It is thus often referred to as the syndrome of **Cerebral Salt Wasting (CSW)**. It results in the excess loss of salt and water from the body and in a decreased circulating volume[553]. Numerous substances liberate **atrial natriuretic peptide (ANP)** such as catecholamines,

endothelin and calcitonin gene related polypetide[200]; whilst calcium channel blockers inhibit ANP[518]. The fact that there are increased circulating catecholamine levels five days after SAH may be relevant in this setting[485]. Interestingly, the most recent member of the natriuretic peptide family, **C-type natriuretic peptide (CNP)**, is most frequently found within the CNS. This agent may have especial relevance to VSM since it **decreases myoproliferation** within the vessel wall[199]. Thus, it is conceivable that CNP remodels the structural changes wrought by acute SAH (Part 1, 1.3.1.5); in addition to explaining why hyponatraemia occurs as a side effect from the second week onwards.

SIADH, by encouraging excess water-retention at the distal renal tubule, will cause the opposite effect. Thus, a significant prevalence of SIADH would be expected to inversely relate to the 'Meyer' effect. However, both SIADH and CSW may contribute to the *worsening* of DIDs by diminishing plasma osmolality and, thus, by encouraging osmotically-driven cerebral oedema. It is important to exclude the (probably) rarer SIADH as a cause for hyponatraemia, since the treatment here is primarily one of fluid restriction. As aforementioned, the majority of patients after SAH develop a relative cerebral hypoperfusion: fluid restriction could, thus, prove calamitous.

2.5 Hydrocephalus

Although the ΔICP experienced acutely after SAH soon falls to near-normal levels, there is often a secondary rise that commences some hours (or even days) later[257, 453]. It is important that the cause of this ΔICP be documented since this may not necessarily be due to hydrocephalus. For example, Hayashi et al—whilst noting that patients with poorer clinical grades tended to have higher ICPs—concluded that this resulted from a dysautoregulation and a subsequent cerebrovascular paralysis[257]. Nornes and Magnaes had also previously noted the latter which, when acute and severe, often served as a harbinger of recurrent haemorrhage[453].

Nevertheless, hydrocephalus frequently does complicate acute SAH. This may be purely obstructive, the result of thick basal clot disrupting CSF flow; but it may also be 'communicating' and develop after some days. The latter is most likely the result of a relative failure of normal CSF outflow mechanisms via the arachnoid granulations. The mechanism is often held to relate to blood debris plugging the CSF outlet: however, this may be far too simplistic. For example, in Cushing's studies, mercury globules released into the CSF easily found their way across this barrier (Part 6, Section I, 1.4.2). Returning to the "irritative" principle of ectopic blood[288, 232], inflammatory changes have been observed to red blood cells trapped in arachnoid villi one week after experimental SAH[543]. That an inflammatory component may be involved is further suggested by the improvement

in hydrocephalus with low dose methylprednisolone[660]. The risk of hydrocephalus appears greater with a larger amount of clot.

Notwithstanding the precise cause, Black has estimated that the **majority** (70%) of patients experience **some degree of HC** following SAH[52]: others concur[632]. However, in only 15-20% does a state of "normal pressure" hydrocephalus (NPH) eventually supervene as a chronic problem[52]. By increasing still further the ICP, the **effective CPP** is **reduced** with hydrocephalus, this aggravating further any CBF compromise that is already extant. But hydrocephalus may be doubly harmful where it relates to diminished CSF drainage, since this will result in an **increased** effective **spasmogen concentration**, with increased risk of VSM.

2.6 Cerebrovascular dysfunction

This has already been alluded to in the previous section. Several non-invasive studies of CBF have demonstrated anomalies in both auto- and metabolic- regulation at the time of DCI[222-224, 257, 453]. Since both processes are crucial to matching CBF with metabolic requirements, such disturbances may both prove critical under even normal circumstances. However, they would clearly serve to amplify any disturbance that has already occurred in regional CBF as a result of VSM.

Although the two processes are entirely different in the way that they affect CBF regulation, metabolic regulation is often confused with autoregulation in the literature, CO_2-regulation often being referred to as 'CO_2-autoregulation'[289]. Their differences, however, become even more apparent pathologically, since one is often affected more than the other. Thus, whilst autoregulation may be lost early[170], and be globally impaired irrespective of the clinical grade[289], **alterations in CO_2 regulation** are usually more **mild** and **local**, and **correlate** more **closely** with **clinical grade**[590, 632]. In consequence, metabolic regulation appears somewhat more robust[615]. This is perhaps not too surprising, since metabolic regulation is a basic one—and absolutely essential for survival. For example, the tissue acidity seen around the time of DCI should result in a paralysis of pressure autoregulation[129], this representing at least one possible mechanism for dysautoregulation after SAH. It is also possible, however, that SAH may disrupt both autoregulation and metabolic regulation, since both are similarly affected after cerebral ischaemia[285]. Clearly, a failure in both may significantly contribute to a failure of 'supply' to satisfy 'demand'.

2.7 Summary

Those surviving either primary or secondary SAH may still succumb to the syndrome of Delayed Cerebral Ischaemia. The cause of this has, ever since its original discovery, been

considered due to a vasospasm of major cerebral arteries associated with the clot. However, since DCI is probably much less common than VSM, the latter must either be particularly severe or be associated with other factors, in order to result in DIDs. Such factors could include global reductions in CPP—in turn related to decreases in global CBF and increases in ICP—since these both occur in the majority of patients. Cerebrovascular dysautoregulation and altered plasma osmolality could also contribute. The cause of VSM itself is divided between those believing in a persistent vasoconstriction, and those believing in a proliferative angiopathy shrinking the vessel as in a wound healing by second intention. In the following chapter we will review various treatment policies that have been devised to combat some or all of the aforementioned and putative contributory factors, and discuss their relative efficacy.

Chapter 3
Treatment policies for delayed cerebral ischaemia

General

Circulating Volume	Hypervolaemia
Hydrocephalus	External ventricular drain

Specific

Functional	Vasodilation
Structural	Transluminal Angioplasty
Blood clot	Clot removal
Free radicals	Antioxidants

3.1 General treatment policies

Certain basic aspects of management such as optimization of oxygenation, analgesia, laxatives, and shade from bright lights, will all be assumed. This section is a necessarily brief review of treatments designed specifically to combat DCI and VSM.

3.1.1 Hypervolaemic hypertensive therapy (HHT)

In view of the findings presented in Part 1, 2.1 above, hypervolaemic hypertension would seem a sensible, albeit empirical, policy. Yet in spite of probable widespread use over several years, broad statistical analysis has failed to show that it has had any obvious influence on either morbidity or mortality[20, 134]. Some have advocated that HHT actually works by principally **improving haemorrheology** via decreased viscosity: particularly when Dextran is used as an expanding agent. However, one can identify several areas where HHT may prove counter-productive:

- Given the prevalence of dysautoregulation post SAH, high levels of MAP may threaten oedema formation and, possibly, capillary damage[115, 285, 360].
- Unless early surgery is undertaken, such therapy risks re-rupture.
- There is a high risk of cardiac arrhythmias in the early post-SAH period. In patients in the poorer clinical grades, with high circulating catecholamine levels, this may become associated with a frankly dysfunctioning heart. "Donor heart dysfunction" is a major cause of subsequently failed heart transplants in patients dying of brain-stem death[458-461, 548]; a high fluid load here risks exacerbating this state.
- An abnormal 'leakiness' of the pulmonary microvasculature—resulting in ARDS—complicates a certain proportion post SAH.
- A state of fluid overload can accompany volume expansion with SIADH.

- Many patients develop SAH in older age where there are ordinarily higher risks of both cardiac and cerebrovascular disease.

3.1.2 External ventricular drain

From first principles, it would seem sensible to reduce the ambient ICP in order to facilitate a greater CPP. This may be particularly the case around DCI, where global falls in CBF appear concurrent with secondary ΔICPs. Because of the nature of the fluid to be drained (contaminated as it is with blood and proteinaceous debris) a standard ventriculo-peritoneal shunt would be unwise, given the risk of coagulum obstruction. Furthermore, as only a small proportion of patients subsequently proceed to develop NPH, a non-permanent procedure would seem even more justified. Some have demonstrated clinical improvement by the use of an external ventricular drain[567]. Against this, however, must be balanced the **risk** of **precipitating secondary haemorrhage** in consequence of increased pressure-differentials across the sac wall[453, 632]. Furthermore, **infection** also becomes an ever-present risk, particularly after 5 days. This is precisely the time when both VSM and DCI are likely to materialize. As a result, EVD insertion could profitably be deferred until the end of the first week.

3.2 Specific treatment policies

3.2.1 Systemic vasodilation

Early attempts to influence VSM centred upon reversing a supposed vasoconstriction. Thus, drugs used to treat conditions such as Raynaud's phenomenon were initially considered: Table 3 lists some of those commonly tried. The use of CO_2 inhalation[436] was probably more theoretical than practical: for example, it would be contraindicated with pulmonary dysfunction. Furthermore, as we have already seen, metabolic regulation is often disturbed after SAH—particularly in the poorer grades, where both VSM and DCI are more likely. Nimodipine was originally developed as a calcium channel blocker, with vasodilatory action specific to the cerebrovasculature: however, it is likely that other of its effects are more pertinent in the sphere of DCI (*see below*).

The major problem with all vasodilators, when given systemically, is that they all risk CBF compromise by virtue of induced **systemic hypotension**. Whilst MAP values of 65 mmHg may not ordinarily be a problem[378], the dysautoregulation apparent after SAH (*see above*) renders such hypotension problematic. This should rarely apply to nimodipine, however, since its action is specific to the cerebrovasculature: but it is an ever-present risk with PPV or SNP. Furthermore, systemic vasodilators dilate the cerebrovasculature indiscriminately: as a result, they risk **vascular "steal"** from areas not able to respond—such as vessels in the region of VSM (Part 6, Section II, 3.7).

3.2.2 Local vasodilation

Because of the risks associated with systemic hypotension, there has been an increase in the use of local therapy in order to obviate systemic side effects. Although most of the drugs listed in 3.2.1 above could be applied, in practice only PPV tends to be given. This can be administered **extraluminally** via an in-dwelling catheter exteriorized following craniotomy[319], or **intraluminally** via the catheter used at angiography. Encouraging clinical responses appear to be obtainable in up to 50% cases[400].

Table 3. Vasodilators used to reverse VSM

Systemic
CO_2 Inhalation
5HT antagonists
Sodium nitroprusside (SNP)
Papaverine (PPV)
Nimodipine
Angiotensin converting enzyme (ACE) inhibitors
Local
Papaverine (PPV)
Lignocaine
β receptor agonists (e.g. isoprenolol)

3.2.3. Anti-oxidant therapy

This is a relatively new concept which targets the deleterious effects of the **degradative** products that accumulate during **clot lysis** (Part 1, 1.4). It is still a mainly theoretical therapeutic option, but some have gained ethical clearance for their use clinically. For example, Asano et al have used the free radical scavenger "Nicaraven" and noted improved outcome at one month after SAH[20]. In this context, the use of **non-steroidal anti-inflammatory agents** (such as indomethacin) may also be described, since they may reduce the ultimate production of prostaglandins and leukotrienes[94].

3.2.4 Nimodipine

This agent must be briefly discussed separately. Although primarily used as a vasodilator, its major action is probably as a **neuro-protective agent**. One cellular event associated with the terminal stages of ischaemia is that of extracellular calcium influx: nimodipine can prevent this. Furthermore, nimodipine can also **prevent atrial natriuretic peptide (ANP)**

release, thus potentially also limiting the risk of CSW. The effectiveness of nimodipine has been established clinically, in a multi-centre trial[492].

3.2.5 Trans-luminal angioplasty

When VSM is considered as largely a more fixed 'fibrotic' state, somewhat similar to atheroma, trans-luminal angioplasty (TLA) has a clearly defined role. Its increased use underscores the concept of a proliferative angiopathy with VSM[449]. Its use, however, highlights many of the paradoxes associated with VSM and DCI. For example, the trauma of TLA should, in principle, act as a trigger toward the further development of VSM (Part 4, 3.1). That DCI, however, only occurs with VSM in the *subacute* stage of SAH, suggests that VSM must be linked to some other process in order to prove critical (Part 1, Chapter 2).

3.2.6 Clot removal

If VSM and DCI are primarily due to the presence of the clot, then its prompt removal should effect adequate prophylaxis. This, however, may have other fortuitous effects, since the hydrocephalus that complicates so many cases may also be fundamentally due to the presence of clot debris. Certainly, the ICP can be lowered by such intervention[453]. Conceivably, clot removal can be achieved by operative evacuation, saline irrigation via cisternal cannula, or fibrinolysis (again by cisternal cannulation).

Operative removal, to some extent, probably occurs in most operations for securing the aneurysm neck. However, its specific performance has been advocated in many studies; particularly within 48-72 hours post ictus[425, 465, 526, 570]. Nevertheless, the practical problem of picking away all of the clot, in piecemeal fashion, is fraught with dangers all of its own. These include further vessel trauma, brain swelling and the need for repeated craniotomies. Furthermore, an early operation specifically performed with this as its major aim will be doubly confounded upon its failure, since fibrinolytic therapy—the current vogue—will then be obviated (*see below*).

To improve upon the dangers and failure of operative clot removal others have resorted to the insertion of cisternal cannulae, and the subsequent use of **saline irrigation**, over the ensuing days. The efficacy of this manoeuvre was mostly demonstrated in animal models[148] where the critical time for this was again established as being within the first 48 hours[457]. In truth, however, saline irrigation is often combined with prior operative removal. This means that such cases are, in reality, statistically ones of *combined* therapeutic intervention. Furthermore, in most animal studies the 'SAH' produced is not always representative of the clinical situation, the experimental clot being far easier to

remove. Perhaps it is not too surprising, therefore, that several groups have since reported upon the lack of efficacy of clot removal[231, 252, 366, 480].

At first glance, **fibrinolysis** appears to be a manoeuvre somewhat fraught with risk since, after all, the rupture site is probably only secured by the very clot to which this therapy may be directed. Nevertheless, fibrinolysis has been increasingly—and successfully—used without this logically-perceived danger being realised[173, 540]. Ordinarily, copious amounts of plasminogen are co-released into the clot at the site of vessel trauma, where they quickly bind to the fibrin polymers. However, in order to effect a subsequent fibrinolysis these amounts of plasminogen need to be activated by a 'plasminogen activator'. Whilst this is the rate-limiting step in most clots, it must be particularly so after SAH, since the CSF is deficient in these 'activator' molecules[173]. The most suitable agent in this regard is that of the genetically-engineered tissue plasminogen activator. This is not only because of the latter' considerably diminished antigenic properties, but also because it is reasonably "clot-specific". Thus, some have argued that it may predominantly dissolve neothrombus formation: in particular that present *extraluminally*. It certainly seems that tissue plasminogen activator is much less likely to provoke a systemic fibrinogenolysis[626]. Of course, its use would be **contraindicated** following any **recent surgical procedure**.

Notwithstanding the resurgence in clot removal, a strong cautionary note is proffered by Alexander and Black. These authors have demonstrated that, in spite of successful clot irrigation, neither the course *nor* the severity of either angiospasm or morphological change, was altered as a result[5, 6].

3.3 Summary and Concluding Remarks

Most treatment policies are empirically geared towards increasing the flow of oxygenated blood to the brain by *generally* improving the CPP. Some are specifically geared towards improving flow *locally* through the VSM territory. This is achieved either by clot removal, or by attempting to dilate the constricted region—mechanically or pharmacologically. However, apart from possibly the singular use of nimodipine, there is scant evidence that either the prevalence, or the outcome, of DCI has been modified as a result of the use of *any* such regimen. Even with nimodipine, there continue to emerge conflicting reports. This, therefore, suggests that factors *other* than those discussed in the current and preceding chapters might be operative. Perhaps because of this, there has been a renewed focus upon VSM qua DCI, with increasing numbers of 'clot-VSM' studies employed to potentially reveal the missing clues (*see* Part 4). Notwithstanding, other factors potentially exist that are regularly overlooked by clinical and experimental studies. These relate to events associated with the acute ictus, and conceivably include the central physiological

disturbance (i.e. systemic and intracranial pressor effects), as well as the properties of the ruptured vessel per se. Such factors could, hypothetically, set in train processes that might prove critical to the subsequent development of VSM and DCI. For example, the assumption made in 'clot-VSM' studies—i.e. that delayed VSM merely results from the chronic effects of clot lysis upon otherwise normal vessels—might not be sound, should it subsequently transpire that the acute ictus itself actually alters cerebrovascular reactivity. This is important, because none of the results of 'clot-VSM' studies have so far led to efficacious treatment strategies. Before testing such a hypothesis, however, a full review of the anatomical, physiological and pharmacological properties of cerebral vessels (i.e. Parts 2&3) is required before an appropriate means of small vessel study is conceived that complements a representative model of SAH (i.e. Part 4).

PART 2

CEREBRAL VESSELS

Chapters 1-4

Chapter 1 Anatomy of cerebral arteries

Chapter 2 Physiology of cerebral arteries

Chapter 3 Smooth muscle cell physiology

Chapter 4 Cerebrospinal fluid and its properties after subarachnoid haemorrhage

Chapter 1
Anatomy of cerebral arteries

1.1 Introduction

The cerebral circulation is fed from two sources bilaterally, each source being surrounded by sympatho-adrenergic fibres principally emanating from the neck. Thus, the two internal carotid arteries (ICAs), both directly deriving from the aortic arch, enter the cranium after passing through the carotid sinus below the floor of the middle ear. They are then intimately related to the VI cranial nerves below the cavernous sinuses, and the II cranial nerves above them. In the neck they are intimately related, at intervals, with the IX, X and XII cranial nerves; and are constantly surrounded by fibres from the SNS. It is a fact that the ICAs conduct the major share of the CBF, this being >70% of the total[592].

The vertebral arteries (VAs), branches of the subclavian arteries, enter the cranium through the foramen magnum after having ascended the neck within the foramina transversaria of the cervical vertebrae. They, again, are intimately associated with fibres from the (cervical) SNS. After a short course on the basi-occiput, they unite to form the basilar artery (BA) at the lower border of the pons. Less than 30% of the CBF is conducted through the VAs[592].

These four sources are normally interconnected by a curious structure found only in the brain, called the Circle of Willis (CoW). Although it must have evolved as a means for the rapid redistribution of arterial input following unilateral neck vessel occlusion, its *relative* significance in this regard has been in question (*see below*). Issuing from the CoW are three major vessels on each side, the cerebral arteries, which finally supply blood to the region of the brain indicated by their names: anterior, middle and posterior. By far the largest flow occurs within the middle cerebral arteries (MCAs), this reflecting the greater role of the ICAs in determining the CBF.

This circle is frequently (50%) incomplete, however, and these anomalies are more frequent in patients who suffer from cerebrovascular accidents[87, 592]. In approximately 30% there is significant hypoplasia of a major vessel associated with it[87]. Such hypoplasia, occurring in the anterior circle, may be especially apparent in patients harbouring an aneurysm of the anterior communicating artery (ACommA), occurring in as many as 85% cases[87].

As described in Part 1, 1.1, the major cerebral vessels ramify over the surface of the brain *before* entering it. An extensive **collateral circulation** is possible through anastomoses between surface pial vessels. These constitute the **leptomeningeal arteries of**

Heubner[100]. However, the vessels distal to this network, which penetrate the cortex in the Virchow-Robin spaces, have little communication with one another. Apart from possible "shunts" occurring in pre-capillary arterioles there is little potential for anastomosis at the intraparenchymal level[87].

The cerebral venous drainage is via innumerable cerebral veins pooling into the cerebral venous sinuses. These, then, drain mainly into the internal jugular system in man. In rats, however, drainage is mainly via the vein of Labbé to the *external* jugular system[139]. Although the cerebral venous sinuses are often considered 'incollapsable', they in fact only prevent 'suction-collapse'. Certainly they cannot prevent collapse from externally-applied pressure. Thus, Cushing in his classical cranial window study of 1901, vividly described the ready collapse of the superior sagittal sinus in even the initial phase of ΔICP, in either dogs or rabbits[115].

1.2 The singular structure of the cerebral arteries

In general, this is similar to that seen in 'resistance vessels' throughout the body. However, there are important differences. The most abnormal layer histologically is that of the tunica adventitia. Deficiencies in each of the three wall layers may, however, play a role in each aspect of SAH. The increased presence of mast cells (MCs) within the vessel wall in cases of aneurysm rupture might possibly suggest their role both in the *development* and *rupture* of berry aneurysms. This is currently considered the case with the rupture of coronary artery atherosclerotic plaques[201].

1.2.1 *Tunica intima*

This consists of the endothelium, the internal elastic lamina (IEL), and the sub-intima. The endothelium is the predominant site of the blood-brain barrier (BBB) (*see below*). Deficiencies in the IEL may represent the *conditio sine qua non* of aneurysm development. The sub-intima is normally thin and insignificant: however it thickens in response to nonspecific vessel trauma (Part 1, 1.3.1.4).

1.2.2 *Tunica media*

This predominantly consists of vascular smooth muscle cells (VSMCs) arranged in (≥ x6) concentric layers[639]. Approximately **70%** of this layer may be composed of **VSMCs**[639]. However, the arrangement of these cells becomes **haphazard** in regions of **acute bifurcations**: regions, in fact, where aneurysms abound. A possible helical arrangement of VSMCs[639], however, may contribute both physiologically and pathologically (Part 2, 3.1.2). It is often held that this layer is thinner than would be expected, in consideration of the active tone and intraluminal pressures normally experienced. However, if Laplace's

law for hollow structures does essentially apply (Part 2, 2.1), the contracted state necessarily signifies wall tensions experienced to be correspondingly the lesser, by virtue of both reduced luminal diameter and correspondingly increased wall thickness. In other words, by virtue of the property of myogenic tone (Part 2, 2.2), cerebral vessels can afford to possess somewhat slender wall mass, because the operating wall tensions would necessarily be the less.

1.2.3 *Tunica adventitia*

This layer is normally thick in systemic vessels. However, it is extraordinarily thin in cerebral vessels. In fact, it essentially disappears altogether in pial arteries with increasing cortical depth[129]. Furthermore, there is **no external elastic lamina (EEL)** present in the major vessels of the SAS. This is a marked departure from normal structure, which, by placing more responsibility on the IEL, may be of the utmost importance in aneurysm development and rupture[499]. Normally, similar-sized vessels possess further tiny vessels—the **vasa vasora**—that course through this layer, supplying blood to the energetic media. However, even the most major branches of the CoW are conspicuously **lacking** in these[95, 101, 275]. Instead, there are numerous **micropores** present—the 'rete vasorum'—which suggest, to many, a significant role for the CSF in both nutrient supply and waste disposal[484, 676].

The latter feature (rete vasorum) lead to one hypothesis that the presence of the thick subarachnoid clot might, by micropore occlusion, disrupt a significant component of vessel wall metabolism. However, Ohta et al subsequently demonstrated that horse-radish peroxidase not only continued to permeate the wall following clot application, but also appeared able to do so more readily[467]. That the addition of powerful vasoconstrictory agents such as barium chloride, also achieved enhanced 'uptake' suggested that blood acted in the same way. That is, vessel wall **constriction** per se appears to effectively **open up** these **micropores**. Thus, rather than limiting CSF metabolite exchange, blood may even increase this. However, by increasing micropore patency, the further penetration of vaso-active agonists into the vessel wall would be facilitated, thus promoting VSM[92, 310, 468].

1.3 The Blood Brain Barrier (BBB)

The BBB essentially derives from the presence of **tight junctions** (*zonula occludens*) between the endothelial cells. Thus, the BBB is mainly due to the membrane properties of this cell, as suggested by its oil-water immiscibility[536]. Such an endothelial cell may often form a complete tube in the capillary, and is itself enveloped by out-projections ("feet") of large nearby astrocytes[536]. The presence of the BBB, by preventing the transudation of plasma under the balance of hydrostatic and osmotic pressure, is the reason why there are

no lymphatics present within the CNS. In likewise fashion, the subarachnoid space is similarly sealed-off from the dura, as both the pia and the arachnoid layers also possess tight junctions[536]. Thus, both CSF and the cerebral blood are very separate from the rest of the brain; and from each other.

The presence of an excessively large number of mitochondria within endothelial cells hints at their intense metabolic properties[536]. Molecules can permeate the barrier by crossing the luminal membrane, traversing the cytoplasm, and finally crossing the abluminal membrane. That such passage exhibits **saturation**, **stereospecificity** and **competition** implies it to be governed by receptor and carrier-mediated mechanisms[536]. Such molecules are often seen to be conducted transcellularly, within intracytoplasmic vesicles[536]. Some have shown that high levels of hypertension, for example, can effectively increase BBB "permeability" by increasing the rate of this vesicular transport mechanism[285].

The most crucial molecules for cerebral metabolism are oxygen and glucose, the latter being transferred as the D-isomer[536]. Amino acids, however, are transported in three essential categories: acidic, basic and neutral[536]. That the transfer of L-tyrosine, for example, competes with that of phenylalanine, was an important discovery in elucidating the mechanism of hyperaminoacidaemia-induced CNS disease. Such is the case with phenylketonuria and hepatic disease, for example, where relative excesses of certain amino acids starve the brain from others by virtue of carrier-mediated saturation.

1.4 Aneurysm formation

Although considered 'congenital', aneurysms rarely occur in childhood. In this sense, berry aneurysms are not truly congenital.

1.4.1 Inheritable factors

It is clear that some aneurysms do appear associated with clearly-recognisable **genetic disorders** such as Ehlers-Danlos syndrome, coarctation of the aorta, and polycystic kidney disease[191, 396]. Nevertheless, few in the past have genuinely felt that aneurysm probands regularly denote familial clusters. However, recent studies have again returned to this theme, one even suggesting that at least 1:5 SAH probands will have an affected first or second degree relative[531]. Most others have found the proportion to be lower[353]. However, a preponderance of fertile women has also been noted in such cases[353], this suggesting underlying genetic heterogeneity[531].

Notwithstanding, the vast majority of cerebral aneurysms do not appear truly 'inheritable'. Furthermore, that aneurysms mainly tend to develop in the third decade[253] in turn strongly

implicates environmental factors, of which hypertension appears predominant[132]. Certainly, some have experimentally created cerebral aneurysms by such MAP elevations[330]. Nevertheless others have singularly failed in this regard, even with pressures up to 600 mmHg![556].

1.4.2 Deficiencies in the wall

Some consider the tunica media to represent the most structurally important layer. Yet, even if this were so, some 31% of 157 random autopsies show major vessels associated with the CoW conspicuously lacking VSMCs at the apex of bifurcations[253]. This tends to support a "congenital" background to the process of medial deficiencies, but it does not explain why only a few actually succumb to aneurysm formation. Quigley et al noted that the IEL was frequently damaged in regions of aneurysm formation[499] (*see* also references in Ref 385). Since the adventitia routinely lacks an EEL in cerebral vessels (*see above*), they postulated that such IEL defects were at least as important as those in the media, the presence of the two compounding each other. Quigley et al, in fact, managed to produce aneurysms by laser coagulation in some 30% of cases which, by causing such defects in *both* layers, resulted in aneurysm-formation without the pre-requisite of increased MAP[499]. There has been a recent proliferation of reports relating IEL defects to abnormally elevated activities of elastase in cerebral vessel walls. However, whilst some demonstrate convincing evidence, many appear experimentally flawed[560]; and all—uniformly—suspiciously fail to quote Hassler's landmark study[384] which categorically refuted such a mechanism[253].

Another possible factor may relate to the presence of MCs within the arterial wall. Their presence not only within cerebral blood vessel walls ordinarily[154], but also more specifically in relation to berry aneurysm rupture[90, 166], have long been documented. Recent interest in their putative role in the "rupture" of a coronary vascular atheromatous plaques[201] raises analogous possibilities with aneurysm rupture. MCs are, of course, pregnant with vasoactive compounds—however, it is with regard to lytic agents that may cause structural weakening of the vessel wall that interest is now being turned to[201].

1.4.3 Infundibula (References in this section are contained in **Ref 385**)

Infundibula (IF) are conical, triangular or funnel-shaped widenings that occur at branch points of major cerebral arteries. Although they rarely occur at other sites, they principally occur at the ICA-PCommA junction. At this location, the IF base—normally defined at a maximum of 3 mm—faces the ICA, whilst the PCommA arises from its apex. IF thus appear as a symmetric bulge without a neck, in marked contrast to an intracranial aneurysm

which bulges asymmetrically from a well-defined neck. They are seen in 7-25% of normal angiograms, although their incidence may be greater in cases of either multiple or familial intracranial aneurysms.

Hassler & Saltzmann were amongst the first to suggest that IF might be precursors of cerebral aneurysms. This was based on the presence of 'aneurysm histology' in IF obtained from seven necropsies following SAH. In these, four IF exhibited IEL fragmentation, one had no IEL at all, whilst five exhibited significant medial defects. Stehbens also demonstrated IEL fragmentation in five out of seven IF, whilst Archer and Silbert demonstrated complete IEL absence in one case with bilaterally symmetrical IF (one having been deemed the site of rupture). However, 'aneurysm histology' is not invariably present in IF. Two in Hassler and Saltzman's series showed no abnormality at all, whilst Sahs demonstrated only one IF with an IEL defect in his extensive series. Furthermore, Epstein et al demonstrated no structural defect at all in all seven of their cases. Based on such findings, numerous authors have denounced the 'pre-aneurysm hypothesis', describing IF instead as merely normal anatomical 'variants'.

Notwithstanding, Endo et al found atypical bulgings on IF strongly suggestive of early aneurysm progression. Indeed, a total of eleven cases have now been reported documenting frank IF-aneurysm progression[385]. Since at least some IF clearly are pre-aneurysmal, one possibility is that such progression only occurs in those with underlying 'aneurysm histology'. Whether this may be congenital or acquired is, of course, the same dilemma that applies to berry aneurysms in general. But it may be the case, however, that IF configurations are hydraulically more disposed toward further dilation. On Bernoulli's principle, sudden decreased velocity—and, therefore, kinetic energy—at IF, necessarily result in increased wall tensions since:

$$p + \tfrac{1}{2}\rho v^2 + \rho g h = \text{constant}$$

where p = fluid pressure, v = velocity of flow, ρ = blood density, g = acceleration due to gravity, and h = height above ground level (the latter three are effectively constants). In the normal situation, flow is of course directed from the ICA into the PCommA: however, this

can be reversed during carotid compression so long as the PCommA is patent. Conceivably, repeated distensions could trigger IEL fragmentation which, in the presence of frequent medial defects, could lead to aneurysm formation. It is interesting that Endo et al found early IF-aneurysm progression to be more likely with enlarged PCommAs.

Chapter 2
Physiology of cerebral arteries

2.1 Resistance vessels in the cerebral circulation

The major level of resistance to flow within the cerebral circulation continues to remain in dispute. That major anomalies in the CoW do not necessarily equate with CBF compromise, particularly following carotid ligation[291], suggests that whatever its teleological function the CoW does not appear crucial toward a collateral circulation[291]. The major resistance appears therefore to lie *distal* to this point, with deficiencies in the aforementioned **leptomeningeal anastomosis** being largely responsible for CBF compromise following carotid ligation[291]. In fact, at least 50% of the cerebrovascular resistance may be contained within these vessels[332]. Nevertheless, it is of interest that CCA ligature results in a 50-60% stroke incidence in animals such as gerbils which completely lack a CoW[379]; and that pressure-falls of up to 30 mmHg have been recorded in human subjects across the circle[291]. Furthermore, the subclavian steal syndrome shows that the CoW may redistribute away from the brain when carotid pressure gradients become reversed[110].

Earlier studies repeatedly suggested that the major level of cerebral resistance resided in the **arterioles**, at diameters less than about 60 μm[185, 378]. Increasingly, however, more recent studies have shown significant contributions from larger vessels, these at least including vessels between 120 μm[627] and **small arteries** of 200 μm outer diameter[138]. An important feature of these vessels is their extent of wall thickness in relation to their luminal diameters, and in the degree to which the medial SMCs function in controlling wall tension. The basic properties may be largely explained by the law of Laplace, which governs the properties of elastic cylinders in response to luminal (radial) pressures. Thus:

$$T = \frac{P.a}{th}$$

Where T is the wall tension; P is the pressure differential across the vessel wall (this is the MAP minus the cranial CSF pressure); and where th is the wall thickness.

However, one must accept the major limitation of this equation in assuming a cylinder containing elastic walls. This, in fact, may not necessarily apply to cerebral vessels as myogenic tone (MT) effectively reduces elastic effects (Part 2, 2.2). Other systemic conduit vessels, however—particularly the pulmonary vasculature—much more closely resemble the 'passive' Laplace requirements (Fig 13). Notwithstanding, it is a useful relationship to bear in mind, if anything because of its simplicity. Thus, a vessel in a

smaller contracted state will necessarily experience smaller wall tensions, therefore they do not need to be 'built' as thick as conduit vessels.

2.2 Pre-capillary sphincters: myogenic tone

The further distally within the microvascular bed, the greater the relative resistance to flow offered. In fact, at some point, arterioles exhibit essentially "sphincteric" function. Such vessels ultimately control both pressure and volume of flow to the vulnerable capillary bed (**capillary regulation**[407]). At this extreme level, the major factors that should dictate flow ought largely to be either metabolic or protective. Thus, an increase in tissue metabolism, through the build up of waste products, should be able to dictate corresponding increases in *local* flow (**capillary 'recruitment'**[407]). In turn, dangerous levels of MAP breaking through proximal resistance vessels should be stopped at the pre-capillary stage, by sphincteric activity.

The latter protective mechanism reflects the innate ability of the contractile machinery of the vessel to respond to luminal distension as a stimulus, it being totally **independent** of **neurogenic control**[663]. This, in fact, is a major property of all resistance vessels, but it particularly applies to **pre-capillary sphincters**. Such vessels can be anything up to 70 to 100 μm in diameter[407], and this property is referred to as **myogenic tone (MT)**. Such vessels characteristically exhibit MT spontaneously, and not just in response to stretch. This is evidenced by a continuous, rhythmic, oscillation in vessel tone in isolated segments @ 3/min in the larger vessels, to 12/min in the smaller ones[22]. It is possibly due to spontaneous electrical activity within the VSMC membrane, and it results form the overall integration of numerous, isolated, phasic contractions occurring throughout a functional VSMC syncitium[407]. Therefore, the SMCs in these vessels possess fundamentally different properties to those elsewhere in the vasculature (*see below*).

Within the systemic circulation, it has been estimated that such MT may amount to up to 70% of the total resting peripheral resistance[407]. Thus, the background "noise" of sympathetic tone is probably less than that of MT. SNS tone is revealed following spinal cord transection above the level of the thoracolumbar outflow, or following the administration of ganglion-blocking drugs, and results in falls of MAP of 40-60 mmHg[663]. However, the SNS becomes more prominent with extremes of MAP within the cerebrovasculature (*see below*). The resting MT within cerebral vessels has been assessed, by some, to represent somewhere between 20%[48] to 40%[475] of the total tone possible. However, this clearly depends upon the degree of distension present and, therefore, upon the intraluminal pressure. It may also depend on the level in the microcirculation, it being

greater the more distally[407, 615]. In consequence, any further agonist-induced vasoconstrictory tone (AT) must be super-imposed upon MT.

2.3 Capillary regulation

Thus precapillary sphincters serve to maintain capillary pressures within a more or less constant range, this being approximately 15-16 mmHg. Without this protective mechanism, and by maintaining MAPs of 125 mmHg for only 15 mins, 50% of the circulating plasma may be lost into the interstitium, this clearly resulting in circulatory collapse[407]. Thus, such vessels offset deleterious sudden surges in MAP. However, they also dilate in response to falls in MAP. Thus, at the capillary level, the majority of tissue beds appear equivalent. Yet in some organs, this property is also apparent in the small arteries as well. As a result, total organ blood flow itself remains regulated to optimum levels, in spite of large MAP variations.

2.4 Autoregulation

The innate ability of some vessels to spontaneously maintain flow to certain regions of the systemic circulation was initially implied by Ostroumoff in 1868[476]. Unfortunately, he could not come to the conclusion that both sensor and effector actually resided *within* such vessels, although this was implicit in his findings. Instead, it was Sir William Maddock Bayliss[35] who first explicitly reported this fact in 1902 (the same year, in fact, that Cushing's Mütter lecture[115] was published—Part 6, Section I, 1.3). Although more famous subsequently for his joint discovery (with Starling) of the first hormone, secretin, his role in developing the concept of autoregulation has become increasingly appreciated, particularly within renal and cerebral beds. The "Bayliss effect" thus serves to maintain *organ* blood flow itself at more or less constant levels, in much the same way that pre-capillary tone maintains capillary flow.

In consequence, CBF is maintained ordinarily at around 750 ml/min[663]. This is approximately 13% of the cardiac output, yet the brain only represents about 2% of the total body mass[663]. Thus, normal levels of CBF approximate to 50 ml/100g/min, being far greater within the grey than white matter[663]. Similarly, in the other major autoregulatory organ, renal blood flow is maintained at levels of 1 200 ml/min: this being some 20% of the cardiac output[663]. Clearly, these are **organs** with **large metabolic activities**. It has been estimated that values between 10-20 ml/100g/min become critical to cerebral metabolism, and that values <10 ml/100g/min result in the onset of ischaemic necrosis[592]. In the presence of autoregulation basal CBF can only be increased by extreme surges in the MAP; or by increases in cerebral metabolism[411]. Thus, only with acute severe hypertension

are cerebral vessels passively dilated and the brain exposed to dangerously increased CBF[33, 428]. Flow itself is approximately governed by the Poissiculle equation:

$$V = \frac{\Delta P \pi a^4}{8 \eta \iota}$$

Where **V** is the volume of fluid flowing per unit time; **ΔP** is the pressure gradient along the vessel length; **a** is the luminal radius; **η** is the viscosity coefficient of the fluid; and **ι** is the length of the tube considered. Once again, as with the Laplace relationship, limitations abound in its translation to the physiological milieu. Thus, it strictly relates only to the **continuous laminar flow** of **homogenous fluids**, flow within **rigid tubes**, and tubes with **wettable walls**. All of these requirements are regularly not met within the systemic vasculature.

Attempts have been made to establish the precise range of MAP values over which autoregulation works. The upper limit has been assessed at around 160 mmHg by Hernandez et al[264]. In consequence, MT must increase proportionately throughout this range in order to maintain functional diameters. Any further AT must, therefore, be superimposed upon this MT. Beyond this limit, then, the cerebrovasculature will be expected to passively dilate. However the upper limit may be 'up-regulated' in essential hypertension.

The lower limit, in turn, has been estimated at around 60 to 69 mmHg by MacKenzie et al[378]. However, this value was derived from haemorrhagic hypotension where reflex sympathetic tone is necessarily induced within larger arteries (*see below*). In consequence, the true value is even lower, as evidenced within the pial vessels within MacKenzie's study; and as produced by various forms of sympatholysis[481]. Thus, CBF can be maintained at least down to MAP levels of 50 mmHg in clinical studies using sodium nitroprusside (SNP)[609]. In similar fashion, the lower limit may be up-regulated in hypertension. Therefore, **between** these two extremes of **50-160 mmHg**, the **CBF** is maintained **remarkably constant**.

2.5 Metabolic regulation

Neither can an increase in MAP, *nor* a release of background sympathetic tone increase the CBF in anticipation of increased levels of demand. The mechanism that can is associated with increased metabolic activity. Thus a dramatic increase in CBF can be witnessed, for example, during an epileptiform convulsion[87]. In consequence, metabolic regulation can readily overcome autoregulation: an important principle (*see below*). Lassen stated, in

1968, that metabolic regulation constituted the most important regulator of CBF, achieving this largely by way of increased tissue acidity[352].

Metabolic regulation is effected by the relative build up in **'waste products'** of tissue metabolism. Such products include potassium (K^+) ion, hydrogen (H^+) ion, carbon dioxide (CO_2), and adenosine compounds[663]. However, it is also effected by a relative *lack* in certain agents. Thus, cerebral blood vessels are extremely sensitive to local pO_2, and any hypoxia will result in vasodilatation. Such substances, individually, can powerfully dilate the vasculature, offsetting autoregulatory tone. Perhaps their effects may even synergize together, since all are likely to accumulate simultaneously[636]. But metabolic regulation does not merely represent vasodilation. For example, an increase in pH (increasing alkalinity) can actually produce a vasoconstriction[129]. This will happen when the local cellular metabolism becomes decreased.

Pharmacological agents can mimic the effects of metabolic regulation. Thus papaverine, for example, can dilate pre-capillary vessels in the systemic circulation[407]. It can thus also abolish autoregulatory MT, and is used as such experimentally. Under these conditions, the microcirculatory bed behaves passively to increases in pressure and flow. Thus, intracapillary pressures of up to 36 mmHg can be experienced, this damaging the capillary bed and resulting in excessive oedema formation[407].

This is not just the case in cerebral vessels, however. Metabolic regulation is, of course, the major influence on increasing tissue blood flow throughout the entire *systemic* circulation. It has been estimated, for example, that, should all the capillary beds within the systemic vasculature become maximally dilated with maximal metabolic activity, the heart would be required to maintain the impossible output of some 40 L/min[407]. With maximal muscular exercise, for example, this output can increase to 25 L/min[663]. With temperatures of 44°C at over 85% humidity, levels of 20 L/min can be reached[663]. The combination of excessive muscular exercise in a hot, humid climate may, ultimately, threaten cardiovascular collapse. Clearly, the development of a mechanism to not only limit the extent of this response, but also to reduce flow to non-vital areas, would be an extremely felicitous one.

2.6 Sympathetic regulation

A major function of the SNS within the systemic circulation is in the redistribution of the cardiac output to various tissues and organs under varying conditions. Another major role, however, may arise under conditions of excessive metabolism. Firstly, by increasing tone in 'non-vital' regions, it can effectively divert more blood to regions of intense metabolism.

In fact it can increase the total peripheral resistance by a factor of five here[407]. This not only increases the head of pressure to those beds still dilated but, by reducing intracapillary pressures in non-active beds to minima of around 7 mmHg, it can also effectively 'suck-back' extracellular fluid from the interstitium[407]. This therefore augments the circulating volume. Secondly, there is evidence that sympathetic regulation may be more potent in those beds most metabolically active. Thus, at the upper level of autoregulatory breakthrough, pre-capillary vessels can be x3 as sensitive to NE when maximally dilated metabolically[407].

Therefore, it appears reasonable that the three regulatory mechanisms (autoregulation, metabolic regulation, and sympathetic regulation), work together in concert at the microcirculatory level; in order to control both the distribution, and the redistribution, of the cardiac output[475].

2.6.1 Role of the sympathetic nervous in cerebral blood flow

Most small arteries and arterioles throughout the systemic circulation possess a dense supply of adrenergic nerve fibres, including even that of the other major autoregulatory organ, the kidney. Although, in comparison, the cerebral circulation is much less densely innervated[663], a reasonable supply is extant to the adventitia of pial vessels in humans and other species[150, 249, 464, 483]. In some this was in evidence even down to the level of the capillaries[504]. However, this is heterogeneous, and maximal only at the level of the CoW[620]. Both innervation and NE-responsiveness fall off markedly within the intraparenchymal vasculature[49].

However, although the cerebral SNS classically ascends the neck from the cervical ganglia, reaching the intracranial cavity upon the large cervical arteries, it also derives from a complicated and largely unknown intracranial source. This involves a source in the **locus coeruleus** which may be activated during stress[663]. The latter nucleus is located dorsomedially in the floor of the rostral part of the IV ventricle. Catecholamine containing neurones thence project via the dorsal tegmental bundle to the hypothalamus, cortex and hippocampus[575]. But they also project caudally to the nucleus tractus solitarius (NTS), and the spinal cord[575]. More importantly, they project directly to the microvessels of the pial circulation[575]. The connexions to the NTS certainly seem to mediate systemic MAP via the thoracic intermediolateral columns. Afferent stimuli, coming from the trigeminal system serving the meninges, may stimulate this system.

Yet several authors have commented upon the relative *lack* of influence of the SNS within the cerebral circulation[140], with even bigger doubts about the cholinergic contribution[23, 122,

[127]. Thus, most have found relatively poor NE responses within cerebral vessels[525, 588, 600, 650]. Although recent comparisons between wire-mounted and pressure-cannulized vessel studies have highlighted important areas of recording bias with adrenergic responses[76, 167], this only refers to response *potency*. Since efficacy, therefore, remains unaffected this cannot account for the lack of effect seen. Moreover, such reduced effectiveness is also observed in vivo[119]. It is possible that this is a feature only with non-human animal vessels. Thus, several authors have noted larger NE responses within human cerebral arteries[600, 607], some suggesting this to relate to a predominance of $\alpha 1$ receptors, $\alpha 2$ receptors allegedly mediating weaker responses. This is a fascinating proposition which, if so, might be explained by the adoption of the upright posture. But a far more likely (and certainly more prosaic) explanation may also relate to a differential responsiveness between the BA and the other cerebral vessels. Thus, the BA tends to be the more frequently used in experimental studies, and yet is known to demonstrate a sparse response to NE[290].

Even so, some have estimated that the cerebral SNS contributes only 5% toward overall cerebrovascular tone normally[481]. This is in marked contrast to its role systemically, where acute falls in MAP of 40-60 mmHg may be observed following sympatholysis[663]. It may be, then, that the cerebral SNS only really comes into play under more extreme circumstances. Thus, in addition to its greater role with metabolic dilation, it may also exert a much greater effect under extremes of systemic MAP. For example, the lower limit of cerebral autoregulation following haemorrhagic hypotension (at 65 mmHg) is significantly higher[378] than that induced under sympatholysis[481]. Thus, CBF can be adequately maintained down to MAP values of 50 mmHg following SNP administration in clinical studies[72], even down to 30 mmHg in experimental ones[609]. Haemorrhagic hypotension is therefore accompanied by a reflex cerebral SNS-activation[358]. Some have estimated its contribution toward overall cerebral vessel tone to be increased fourfold to around 20% under such circumstances[481].

Similarly the cerebral SNS may exert a greater influence with increases in MAP, with several documenting increased recruitment here. This may serve to offset dangerous surges in CBF[51, 260, 428]. Therefore, with systemic hypertension the cerebral SNS appears to augment the underlying protective autoregulatory mechanism. In contrast, under hypotensive conditions, it appears counterproductive to this mechanism. Perhaps this is because the SNS must necessarily be brought into play as part of a generalized response to circulatory shock: a primitive reflex to generally maintain systemic pressure.

Whatever its role there is much evidence suggesting this to be largely experienced upon the larger extra-parenchymal cerebral vessels within the subarachnoid space. Thus, cerebral

angiography undertaken during periods of SNS activation frequently show larger arteries to be constricted[222, 223] concurrent with pial dilation[378]. This is compatible with a greater extraparenchymal adrenergic innervation[153]. This observation has given rise, then, over the years, to the **"Dual effects" hypothesis**[378, 481].

2.6.2 Ischaemic pre-conditioning?

An interesting alternative role for the SNS may be implied from consideration of its effects within the myocardium. Thus, by α1 adrenoceptor activation[594], (or by somehow increasing *Gs* stimulation relative to *Gi*[450]), NE at especially high levels around 56 μM[29] appears able to lengthen the ischaemic tolerance of cardiac muscle to subsequent decreases in coronary blood flow[135, 612]. It may be that a similar role for the cerebral SNS pertains within the cerebrovasculature since, teleologically, the cerebrum is particularly prone to the occurrences of decreased blood flow. The fact that this may be particularly so with the upright posture of humans, may possibly reflect its greater role here[607]. It is interesting, however, that nitric oxide (NO) has also been implicated in ischaemic pre-conditioning. In one study, NO produced from *e*NOS mediated ischaemic pre-conditioning by a mechanism that did not alter mitochondrial hyperoxidation induced by subsequent reperfusion[85].

Chapter 3
Smooth muscle cell physiology

3.1 Introduction

SMCs are spindle-shaped, 50-400 μm long, and 2-10 μm thick[519].
In general there are two types of SMCs defined by their responses to stretch or to neuronal input.

3.1.1 Plastic compliance and myogenic tone

After an initial rise in tension following stretch, due to inherent visco-elastic properties, most SMCs exhibit **plastic compliance**. In the post-stretch phase, tension decreases abruptly at first, and then ever-more slowly. Thus, such types can be completely relaxed in both the shortened and stretched states, and feature largely in structures required for storage, such as the urinary bladder[519]. However, some SMCs also respond with a **stretch-activated contraction**, this being superimposed onto the aforementioned visco-elastic properties. Thus such SMCs behave similarly to cardiac and skeletal muscles, and are prevalent within the 'autoregulatory' vasculature. Intriguingly, the two types may not be fixed. For example the urinary bladder, following cord transection, can change from having one having distinctly visco-elastic/plastic properties, into one with a stretch-activated response over a period of several days. Hence the 'automatic-emptying' of the bladder in the acutely paralyzed patient[519].

Some SMCs in the body are **spontaneously active** and do not require any stretching. Thus the ureter, the intestine and the uterus spontaneously contract in rhythmic fashion (e.g. Braxton-Hicks contractions within the uterus). This is truly myogenic, as in the heart. Excitation can rapidly spread from "pacemaker" regions throughout the muscle due to electrotonic spread across low-resistance tight junctions at intercellular nexi (i.e. a functional syncitium[519]). In many ways, autoregulatory vessels resemble these tissues, with spontaneous rhythmic activity of between 3 and 12/min[22]. However, even with such myogenic SMCs some degree of plastic compliance is still apparent, as is reflected during the mounting procedure of cerebral arteries in wire myography (Part 4, 5.2.1).

3.1.2 Vascular smooth muscle cells

It is not known where the stretch sensor or effector resides within the autoregulatory mechanism. Some empirically accept that the sensor must represent stretch-sensitive ion channels within VSMC membranes[407]. However, others have demonstrated the endothelium to be necessary, certainly within cerebral arteries[226, 240, 320, 321]. This is also in evidence in experiments where vessel compliance was greater following endothelial

denudation[326]. Although the latter authors assumed that all MT had been abolished with 100 μM PPV, this is not invariably the case (Part 5, Section II, 3.8); and, thus, the greater compliance seen with endothelial denudation may have been due to a "release" of MT. Thus, the endothelium not only may sense the degree of luminal stretch, it may also enact the VSMC response via an endothelial-dependent contracting factor[226, 240, 320, 321] (EDCF). Such endothelial-VSMC electromechanical coupling is a common event physiologically. Thus, there is a similar release of an endothelial derived relaxing factor (EDRF) in response to a shear stimulus (Part 3, 1.8). Whilst the EDCF is probably a prostanoid, the EDRF can be either prostacyclin, nitric oxide (NO) or, even, some other substance with hyperpolarizing properties (*see below*). There is some reason to believe that the endothelium and the VSMCs, by virtue of tight nexi between them, act as a kind of functional syncitium[506]. Certainly, this would explain their tightly linked functions.

Based upon a circumferential arrangement of VSMCs in arteries, Walmsley et al[639] have assessed that a 58% decrease in length of the cells would be required in order to afford luminal closure. Whilst this is possible, a dual-assumption of a helical VSMC arrangement of along with essentially fixed vessel lengths, much better explains vessel closure[639]. In this hypothesis, much less than a 58% degree of VSMC shortening would be required. Even based upon a helical arrangement, it seems likely that the **inner layers** of VSMCs only contribute to the **initiation of contraction**, with the **outer layers** the **maintaining this**[639]. If so, it may be that this is the reason why medial myonecrosis largely appears within the outer layers post SAH[669], as this would represent the most physiologically active.

3.2 Mechanism of contractile force

The actual contractile mechanism within SMCs is probably fundamentally that as described for skeletal muscle[280]. Thus, it is essentially due to the flexing and rowing movements of myosin (thick filaments) along a double strand of actin (thin filaments). However, in SMCs the actomyosin filaments are not arranged as regularly as in skeletal muscle, and so lack the striations which are so much a feature of skeletal muscle[519]. The rate of filament sliding is, furthermore, approximately x100 to x1000 *slower*, so allowing for prolonged, maintained contractions without fatigue, and with little energy consumption[519]. The contractile tension per unit area is, nevertheless, of the same order as that of skeletal muscle (about 30-40 N/m^2). So, in the long term, VSMCs can support as great a load. Yet the energy consumed in the process is some x 100 to x 500 smaller. This is an important point to bear in mind when considering persistent vasoconstriction as a cause of VSM.

The elastic "pull" of an acto-myosin flexing movement, however, can actually generate a force without any sliding, this constituting **isometric tension** (*see below*). It has been estimated that a billion of these actomyosin-induced elastic tensions, arranged in parallel, would constitute a 1 mN force in that muscle[519]. But each is not an isolated and fixed reaction. In reality, there is a continuous 'attachment-release' cycle of these bonds @ 5-50Hz (with attachments lasting only 1/100 or 1/10 second). Therefore, so long as there is a normal level of cellular metabolism (*see below*), acto-myosin bonds are being continuously made and dissociated following activation by the appropriate switch mechanism (*see* **second messenger systems**). Yet, overall, the contraction itself appears *smooth* because, statistically, a certain number of units will be attached at any one time: the actual number being dependent upon the extent of the stimulus[519]. Because of these repeated cycles, a certain amount of energy is lost as heat: the maintenance heat of contraction. This is essentially because only about 40-50% of the chemical energy associated with ATP can actually be converted into mechanical energy[519].

3.3 Muscle energetics

A major finding was that the addition of ATP to a solution of actomyosin resulted in the dissociation of actin from myosin[564]. That is, ATP is required to separate these two structures. Thus, when the two are connected there can be **no relaxation of tone unless ATP is present**[564]. This is, in fact, the situation in *rigor mortis*. Thus, if there had been, for example, an excessive amount of muscle activity pre-mortem (as in a violent struggle), then rigor mortis will occur all the more sooner post-mortem, as all the ATP may have been used up. This can prove important forensically. It may also be relevant to the possible mechanism of VSM (*see below*).

Thus, in the first "act" of the cycle, the ATP molecule separates the two filaments. Since a flexing movement *had* already occurred in the previous cycle, this release allows the myosin head to 'spring back' to its resting position. Almost immediately thereafter, the ATP-cleavage reaction commences, but it only remains half-complete:

$$\text{MyATPase} + \text{ATP} + H_2O \rightarrow \text{MyATPase-[ADP} + Pi]$$

this is because although the myosin 'head' region actually functions as an ATPase, it can only function as such by a subsequent re-attachment to actin (with Mg^{2+} as cofactor)[519, 564]. Thus the ADP molecule effectively obstructs the catalytic centre of the MyATPase, thus preventing it from completing its task. Subsequent fusion with actin, by allowing for the discharge of the [ADP + P*i*] bundle, thus permits the reaction to go to completion[519, 564]. The stored energy is, then, only released at this stage, and is reflected in the further flexing

of the myosin head upon its neck[519]. Thus the **stored energy** is **released** only with the '**power stroke**'. Another molecule of ATP may then come in order to commence another cycle. If the muscle as a whole is allowed to change its length, then this will result in a progressive shortening with each cycle. If, however, this is prevented, then tension will be maintained without shortening: an isometric contraction.

3.4 The implications of intensive and prolonged vasoconstriction: physiological sphincters

It would be prudent at this stage to consider the possible effects of prolonged vasoconstriction, as this represents to many the major mechanism behind VSM *qua* DCI. What is the evidence that such a profound and prolonged state is, actually, detrimental? Certainly, the complete physiological closure of a major vessel for any significant length of time would be, in the limit, terminal for the tissue supplied. However, is it necessarily so for the vessel wall itself? As already explained, SMCs are by nature able to maintain prolonged states of contraction with minimal energy consumption. Indeed, the pre-capillary sphincters, to which MCAs bear many functional resemblances, are quite clearly able to maintain prolonged constrictions particularly with hypertension. But perhaps the SMCs that display this camel-like endurance par excellance must be the sphincters of the urinary and gastro-intestinal tracts. Here, apart from brief episodes of relaxation, a continued state of near-maximal contraction can be maintained, it would seem, indefinitely.

However, even here there are degrees. Thus, although seemingly maximal, both urinary and anal sphincters can be voluntarily 'squeezed' by superadded contractions. It would seem that in the latter, at least, complete obstruction of the lumen is effected by expansion of the haemorrhoidal cushions. Indeed, in certain conditions such as anal fissure, an abnormally increased resting tone is the rule: this sometimes resulting in a lack of compliance known as anal stenosis. It would seem that in these cases the neuronal input is predominantly present to afford a relaxation from this natural constrictory tendency. This is evidenced both in Hirschprung's disease of the recto-sigmoid, and in achalasia of the cardia[98, 490]. Indeed, achalasia means "failure to relax". In both conditions, there is an aganglionosis of gut wall plexi, this permitting the development of pathologically-increased tone[98, 490]. Interestingly, with **Hirschprung's disease** there is both an **increased wall rigidity** as well as a **failure** to relax with smooth muscle **dilating agents**[490]. This is clearly an analogous state of affairs to that seen with VSM. Yet, in contrast, a myonecrosis is not in evidence. In fact, at least with achalasia of the cardia, a SMC hypertrophy is apparent[98].

Perhaps because major cerebral vessels are conspicuously lacking in vasa vasora (Part 2, 1.2), the limiting factor is dependent upon luminal diffusion to the vessel wall. Such a lack of vasa vasora seems surprising, as one might expect myogenic SMCs to be more energy-requiring. Nevertheless if the major energy source then derives from the lumen, such prolonged luminal narrowing may indeed result in medial ischaemic change.

3.5 Second messenger systems

These are agents that act as intermediaries relaying the membrane-signal to the active intracellular mechanism. They are thus **membrane-transduction agents,** acting as *switches* to the contractile mechanism[564]. They were first identified as intermediaries in the actions of hormones. In the same way, they are now established as the major intermediaries in the action of drugs. Probably only one, however, mediates the neurogenic skeletal muscle response: calcium (Ca).

In spite of there being an enormous variety of drugs and hormones, and therefore in their corresponding receptors, there are only a *few* types of second messenger[564]. This is an important principle because the **second messenger** is usually a common and **ubiquitous** molecule, capable of being present in large supply at short notice. Clearly, an infinite variety of second messenger would be unfeasible in this context. Because of this, one hormone combining with one receptor, for example, can result in the liberation of many more molecules of second messenger intracellularly. In turn, the latter may activate many other messenger molecules, so resulting in a **cascade reaction**. This greatly amplifies the cellular response. For example, the concentration of many hormones in the plasma is around the 10^{-10} M level, yet intracellular Ca and cAMP are both considerably higher at 10^{-8} M[564]. But not only must there be a **large potential pool** of second messenger at hand, there must also be **efficient mechanisms** for either its removal or **de-activation**. After all, a switch must be able to be turned-off as quickly as it is turned-on.

3.5.1 Vasoconstriction: Calcium

The essential "switch" to elicit neurogenic contraction is, classically, due to elevations in intracellular calcium (*i*Ca) levels. Thus, transient elevations from the resting intracytoplasmic level of **10^{-8} M to** that of over **10^{-6} M**, effectively switch-on the contractile machinery[519, 564]. In fact, there appears to be a **dose-dependent effect** of graded elevations in *i*Ca with contractile force generated, and with levels of ATPase activity[519]. Furthermore, if the level is maintained multiple single twitches will fuse into a tetaniform spasm, this maintained as long as the increased *i*Ca is maintained. Such contraction is **maximal** at *i*Ca levels of **10^{-5} M**[519, 564].

Precisely how *i*Ca switches the mechanism on, however, remains in dispute. With skeletal muscle, it involves conformational changes in troponin molecules which somehow force movements in long tropomyosin molecules lying parallel to actin filaments. Since tropomyosin ordinarily overlies S1 myosin filament binding sites, such movement therefore exposes the 'active' region, so allowing acto-myosin bonds to form. In SMCs, however, another structure—**Calmodulin**—is involved in the Ca switch[519, 564].

Although the resting *i*Ca is around 10^{-8} M, skeletal muscles actually contain considerably more. In fact, they contain about 1 μM Ca per gm weight[519]. Therefore, in order to prevent continual (tetaniform) spasms, this excess of second messenger requires ordinarily to be locked away. The major way by which this is achieved is by an *active pumping* of Ca ions *across* various *membranes*. The latter include not only the plasma membrane itself, but also membranes associated with intracellular storage: (the endoplasmic reticulum, mitochondria, and possibly others). The pump itself is a Ca-ATPase complex attached to the intracellular membrane in question, and it is this complex that maintains *i*Ca homeostasis[519, 564].

Thus, when the cell is appropriately stimulated, the *i*Ca rises. This may simply be achieved by a reversal of the above *i*Ca-extruding mechanisms. However, the major mechanism is via the influx of extracellular Ca (*e*Ca) across the plasma membrane. There are many ways that this can be achieved. There are **potential-specific Ca channels (PSCs)** within the membrane, for example: these can open upon depolarization of the membrane. Similarly, there are **receptor operated channels (ROCs)**, which can either themselves affect the membrane potential, or even open Ca channels directly. It is also possible that other second messengers can cause *e*Ca influx, such as perhaps PKC. Rapid release of Ca from intracellular stores may be an important feature of action of various agonists (*see below*). Finally, there may be reciprocal interactions between these mechanisms: that is, between *e*Ca influx and *i*Ca release. Thus, the initial release of *i*Ca pools may then trigger *e*Ca influx; the *e*Ca influx may simultaneously maintain actomyosin activation at the same time as replenishing the *i*Ca pools; some ER stores may directly communicate with the plasma membrane (and so become directly replenished along with *e*Ca influx); there may even be present Ca-stimulated Ca membrane channels; and it is also possible that *e*Ca influx itself may release *i*Ca stores[71, 77, 421, 519, 564].

3.5.2 Vasoconstriction: Phosphatidylinositol system

However, a SMC contraction can also be stimulated by another process completely independent of the Ca-calmodulin mechanism. This is achieved by **Protein Kinase C (PKC)**, which works largely by an unknown mechanism. It is quantitatively and

qualitatively different from a "classical" Ca contraction[519, 564]. Thus, it is **slower** to build up yet **more persistently maintained**. It also uses much **less energy** in the process[565]. Some recent findings have suggested that receptor-mediated contractile (RMC) agents, such as nor-epinephrine (NE), can 'reset the bias' in the Ca-calmodulin mechanism[292]. Thus, for example, NE may serve to 'up-grade' the ca/calmodulin mechanism at each particular level. This could possibly be due to a direct effect of PKC upon the Ca-calmodulin mechanism[138].

However, whatever its precise mechanism, this represents a major way by which receptor-mediated contractile (RMCs) agonists work. The receptor itself is largely biased toward the outer side of the plasma membrane[564]. On its inner aspect it is associated with a G protein, so-named because it can split guanidine triphosphate[564] (GTP). The **agonist-receptor complex**, by a conformational change, **activates** the **G protein**. This then phosphorylates phosphatidylinositol diphosphate (PIP_2) within the plasma membrane, generating both **inositol triphosphate** (**IP_3**) and **diacylglycerol** (**DG**). Both of these agents subsequently play a crucial part in activating the intracellular contractile machinery[564].

IP_3 liberates of ***i*Ca** from intracellular stores: therefore, this pathway activates the Ca-calmodulin mechanism (*see below*). Since IP_3 may, in turn, become successively dephosphorylated into IP and IP_2, and since these also achieve *i*Ca release, the effect may be perpetuated. Furthermore, IP_4 may similarly be formed as a result of a further phosphorylation, and this effects an elevation in the *i*Ca by encouraging *e*Ca influx[519, 564].

However, DG—by combining with a membrane associated phorbol ester—activates **protein kinase C** (**PKC**). This effects the phosphorylation of numerous intracellular proteins in the same way that all protein kinases do (*see below*). In this context it also activates the actomysosin mechanism, but by a process not involving Ca-calmodulin. It is also possible that an amount of *i*Ca released (perhaps by the IP_3 pathway) which also serves to liberate the PKC pathway. Therefore, the phosphatidylinositol system potentially has much greater scope to effect vasoconstriction than the Ca-calmodulin pathway alone[519, 564].

3.5.3 Vasodilation: Cyclic Adenosine 3',5' Monophosphate (cAMP)

cAMP was first discovered in association with its role as second messenger to several hormones (particularly the catecholamines). It developed phyllogenetically as a "hunger signal". Thus in bacteria, it signals a relative 'glucose lack' to the cell, and thus triggers the de novo production of other enzymes, more suitable for the catabolism of alternative food substrates[564]. Whilst continuing to mediate this signal in mamalian cells (for

example, epinephrine β-mediated hepatic glycogenolysis), it does so by the phosphorylation of protein kinases rather than by genetic transcription[564]. It is formed by the reaction catalyzed by the enzyme **adenylate cyclase (AC)**:

$$ATP \rightarrow cAMP + PPi + H^{+}$$

Thus, cAMP derives from a potentially large source since ATP is a ubiquitous molecule. Though derived from a substance at the centre of cellular metabolism cAMP has nothing further to do with this process, and so can be independently controlled[340, 564]. Moreover, it has a number of functional groupings capable of binding numerous protein kinases, so allowing for their activation. It functions by activating the normally unstable **Protein Kinase A (PKA)**. This molecule consists of two regulatory subunits (R) and two catalytic subunits (C), and these are cleaved by cAMP in the following reaction, at cAMP concentrations around 10^{-8} M[340, 564]:

$$R_2C_2 \text{ (unstable)} \rightarrow R_2 + \mathbf{2.C}$$

Thus one molecule of cAMP liberates two catalytic subunits from PKA upon activation[340, 564]. These then phosphorylate cellular proteins, thus activating them.

Adenylate cyclase is activated in similar fashion to that described for PIP_2 (*see below*). Thus an agonist-receptor coupling results in the activation of an inner membrane-bound Gs protein, which then cleaves GTP. It is this latter reaction that activates AC: again on the inner membrane wall. However, AC may also be directly inhibited by an analogous inner membrane-bound G*i* protein (which again achieves this effect by cleaving GTP). Thus β adrenoceptor stimulation characteristically elevates cAMP levels by way of **Gs →AC activation**, whilst **α2** adrenoceptor stimulation, on the contrary, *decreases* cAMP levels by **G*i* → AC inhibition** (*see below*).

cAMP itself can be switched-off by hydrolysis in the reaction catalyzed by **phosphodiesterase (PDE)**:

$$cAMP + H_2O \rightarrow AMP + H^{+}$$

The activity of both AC and PDE can be subject directly to negative feedback mechanisms[340, 564]. This may play a major role in agonist-induced desensitization.

The mamalian brain has a enormous capacity for the metabolism of cAMP[543]. Thus, levels of both AC and PDE are considerably higher in the CNS than they are within most other tissues[579]. Almost certainly, it plays a **major role** in mediating **cerebral metabolism**, irrespective of its role in mediating cerebral vasodilation. Thus **cAMP** may cause an **increase** in the **CBF** both directly—by **vasodilation, and** indirectly **by stimulating $CMRO_2$** (metabolic regulation)[411, 579]. cAMP achieves vasodilation largely by enhancing the clearance of *i*Ca. One possible mechanism proposed is by stimulation of membrane Na/K-ATPase[359]. By decreasing *i*Na levels, lesser amounts are then available for Na/Ca exchange; and thus *i*Ca levels become diminished in consequence[359].

3.5.4 Vasodilation: Cyclic Guanosine 3',5' Monophosphate (cGMP)

In similar fashion, cGMP is formed by receptor-G*s* stimulation of the membrane-bound enzyme **guanylate cyclase (GC)**: this then catalyzes the reaction

$$GTP \rightarrow cGMP + PP_i + H^+$$

In similar fashion, cGMP exerts its effect intracellularly by the activation of a protein kinase: in this case, by activation of **Protein Kinase G (PKG)**[340]. VSMC relaxation is achieved as with cAMP, by decreasing myofilament sensitivity to calcium[544] and by decreasing *i*Ca levels[544]. However, PKG is activated without necessitating its dissociation into R and C subunits[340]. Whereas PKA is a ubiquitous enzyme, present throughout most species and phyla of the animal kingdom[340] PKG, by contrast, is scarce in mamalian tissues, although comparable concentrations exist in the *brain* (cerebellum) and VSMCs[340]. Nevertheless, it has otherwise similar molecular and catalytic properties to that of PKA, and this suggests a similar phyllogenetic ancestry[340]. Perhaps not surprisingly there is much evidence to suggest the two systems, **cAMP** and **cGMP**, to **interact** at various levels. Thus cAMP and its derivatives are capable of maximally-stimulating PKG activity[340]. Similarly NO (cGMP) and PGI_2 (cAMP) synergize markedly in platelets to inhibit aggregation[500] at doses where both agents alone are completely without effect. More specifically, it has been reported that stimulation of cAMP in VSMCs augments the response to NO[550]. Recently, it has been shown that cGMP and cAMP may directly interact to produce vasodilation via a common mechanism, with both agents potentiating one another[666]. cGMP (e.g. via NO release from SNP) can elevate levels of cAMP by inhibition of Phosphodiesterase III[397].

It is important to note the other cellular mechanisms which cGMP mediates at this stage. Thus **decreased levels** of **cGMP**, for example, are associated with the **stimulation of myoproliferation** in the vessel wall[200]. Therefore, decreased EDRF-NO, for example

resulting from endothelial denudation, may result in the development of SIT. Whilst the administration of sodium nitroprusside, for example, can inhibit this response (*see below*)

Chapter 4
Cerebrospinal fluid and its properties post subarachnoid haemorrhage

4.1 Normal cerebrospinal fluid

The CSF is largely formed by the choroid plexus, where fenestrated endothelial cells abound. Thus the BBB is relatively 'deficient' here. However, a significant proportion (perhaps 30%) derives from the ependyma, and interstitium, of the brain[536].

Ordinarily, there is a gradient for amino acids which favours flow from the plasma and the interstitium, toward the CSF. The CSF, therefore, acts as a 'sink' for these molecules. This is because the choroid plexus constantly extrudes these against an 'uphill' gradient in addition to limiting entry from the plasma. Thus the concentration of, for example, L-arginine is around the 20 ηM level within the CSF, but yet is considerably higher within the serum at up to 50 μM. Similarly, the CSF protein and glucose levels are considerably lower than in the plasma. However, this was not always the case ontologically, since foetal CSF protein levels are up to x 40 that experienced in adult life[536]. Contrary to early reports this is unlikely to be due to developmental differences in the BBB, but more likely instead to the rapid development of aforementioned ependymal protein-uptake mechanisms, thus forming a 'CSF-brain' barrier[536].

4.1.1 Sampling errors

It is important, however, to note that various **CSF concentrations vary** from the **site** of CSF **sampling**. Thus protein levels progressively increase from the ventricles to the lumbar CSF[536]. It is also important to note that measurements of CSF pH are poorly representative of that pertaining in the immediate neuronal environment: (the latter being more acidic than the former). In this context, it must also be stressed that the assessment of neuronal metabolism based upon CSF sampling may also be fallacious: spinal CSF sampling, therefore, merely reflecting spinal neuronal metabolism[536].

4.2 Post subarachnoid haemorrhage cerebrospinal fluid

The CSF of patients following acute SAH (SAH-CSF) is considerably different from that of normal. This fact has been used both diagnostically and prognostically. Thus, a CSF **lactic acidosis**[632] and **fall in glucose level**[630] are often apparent at around the time of DCI. Although both may be epiphenomena, they may both directly impair cerebral function and thus cause further deterioration[487]. However, aforementioned sampling errors must also be borne in mind. Furthermore, temporal factors also introduce further sampling errors, the CSF varying with the time interval post SAH. As a result, this aspect of VSM-DCI study,

as with so many others, has not always been a very precise science; the comparison of 'like with like' frequently being called into question (Part 6, Section III). Nevertheless, several interesting findings have accrued.

4.2.1 Vasoconstrictory phenomena

Many authors have shown, for example, that SAH-CSF is **vasoconstrictory**. Some have shown that this is associated with massive influxes of extracellular $Ca^{317, 529}$ (*e*Ca) into VSMCs[382, 438]. That *e*Ca influx can also be achieved by the mere application of surface blood[124] might suggest the substance to be blood-borne. Bilirubin, for example, can also causes *e*Ca influx[144]. Another mechanism relates to the SAH-CSF breakdown of natural inhibitory mechanisms favouring vasoconstriction: for example, by inhibition of EDRF PGI_2[53]. A similar proposition was mae by Kanamura et al[308], who also noted that this effect was indistinguishable from that of the addition of mere surface blood. Nevertheless, studies that have specifically measured **Hb levels** in SAH-CSF have shown that these **do not correlate** with **VSM**[655]. The overall consensus, therefore, is that the 'unknown substance' is merely that of Hb, and that this per se does not directly correlate with VSM or DCI—although it certainly causes vasoconstriction.

In a slightly more ordered experiment, Sasaki et al (1984) systematically added various antagonists to canine basilar strips pre-constricted with SAH-CSF (Type V VSM model; Part 4, 2.5)[528]. In so doing, they seemingly eliminated agonists such as 5HT and NE in this search, finally concluding the vasospastic substance to be either a "....**prostanoid** or a **lipid peroxide**". Several others have arrived at similar conclusions[58, 509, 539]. Yet many have shown the presence of 5HT and NE—in addition to prostanoids—within SAH-CSF[108]; and VSMC culture with catecholamines has also resulted in the aforementioned *i*Ca overload[357]. Furthermore, HbO_2 can also enhance agonist responses (Part 1, 1.4).

Hardly surprisingly, then, there is much non-uniformity in the literature regarding the characteristics of the SAH-CSF vasoconstrictory response. For example, SAH-CSF may cause an abrupt response when applied, or cause one only after temporal delay[57, 64]. The response obtained may exhibit rapid decay, or be somewhat more sustained[655]. Such variation strongly suggests a failure to compare 'like with like' (Part 6, Section III). In summary, there appears to be **no strong correlation** between SAH-CSF **vasoconstrictory activity** and the **development of VSM**. Indeed, the former is often maximal earlier on when the incidence of VSM is remote[527]. Even if there were a strong correlation, it would be hard to deny that such enhanced tone ought to generally persist throughout the cerebrovasculature; throughout the post-SAH period. This, however, is clearly not the case.

4.2.2. Myofibroblasto-proliferative phenomena

In marked contrast to the aforementioned, Yamamoto et al[668] demonstrated the intriguing finding that SAH-CSF could cause the **de-differentiation** of human **SMCs** in culture **into myofibroblasts**. This is both interesting and important because it suggests that agents acting as *cytokines* are present. Such results endorse the 'structuralist' hypothesis of VSM. For example, both of the cytokines interleukin 1 α and β (IL-1 α and IL-1 β) can induce fibroblastic proliferation in culture[89], and there is evidence accumulating indicting interleukins to be present within the SAH-CSF (Part 6, Section I, 1.6).

PART 3

PHARMACOLOGICAL AND PHYSIOLOGICAL AGONISTS TO BE USED

Chapters 1-3

Chapter 1
Agonists to be used and their properties

1.1 Introduction

It is proposed to use a combination of physiological and pharmacological agents in order to obtain an array of MCA attributes in controls, shams and after SAH. Thus, certain physiological agents will be chosen that are either explicitly, or implicitly, connected with either VSM or DCI. Similarly, certain agents used therapeutically will be used to gain further characterization. It will be pointed out from the outset that there are severe practical limitations to the *number* of agonists that can be studied or their combination (Part 3, Chapter 2).

1.2 Potassium Chloride (KCl)

KCl is regularly used in small vessel agonist studies to primarily 'test' initial vessel viability—and at intervals thereafter. In **low dose** (up to about 10 mmol) KCl acts as a **vasodilator**[635]. In this regard, KCl functions as an important mediator of metabolic regulation (Part 2, 2.5). With **increasing concentration** (>20 mM), KCl causes an ever-increasing VSMC **depolarization** and **vasoconstriction**[635]. In most excitable membranes, the potential across them is largely dictated by an asymmetrical K distribution[59, 519]. This is because greater amounts of K^+ ions must accrue intracellularly to balance an excess organic cations. A negative transmembrane potential around –94mV is created[519], this approximating to the overall membrane potential[519]. Thus, increasing the eK^+ will reverse transmembrane polarity. However, only a small change in this potential is required to activate smooth muscle tension. For example, there can be a 30% increase in tension consequent upon only a 4 mV change in potential, and an 80% increase can follow a depolarization of 14 mV[274]. Such a depolarization may be apparent following either denervation or acute SAH (*see below*).

The K^+ contraction characteristically consists of two components: a **peak (phasic)**, followed by a **plateau (tonic) response** usually smaller in magnitude (Fig 15). It is thought that both responses are largely due to the opening up of potential-specific channels (**PSCs**) within the VSMC membrane (Part 2, 3.5). Thus KCl's action is totally dependent upon the presence of adequate amounts of external Calcium (***e*Ca**). However, unlike the case with receptor-mediated contractions, the K response is independent of the endothelium and the co-release of any EDRF[197, 326].

Although producing reliable and characteristic vasoconstrictions there are several reasons for not wishing to use KCl as a pre-constrictor of vessels for a subsequent vasodilator

study. Thus a **prolonged KCl** depolarization can be **irreversibly detrimental** to vessel function. As a result, endothelial-dependent vasodilations may be less efficacious following KCl vasoconstriction[197]. Indeed, KCl can cause significant endothelial damage[542]. Because some vasodilator studies require over half an hour, such prolonged KCl contact could be detrimental. Moreover, the KCl response may be affected by SAH[274, 635, 641]. Thus KCl was not to be used as the pre-constricting agent in vasodilator studies in this thesis.

1.3 Norepinephrine (NE)

The catecholamines epinephrine and norepinephrine (NE) have widespread effects throughout the body. NE, in particular, may be important after SAH, as not only is it a prime **neurotransmitter** within the SNS, but it also present in significant amounts within **platelets**. The latter are, of course, in plentiful supply within the SAH clot. NE is synthesized intraneuronally from the substrate tyrosine. This is first hydroxylated into DOPA (dihydroxyphenylalanine), and then subsequently decarboxylated into dopamine (3-hydroxytryptamine). The latter is then finally β-hydroxylated into NE[73]. NE, in turn, may be catabolized by two major mechanisms. One involves **catechol-o-methyl transferase (COMT)** extraneuronally; the other, a **mono-amine oxidase (MAO)** largely found intraneuronally[73]. Even so, the vascular endothelium can also take up and destroy both NE and 5HT by MAO mechanisms[618]. Although **most NE released** (probably up to 90%) is subject to **re-uptake into the SNS neurone**, and thus made available for re-use[73]; this does not appear to be the case with cerebral vessels[50].

Adrenoceptors hold a classical place in pharmacology in that they were the first to be studied and classified. The first major categorization was by Ahlquist in 1948 who suggested the classical division into α and β **adrenoceptors**. The β receptor was clearly distinguished by its insensitivity to the ergot alkaloids and, ultimately, by its selectivity to isoprenaline. Therefore, Ahlquist's classification was based purely upon drug sensitivity and *not* physiological effect. As a result each receptor could, depending on the tissue, still actually mediate stimulatory *or* inhibitory effects—it was only the drug-binding character of the receptor that mattered in this classification. The three major physiological agents epinephrine, norepinephrine and dopamine could, however, be effective at both receptor types, with different potencies[71, 77, 421].

A further contribution came in 1967, when Lands et al showed that there were two subtypes of β receptor. These were distinguishable by their different tissue distributions; and by their differing affinities to NE. Thus the **β1** receptor was found mainly in the **heart** and adipose tissue, and appeared equally sensitive to both agents. However, the β2

receptor was mainly associated with the smooth muscle of the **blood vessels**, the **airways** and the **uterus**; and was considerably **more sensitive to epinephrine** than to NE. In 1974 Langer subdivided α receptors into α1 and α2 subtypes, initially based on anatomical grounds (α1 receptors were mostly post-synaptic, α2 receptors pre-synaptic). However, this was soon extended into a physiological subdivision, the drug prazosin specifically combining with α1 receptors (α2 receptors could also be post-synaptic). More recently, the β3 receptor has also been identified, this being associated with neonatal brown fat stimulation[71, 77, 421].

However, it soon became clear that the α receptor heterogeneity represented no mere subdivision as with β receptors. Thus the α1 and α2 receptors were as distinct from each other as they were from the β receptors. In consequence, it is far more useful nowadays to **classify** the **adrenoceptors** into **three main groups** **α1**, **α2** and β, for three main reasons:

- Differences in **agonist affinity** vary most between these groups, rather than within them.
- The **second messenger systems** differ only between these groups.
- **Amino acid sequencing** suggests three groupings rather than two.

In fact, this has now set the precedent for the classification of all receptor types: namely **drug-related properties, signal transduction mechanisms,** and finally **genetic/amino acid profile**[71, 77, 421].

1.3.1 α1 receptors

Responses can be summarized as[71, 77, 421]:

- Epinephrine and NE equally effective.
- All labelled with ^{3}H Prazosin.
- Tend to mediate SNS responses.
- All associated with **increased *i*Ca.**

The mechanism of action may actually vary between agonists. The most established mechanism is that of G*q* activation leading to IP_3-*i*Ca release from intracellular stores, and PKC activation. Additionally there are direct G protein linkages to membrane PSCs, thus causing *e*Ca influx. There may, in fact, be two G proteins associated with α1 receptors. One links to PIP_2, the other to PSCs. It may be that only activation of both reflects **full agonist** activity at these receptors. Activation of only one (the PSC) is indicative of **partial agonist** activity. The fact that *low dose* ($<10^{-8}$ M) NE vasoconstriction is *not* associated with a *membrane depolarization*, whereas higher doses are indicate such channels to not be opened at lower doses. However, the situation may be more

complicated as **stimulation** of **cyclic nucleotides**, **phospholipase A_2** or even **phospholipase D** may also result form α1 activation[71, 77, 421].

1.3.2 α2 receptors

Responses can be summarized as[71, 77, 421]:

- Epinephrine and NE equally effective.
- All labelled with ^{3}H Yohimibine.
- Tend to mediate circulating catecholamine responses.
- All associated with **inhibition of adenylate cyclase**: (i.e. 'anti-β' effect).

The major mechanism of action here is G*i* (inhibitory) activation inhibiting AC, this depressing intracellular cAMP. It is unlikely, however, that there is a basal cAMP release in resistance vessels for which to inhibit. Therefore, other mechanisms are required to explain the pressor effects of α2 stimulation here. Thus, α2 receptors may also be directly linked to PSCs, α2 stimulation thus enhancing *e*Ca influx. Again, the situation may be complicated further still by activation of K channels, **phospholipase A2** and increased Na/H exchange[71, 77, 421].

1.3.3 β receptors

Responses can be summarized as[71, 77, 421]:

- Epinephrine x100 selectivity at β2 receptor
- NE more potent at β3 receptor
- Propranolol β1, β2 >> β3 receptors
- All **activate adenylate cyclase**

The common mechanism here is straightforward G*s* activation of AC with elevation of cAMP. Again, there may also be direct connexions to membrane PSCs, especially in cardiac muscle.

It is known that all three receptor types can be present upon the same cell, but that usually one predominates in any particular tissue[77]. The permutations increase even further when we contemplate that all three types can appear in all three major locations (VSMC membrane, pre-synaptic SNS membrane, and endothelium). Such **receptor combinations** could mediate **opposing** (α2 versus β); **redundant** (β1 versus β2); or **synergistic** (α1 and β especially in the heart)[77]. There is some evidence that the α2 receptor is both more common in the non-human animal kingdom, and that it mediates less potent vasoconstrictory responses[607]. However, the apparent association between the α2 receptor diminished responses may well be because of α2 endothelial presence[607]. Here α2

stimulation results in simultaneous EDRF release: either PGI_2[321, 679] or NO[243-245, 679]. Jones et al noted that, within the coronary circulation, α1 receptors predominated on the small arteries, whereas the α2 receptors were more present upon the arterioles[298]. They noted a significant EDRF component to NE agonist tone associated with α2 endothelial receptors, this being 'unmasked' by L-NAME. This explains why NE responses are diminished in certain locations (e.g. the coronary circulation) where it would be undesirable to have adrenergic influence. Perhaps this is also the case, then, within the cerebrovasculature[50]?

The NE response, like KCl, is composed of **phasic** and **tonic components.** However, the phasic response is neither as abrupt nor as large. Although it has always been hoped that clearly-defined mechanisms would explain these two components, no such clarity exists. Certainly in some vessels, the phasic response appears more dependent upon *e*Ca; yet in others the converse is true[71, 77, 421].

1.4 5 Hydroxy Tryptamine (5HT) (Serotonin)

1.4.1 Introduction

5HT is a widespread physiological agent; with widespread functions. Probably its most important is as a **neurotransmitter** within the brain. But it also has widespread effects upon smooth muscle throughout the body. Of particular relevance are its **cerebrovascular effects**, its release from **mast cells**, and its release from **platelets**. It has long been considered to be instrumental in the development of VSM. This is probably because it has been similarly implicated in various other 'vasospastic' states such as Raynaud's phenomenon, Prinzmetal's angina and migraine. It is also present in the CSF of patients following SAH[87, 676]. Virtually all of the circulating 5HT is locked up in platelets[276, 323, 610]. Since reserpine also depletes 5HT from both platelets[649] and neurones[405], it is possible that reserpine exerts its VSM-protective effects by 5HT inhibition in addition to NE inhibition[555, 584] (Part 1, 1.3.2.2). More interesting still, 5HT possesses VSMC myo-proliferative effects[632]. Thus, 5HT may play a role in both structural and functional mechanisms associated with VSM.

1.4.2 Receptor heterogeneity

With NE one may reasonably derive the impression that adrenoceptor subtypes developed as genetic polymorphisms, which subsequently became established as the result of evolutionary pressures. If so, then the situation with 5HT denotes an extreme one. Thus, in excess of **fifteen receptor subtypes** have evolved to cater **for**—seemingly—**one agent**[273].

Three major 5HT receptor **divisions** contain **further subtypes**. The first major division (**$5HT_1$**) has five subtypes: $5HT_{1a-e}$. This first grouping is quite a heterogeneous one. Certainly the $5HT_{1c}$ receptor appears to have more in common with the second group ($5HT_2$), and there have been some calls to re-classify it as such. But as a group, their common feature is their transduction of **decreased intracellular cAMP levels** via a negative (G-protein → adenyl cyclase) coupling. Cerebral vessels contain $5HT_1$-like receptors in the **MCA**, **BA** and **pial vessels**. These may be directly linked to PSCs as with α receptors: certainly this may explain their strong contractions. Sumatriptan acts as an agonist, and methiopin as a non-specific antagonist, at these receptors[273].

$5HT_2$ receptors activate platelets, and mediate bronchoconstriction and non-cerebrovascular function ($5HT_{2a}$). They commonly transduce by **stimulating PIP_2 breakdown**. The $5HT_{2c}$ receptor has also been shown to mediate the composition and volume of CSF. Ketanserin effectively antagonises at these receptors, and was formerly used therapeutically in the treatment of another 'vasospastic' condition (Raynaud's phenomenon)[273].

$5HT_3$ receptors mediate **cellular depolarization** and therefore $_e$Ca influx: this is important in triggering the Ca-calmodulin dependent nitric oxide system, and can result in vasodilation via increased cGMP (*see below*). They do not couple to membrane G proteins. Ondansetron (a potent anti-emetic) is the antagonist at these receptors[273].

$5HT_4$ receptors stimulate adenyl cyclase and **elevate intracellular cAMP levels.** Metoclopromide can exert agonistic effects at these receptors. The transductional characteristics of the $5HT_5$ receptors are unknown: these receptors mediate the effects of LSD (lysergic acid diethylamide), for example[273].

1.5 Prostaglandin F2α (PGF2α)

1.5.1 The prostaglandins

The biochemical activity ultimately associated with prostaglandins (PGs) was actually discovered as long ago as 1930 in a sample of human seminal fluid. It was not until 1957, however, that Bergstrom and Sjovall successfully purified and isolated the first of these lipid acids[105]. Thus, PGE and PGF were identified based upon whether they were extractable from ether (E) or not (F). Von Euler later coined the term "prostaglandin" in reverence to the organ of their original discovery (prostate gland). The chemical structures of these two agents were not elucidated, however, until 1962. They were then formerly identified as PGE_1 and $PGF_{2\alpha}$: lipid acids with peculiar cyclopentone rings. Since then the main family has been designated alphabetically from A_2 to H_2. The **letter** characterizes the

ring structure; the **numerical subscript** the *number of double bonds* in the *side chains*[105]. This classification has now been extended to include two rather unstable compounds with prostaglandin-like activity: the "prostanoids" thromboxane (TXA_2) and prostacylcin (PGI_2). Both are vitally important to haemodynamics. PGs are synthesised by the action of cyclo-oxygenase on the precursor **polyunsaturated fatty acid (PUFA)**: 5,8,11,14 eicosatetraenoic acid: **arachidonic acid**. At an early stage, an alternative reaction pathway can lead to the formation of leukotrienes[105].

A feature of PGs and receptor-interaction is that the latter 'autoregulate'. Thus, high local concentrations of agonists result in **rapid 'down-regulation'** of transduction mechanisms, or in decreased receptor binding sites[105]. This resembles the **tachyphylaxis** of CAs. The second messenger mechanisms vary between each group: these will be discussed with each subtype. PGs can be released in vivo by both NE acting at $\alpha 1$ adrenoceptors, and by HbO_2[183, 601] acting on the endothelium.

1.5.2 Prostaglandin receptors

The action of the five naturally occurring prostanoids, PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and TXA_2, are now known to be mediated by complementary receptors thus designated DP, EP, FP, IP and TP. Therefore, PGE has its greatest affinity at EP receptors; PGF at FP receptors; and so on. As might be anticipated, there can be cross-reactions within this scheme. Yet in comparison to other hormones receptor-selectivity is impressively conserved. This is in marked contrast to the catecholamines, tachykinins, and even the related leukotrienes[105].

DP receptors are present on platelets, VSMCs and in the CNS. Apart from mediating painful stimuli (hyperalgesia), they are generally 'inhibitory' in nature. Thus, they are anti-aggregatory and vasodilatory agents. The transduction mechanism involves a Gs protein mediated elevation of cAMP by the stimulation of AC[105].

The EP receptors are the most complicated. The most relevant are the EP_3 receptors which mediate VSMC contraction and aggregate platelets. They interact with two G proteins. Thus G*i* coupling decreases intracellular levels of cAMP by the inhibition of AC, and G*q* coupling induces the breakdown of PIP_2[105].

The IP receptors are mainly associated with VSMCs and blood platelets. They mediate a profound hyperalgesia, but otherwise are quite 'inhibitory'. Thus, they mediate vasodilation and are anti-aggregatory. They are present mostly on arteries: rarely on veins. The transduction mechanism involves G*s* stimulation of AC to elevate intracellular cAMP.

However, it has become apparent recently that, in some cases, they can mediate a vasoconstriction: in this case they elevate *i*Ca levels by some unknown mechanism[105].

TP receptors are predominantly present upon platelets and VSMCs: they are thus the most 'specialized' of the series. TXA_2 acting on these does so in an opposing fashion to that of PGI_2 at the IP receptors. Therefore they tend to elicit vasoconstrictory and platelet aggregatory phenomena. TP receptors are far more widely distributed in the vasculature than IPs, and may play a major role in pathophysiology. With perhaps particular reference to the process of VSM, there are TP receptors present upon myofibroblasts: thus, TXA_2 ordinarily mediates the process of wound healing[105].

1.5.3 PGF2α

Whilst PGF2α is a potent FP receptor agonist, analogues were not formerly particularly selective, there being appreciable agonistic activity at both EP and TP receptors. However, current PGF2α analogues are increasingly selective, with little or no 'cross-reactivity'. Moreover, the FP receptor itself is 'pure' in that there have been, as yet, no subdivisions of this receptor found. FP receptors are widespread throughout the body, but are especially present upon VSMCs[105]. PGF2α may mediate uterine contractions, as well as the constriction of the long spiral arteries of the uterine wall causing menses (Part 1, 1.3). The transduction mechanism is predominantly via G*q* membrane protein activation of PIP_2[105]. Thus, PGF2α results in contraction by both PKC and *i*Ca mechanisms. In addition, there is some evidence that a second and separate phase of Ca elevation occurs as a consequence of *e*Ca influx. Thus, PGF2α produces reliable and prolonged contractions for indefinite periods, it therefore being the ideal contractile standard in most agonist studies[571, 652]. It is one of the strongest vasoconstrictors known, and can maintain constriction even in a Ca-free medium.

1.6 Uridine 3', 5' Triphosphate (UTP)

The major nucleotides usually studied are the purines, in particular adenosine. Adenosine is a significant vasodilator within the microcirculation which, as a 'waste product' of tissue metabolism, functions in metabolic regulation (Part 2, 2.5). However, UTP, a pyrimidine, acts as a vasoconstrictor in cerebral arteries and, therefore, is not likely to play a role comparable to adenosine. Whatever its precise role, this remains relatively unexplored within the CNS. This is in spite of the fact that the **brain** represents a rather **rich source** of this nucleotide (30μM/100g tissue)[614]. The apparent disregard for UTP becomes more surprising when one considers that UTP may be relatively *specific* in function toward the cerebrovasculature. Thus, systemic injection fails to elicit any pressor response at all[614],

whilst negligible contractile responses are achieved in isolated coronary *or* mesenteric vessels[614].

The attraction of UTP to the current experiment becomes heightened upon consideration of its contractile attributes. For example, UTP produces a *prolonged* vasoconstriction of cerebral arteries that lasts up to 7 hours dogs, or 20 hours in humans[614]. Such a result can also be achieved in vivo, thus defying the principle that most spasmogens are rapidly inactivated in free-form within most biological fluids[313]. This **relative absence of tachyphylaxis** distinguishes UTP from other VSM contenders such as 5HT[652-655], and is in marked contrast to the often rapid tachyphylaxis seen with catecholamines (Part 3, 3.2). In addition, its immediate metabolite, uridine diphosphate (UDP) may share similar properties[614]. UTP is, of course, stored in significant amounts within blood platelets, along with serotonin (5HT) and norepinephrine (NE) [614].

There are specific receptors for the pyrimidines[614] although these remain under-explored. Such pyrimidoceptors would provide as targets for therapeutic antagonism should UTP be shown to influence VSM. However, UTP may exhibits receptor heterology, cross-reacting with purinoceptors[614]. This must be taken into account when developing the appropriate antagonist.

In cerebral arteries, UTP also produces **phasic** and **tonic responses**. The intracellular 'second messenger' systems are probably similar to those of other receptor mediated agonists. Thus, it has been concluded that UTP can result in the opening of both ROCs and PSCs in the cellular membrane, as well as the quick release of membrane-associated *i*Ca[614]. The latter may quickly trigger the former, and this may be the mechanism behind both the rapidity, and the increased magnitude of the phasic to the tonic response[614].

1.7 Papaverine (PPV)

Papaverine (PPV) is an example of a **pharmacological agent** to be used in this study. It is an opiate alkaloid, with potent vasodilatory actions in a wide range of vascular beds[13]. However, it can also dilate other smooth muscles, and may cause a mydriasis if infused close to the origin of the opthalmic artery[262]. It is thought to act directly on VSMCs by **inhibiting phosphodiesterase** in the sarcoplasm: the build-up cyclic adenosine monophosphate (cAMP) then reduces *i*Ca by a number of mechanisms[73]. However, it **also** may **directly interfere** with the **Ca-calmodulin** machinery. Because it is moderately lipophilic it should be able to exert its effects both intra- and extra- luminally; as is indeed clinically apparent. However, PPV may have a longer half-life when administered extraluminally into the cerebrospinal fluid (CSF)[463, 537, 634], and it may be even longer in the

region of a clot where the 'washing-action' of CSF may be deficient[614]. It is frequently used extraluminally at craniotomy by topical application onto vessels within the operative field. The intraluminal route is used more specifically to reverse angiospasm following SAH. Maximally effective concentrations are in the 10^{-4} M region[608].

1.8 L-arginine (L-ARG)

The importance of the endothelium with vasodilation by agents such as acetylcholine (ACh), was first hinted at by Furchgott in 1980. By 1984, evidence for a diffusible substance that passed from the endothelium to the VSMCs had been obtained[197], this agent being referred to as an **endothelial derived relaxing factor (EDRF)**[219]. It appeared to be a very unstable substance, particularly in the presence of superoxide anions, hyperoxia, methylene blue, and (of great relevance to VSM) Hb. The latter principle stresses the importance of the tight nexi between the endothelium and the VSMC[506]. By 1987 there was much evidence accruing identifying **nitric oxide (NO)** as the EDRF[478]. It soon was demonstrated that NO was produced from L-arginine within the endothelial cell by the activity of the enzyme **nitric oxide synthase (NOS)**[478]. It was evident that, at least with systemic vessels, NO was **constantly released** under **basal tonic conditions** in vivo, this sometimes amounting for up to 20-40% of the total NO-release possible[75]—a shear stress representing the stimulus required for its release.

NOS was soon found in other tissues such as in the brain (where it could also function as a neurotransmitter), and within platelets. It only required NADPH as cofactor, and released NO in picomolar amounts. However, this process was dependent upon the activation of the intracellular calcium/calmodulin system[187]. It was found to be a **constitutive** enzyme **(cNOS)**, present in fixed amounts within tissues, the cNOS present within **neurones** and **blood vessels** being distinguished as **type I** and **type II** respectively[318]. Thus:

$$\text{L-ARG} \rightarrow \text{NOS} \rightarrow \text{NO}$$

Upon diffusion to adjacent VSMCs, **NO** then becomes the **substrate** for **GC** to produce cGMP[426]. Thus, all NO-forming agents—such as the organo-nitrates—similarly function by the formation of intracellular cGMP.

However, it had been known since 1983 that rats treated with *E.Coli* lipopolysaccharide (LPS) had a high urinary nitrate output which correlated with the degree of the pyrexia[426]. Even further back, it had been known that macrophages stimulated by cytokines (from sensitized lymphocytes) exhibited **non-specific cytotoxicity** toward micro-organisms[426]. In fact, they were also cytotoxic to neoplastic cells. In neither case, however, was this dependent upon phagocytosis[426]: rather it involved target cell inhibition of DNA replication[426]. Granger and Lehninger then showed[216] that such non-specific cytotoxicity

involved an **inhibition** of **target cell mitochondrial respiration** at the levels of complex I and II in the **electron transmitter system.** Others, in turn, showed that the **citric acid cycle** enzyme aconitase was similarly inhibited by a soluble factor from appropriately stimulated macrophages[426]. Formerly identified as NO, potent inhibition of cytochrome oxidase was obtainable in the (higher) nanomolar range[426]. Biochemically, NO appeared to combine with (non-haem) iron-sulphur groupings at the catalytically active site, forming iron-nitrosoyl complexes which inactivated the enzyme[426]. This effectiveness clearly varied between cells, with some being cytotoxic and others cytostatic.

A clue to this 'other side' to NO came from Rees et al in 1990[503]. They showed that endothelium-depleted rat vascular rings incubated with **lipopolysaccharide (LPS)** produced **de novo NOS**, predominantly within the **VSMCs**[503]. This, therefore, tied in with the findings of Wagner et al 1983, and appeared inextricably linked with the 'macrophage' findings. Thus, NO could also be formed by other cells in response to "infection risk" stimuli. It was soon apparent that this de novo NOS **differed markedly from its constitutive relation (cNOS):**

- It was Ca/Calmodulin independent.
- It could be induced either by **LPS** or directly by **cytokines**.
- It requires NADPH, tetrahydrobiopterin, flavine adenine nucleotide, and reduced glutathione.
- It was **not subject to** any cellular **control** mechanisms: i.e. it will continue to form NO from L-ARG unless either the substrate runs out, or the cell dies[426].

The distinct form of NOS was therefore referred to as ***i*NOS: (*inducible* NOS)**. It may be of supreme importance to the CNS as the appropriate cytokines can be stimulated, in great quantities, by processes as seemingly unrelated as acute bacterial meningitis and SAH. Thus, widespread release of excess NO in these circumstances can be cytotoxic both to foreign cells as well as to host cells; *i*NOS producing indefatigable amounts of this cytotoxic agent. For example, IL-1β has been shown to stimulate ***i*NOS** production in **VSMCs, endothelial cells, microglia, astrocytes,** and **fibroblasts**[420, 443].

As aforementioned, Hb has a strong affinity for NO: a fact known since 1865[263]. In 1957 it was shown that Hb combines with NO with x1500 greater affinity than with carbon monoxide (CO)[209]. It has also been shown that topical application of blood to cerebral vessels effectively inhibits tonic NO activity, leading to vasoconstriction. This has therefore been proposed as a mechanism for VSM. However, **Hb predominantly inhibits extracellular NO** since Hb cannot gain easy access to the intracellular milieu[611]. A priori

Hb may not readily affect a VSMC source *i*NOS. **Hb** may, in turn, actually **augment IL-1β production** of **more NO**[318, 573].

NO has more diverse effects still. Its role as a **neurotransmitter** has already been mentioned—particularly when released from nitroxidergic neurones[486]. It also has important **anti-platelet** and **anti-thrombotic** properties, which it shares with PGI_2 (*see below*). It may even interact with PGI_2 in this regard[198]. NO particularly appears protective against injury-induced arterial thrombosis[426]. It is also **mast cell stabilizer**[201, 389]; and, as such, would be expected to possess marked anti-inflammatory properties, especially in the context of ischaemia-reperfusion injury[311, 341]. Particularly important in this regard would be the preservation of the BBB in the presence of platelet activating factor release from endothelial cells[261]; and from mast cells[201]. In fact **NO** has been shown to be positively **beneficial** in **stroke**[677].

Finally, NO has potent **anti-proliferative effects**. Basally released NO is essential not only for counteracting vessel tone but also for damping down the release growth factors[208]. Chronic administration of L-ARG effectively reduces the SIT that develops in response to non-specific vessel trauma[203, 234], whilst impairment of EDRF release correlates with the degree of SIT[286]. In this context, **basic fibroblast growth factor (bFGF)** appears important in **restoring** any endothelial damage and increasing **NO production**, following vessel trauma[414].

It should be pointed out that "EDRF" is now known to actually be more than one agent. In fact, it is probably at least three. For example, prostacyclin, PGI_2 (*see below*), is also an EDRF which shares many of NO's other properties (*see below*): the two agents possibly interacting in some of these aspects. Another EDRF is associated with hyperpolarization of the VSMC membrane (endothelial dependent hyperpolarizing factor, EDHF). However, there is some evidence that NO itself may be associated with a hyperpolarization of the VSMC membrane.

There may be bountiful amounts of the substrate L-arg in the blood in certain conditions (up to 45-148 μM)[426]. Its concentration within the CSF is normally around 20 nmol/L: but this may increase to 80 ηmol/L with the presence of subarachnoid blood[304], as do most amino acids except taurine.

1.9 Nω-Nitro-L-Arginine Methyl Ester (L-NAME)

Nω-nitro-L-arginine methyl ester (L-NAME) acts as an analogue of L-ARG which competitively interacts with NOS. Instead of forming NO, however, it results in the

formation of an inactive complex. Thus, it antagonises NOs effects[503]. When injected systemically into normal rats, it causes an elevation in MAP concurrent with a marked decrease in VSMC cGMP[15]. This, therefore, suggests that NO is 'basally' released within the systemic circuit, tending to constantly counteract background tonic activity (*see above*). Such basal release may also be present within the cerebrovasculature, at least within the BA. Thus, addition of L-NAME in vivo diminishes CBF, at least transiently[503]. L-NAME is, therefore, predominantly used to assess 'basal' release: either in vitro or in vivo.

1.10 Sodium Nitroprusside (SNP)

Sodium Nitroprusside is another example of a **pharmacological** agent to be used in this study. It is an 'organo-nitrate' like isosorbide nitrate (ISN) and glyceryl trinitrate (GTN), and is thought to produce its effect primarily by the 'spontaneous' liberation of NO *within* VSMCs. This concept derives from the practical observation that solutions of the compound are very unstable experimentally, particularly in the presence of light[16,17]. Perhaps it is too simplistic then to refer to such 'spontaneous' liberation. Nevertheless, the presence of reducing agents do greatly promote this effect (even the presence of red cell or VSMC membranes achieving this)[31]. Whatever the precise mechanism, somehow integral to this process is the **simultaneous release of cyanide** within VSMCs[31]. Although cyanide potentially accumulates as NO is released, this is not ordinarily a problem as NO is so potent it is effective at lower concentrations where little cyanide toxicity would accrue[16,17, 426]. But with higher doses, and particularly following prolonged usage, this becomes an ever-present danger[426]. Such toxicity could also be a theoretical problem within the context of the current experiment. Because of this, the duration of exposure at each dose was kept to a minimum, and the bath copiously washed out with saline solution at the end of each study.

Therefore most of **SNP's effects** ought to **resemble** those of **NO** or other NO-donors (*see above*). Importantly, this does not require the presence of the endothelium. Therefore, SNP assesses NO activity independently of endothelial release. There is some confusion as to whether Hb may actually interfere with NO release from SNP (or with other nitrosovasodilators). Kim et al found that Hb in CSF could inhibit the effect of the nitrosovasodilators, by suppressing GC activity[325]. Feelisch and Noack, however, found the opposite result[169]. The situation may be different with the cerebral vessels: particularly in vivo within the CSF.

SNP is most often used in neurosurgical practice to obtain **controlled systemic hypotension** immediately **prior** to **aneurysm manipulation**. However, it has also been used to directly reverse clinical and angiographic VSM[268]. Naturally, the risk of an acute

fall in MAP has tended disfavour such use. Nevertheless, some have found that this need not necessarily occur[617], whilst others suggest that regional CBF may only suffer in the telencephalic or neocortical regions[609].

1.11 Histamine (HA)

A major source of HA is within **mast cells (MCs)**. MCs are present within the systemic and cerebral vasculature[112, 154, 202]. However, they are said to be **more prevalent** in association with **vessels** having **ruptured** from a **berry aneurysm**[90, 166]. Thus, they may have been present in increased amounts prior to SAH (Part 2, 1.4.2). HA may, however, also be associated with **non-MC sources**. There have been several allusions to these[309], some authors demonstrating MCs to be present within medial VSMC layers[159]. HA is also an important neurotransmitter[220, 259].

There are non-uniform reports in the literature as to the type of response that HA effects within the cerebral vasculature. Ultimately, HA responses may depend upon the concentration used, the systemic vascular bed, or the species: the latter being particularly important. Thus, many have found HA to be vasoconstrictory in dogs, cats or rabbits[11, 447, 568]. In contrast, in mice HA may have no obvious effect[512], whilst in primates[309, 643] and humans[595] HA is a cerebrovascular dilator. HA is also a cerebrovascular dilator in rats.

Whatever its ultimate cellular response, HA achieves this effect via three different kinds of membrane receptor: HA1, HA2 and HA3. The HA1 receptor classically mediates capillary 'leakiness' via endothelial receptors and cGMP mechanisms. However, HA1-mediated vascular permeability is conspicuously absent in the CNS, even when applied topically[388]. This may result from the fact that although the capillary endothelial cells take up HA from both sides the vessel wall, HA is subsequently released *only* on the luminal side[277]. The HA1 receptor may be blocked by agents such as chlorpheniramine.

HA2 receptors mediate gastric acid secretion and can be blocked by cimetidine or ranitidine. However, they also appear to mediate most of HAs effects within the cerbrovasculature, this being achieved via cAMP mechanisms[388, 452]. HA can also release EDRF via endothelial HA1 receptors, thus effecting vasodilation via cGMP mechanisms[309]. Cerebrovascular responses in most cases are HA2-mediated[259]

1.12 Hypoxia

Hypoxia can be achieved in in vitro studies by bubbling a mixture of 95% N_2 and 5% CO_2 over several minutes. The effective pO_2 can then be directly ascertained from the saline bath. CO_2 responses are more commonly used in cerebral vessel studies, since CO_2 is

ultimately a therapeutic weapon. Nevertheless, it is difficult with in vitro studies to isolate the effects of CO_2 from that of the acidosis necessarily created. Furthermore, hypoxia is able to dilate MCAs from baseline MT without any preconstriction being required, this being achieved via non-NO EDRF release[193]. Hypoxia may thus serve the dual function of measuring MT as well as confirming endothelial function. The EDRF released is probably a prostanoid[193].

Chapter 2
Permutations and combinations

It is important to appreciate that agonists do not, of course, work in isolation within the physiological milieu. This may be considered both a limitation as well as an advantage of in vitro models (Part 4, 4.2). Thus, agonists occur physiologically in combination with others with which they may ordinarily **interact**[572, 655]. Such interactions may be either **additive**, **synergistic**, **inhibitory**, or **non-existent**. Furthermore, effective agonist concentrations may vary dependent upon local concentrations of catabolic enzymes, for example. Even the ambient temperature may vary in vivo between regions. Thus, in cooler regions (e.g. fingers and toes) agonists may effect different responses, such temperature-dependence explaining the distribution of diseases such as cryoglobulin-induced Raynaud's phenomena and Leprosy, for example. Furthermore, although homeostasis ultimately supervenes in vivo, this is often only after significant local fluctuations.

In vitro studies therefore circumvent these problems by, as far as is reasonably possible, controlling all such variables (Part 4, 4.2). This allows for the identification of **isolated** vessel properties. Between the two extremes of in vivo and in vitro there are obviously a vast number of intermediate agonist permutations possible, all potentially analyzable in vitro. Thus, although one must start by noting isolated characteristics, one must, ultimately, build up a picture of how these may **interact** cumulatively.

The problem is, of course, that the number of possibilities is prohibitively vast. For example, in the current study it is proposed to analyze the isolated properties of up to 10 agonists: a mixture of vasoconstrictory and vasodilatory substances. Since each requires, following suitable periods of wash-out and equilibration, about one hour in order to be completed, up to 10 hours (Part 4, 7.4) would be required to study all 10 agonists. This, in turn, would be additional to the time taken to obtain the vessels (1½ hours in controls, and up to 6 hours following operation!). Since all current in vitro studies follow directly on from any operative model—no vessels were fridge-kept for use on subsequent days—this all has, therefore, to be accomplished within the confines of one day. As a result, this obviously places a great limitation upon any proposed further study of agonist *combinations*.

Such agonist combinations can be numerized. For example, the choice of *r* items from a total of *n* possibilities (where the order is unimportant, i.e. a combination) is governed by the equation:

$$nCr = \frac{n!}{(n-r)!\,r!}$$

the sign ! denoting the *factorial* of that integer chosen (i.e. 4! = 4x3x2x1).

This would apply to combinations such as [NE + PPV], [HA + SNP], [5HT + L-NAME] etc. Therefore, if we were to repeat the study using **two agonists** at a time (from a total of ten), instead of using each in isolation, then one could do this in:

$$\frac{10!}{8!2!} = \frac{10x9}{2x1} = 45 \text{ways}$$

Similarly, one could choose a combination of **three** agonists in **120** ways; **four** agonists in **210** ways; and **five** agonists in **252** ways.

Thus, although conceptually attractive, the concept of using sequential agonist combinations in vitro is not really tenable. One combination that is necessarily used in the current study is, of course, that of [*vasodilator* + PGF2α] (*see* Part 5, Results). Nevertheless, other combinations were attempted, in particular that of PPV with SNP (*see below*). The concept of permutations and combinations will be referred to again later, in the assessment of the nature of receptor-mediated responses. It is quite likely that VSM may be much better explained by such interactions[345].

Chapter 3
General properties of agonists relevant to vasospasm

3.1 Introduction

Here we discuss the effects of an abnormal increase or decrease in the amount of agonist upon 'end-organ' responses. The main example is that of NE acting as neurotransmitter between the SNS and VSMCs, but the principle may be more widespread. Such consideration leads us frequently to regard the relationship as one of a **'homeostasis of effector-cell function'**.

3.2 Agonist-induced desensitization

A feature of some agonist-receptor interactions is that of a rapidly-developing **tachyphylaxis**. This is a particular property of catecholamines, particularly when acting in a hormonal capacity: but it may also be apparent with PGs[105]. It is particularly well developed within the **pineal gland**[181]. In some cases agonist incubation for only 30 mins or so will result in an 80% loss of effect. This is not completely explained by a decreased receptor density as decreases here may only amount to 17%[664]. In many cases the subsequent recovery of normal responsiveness requires de novo protein synthesis. Alternatively, de novo protein synthesis may be required to effect receptor 'down-regulation' in the first place. Whatever the mechanism, adenyl cyclase alone is a prerequisite[664]. Ultimately it entails two processes separated by time. The **acute** desensitization is effected by **receptor uncoupling**. Up to 50% can be uncoupled in this way, this being achieved by a disconnection of the receptor **from** the membrane **G protein**. Thus, any agonist-receptor combinations are functionless. Eventually in the chronic situation, however, the **receptor number** itself becomes **reduced**[664].

3.3 Denervation Supersensitivity (DSS)

Denervation can have many differing effects, this dependent upon the particular nerve, its situation, and transmitter substance released. It also depends heavily upon the completeness of the denervation. The main principle to be discussed here is with regard to VSMCs and transmitters such as NE, 5HT and UTP. In 1949, Cannon and Rosenbleuth formally described the gradual development of a supersensitivity of the skeletal muscle membrane following acute denervation[79]. This was particularly so to the corresponding transmitter, acetylcholine, and it involved an **increased number** and **distribution** of nicotinic **receptors,** as well as **diminished transmitter degradation** within the synaptic cleft[79]. But it also involved a certain non-specific increase in membrane sensitivity. This is clearly apparent with the endless fibrillations observable in the denervated muscle

clinically[79]. The latter principle is even more apparent with smooth muscle denervation (*see below*).

In theory, there are two basic kinds of DSS[181]. Following denervation where neuronal reuptake forms a major route of transmitter 'disposal', maximal concentrations of transmitter may therefore pertain within the synaptic cleft. This constitutes **deviation supersensitivity**. Here there is no genuine increase in the efficacy of the response, the latter being maximal merely because of **maximal agonist concentrations**. However, within the CNS, there are ample means with which to dispose of this accumulated transmitter, so this may be of limited potential in this situation. In any case, continued maximal NE would soon lead to agonist-induced desensitization (*see below*). Therefore the other major type of DSS, **adaptive** supersensivity, will be the one considered instead. In this "truer" form there is a genuinely increased target cell responsiveness (*see below*).

3.3.1 Optimum level of target cell activity

Here the target cell appears to possess some form of **homeostatic 'set-point'**. Thus, the target cell possesses a certain level of optimal function to which, following under-stimulation, it strives to return to[181]. Following skeletal muscle denervation *any* form of electrical activity within the sarcolemnal membrane will maintain the functional integrity of that muscle: hence the use of faradic stimulation in states of reversible paralysis. This suggests that membrane stimulation is the only requirement here. But the organ that demonstrates homeostasis of function par excellance, however, is that of the pineal gland[181, 664]. Here, following any apparent increase in glandular stimulation mechanisms are immediately set in motion to decrease the target cell response. Alternatively, periods of decreased stimulation result in the gland developing a supersensitivity to subsequent stimulation. The target cell appears to largely achieve these aims by variations in β adrenoceptor density, variations in the degree of adenyl cyclase activity, and variations in the levels of cellular phosphodiesterase activity. Whatever the case, it would appear that some target cells appear to possess 'memory' of optimal activity levels required[181].

3.3.2 Inhibitory trophic substance

In other cases it appears as if some **substance**—either co-released with the neurotransmitter or actually that of the **neurotransmitter itself**—acts as a **continuous inhibitor** of **maximal target cell function**[181]. Thus, following removal of neurotransmitter from the scene, the target cell response becomes 'up-graded' to any subsequent stimulation. For example, the injection of high doses of botulinum toxin (BT) into skeletal muscle results in a complete denervation and, therefore, in a full DSS. Modest doses of BT, however, result in much lesser degrees of DSS. Modest doses are

used clinically to suppress the over-stimulation of certain muscles, such as the facial muscle in hemi-facial spasm. In moderate dose, BT still allows the basal release of neuronal quanta in order to maintain background levels of MEPPs (miniature end-plate potentials). Since DSS is less pronounced, this therefore implies that basal amounts of trophic inhibitor are continuously delivered. In another example, following *adrenergic* denervation of the cat nictitating smooth muscle membrane, the status quo can subsequently be restored upon re-innervation with even cholinergic neurones! Thus, any transmitter will suffice in this case! Such a promiscuity of effector cell sensitivity following denervation is a frequent finding in VSMCs[181].

In other situations the trophic substance is clearly *not* the neurotransmitter released. The application of either colchicine or vinblastine to a neurone, for example, specifically inhibits axonal transport without affecting transmitter release. The DSS that subsequently occurs, then, is clearly *independent* of neurotransmitter release. An interesting analogue of this that of hepatic function following adrenalectomy. Normally, epinephrine stimulates hepatic glycogenolysis via β receptor-adenyl cyclase activity. Adrenalectomy results in an increase in receptor density and in enzyme activity. However, in order to decrease the latter back to normal, one has to administer cortisone and *not* epinephrine. Thus, adrenal steroids act as the trophic substance in this case. This is not as fanciful an analogy as it might seem as the adrenal medulla is, in reality, an extremely enlarged and elaborated synaptic bouton within the SNS[181, 664].

The latter example reflects an important principle because, theoretically, denervation will result in the gradual development of **DSS** in spite of the **continued presence** of the **'transmitter'** from an **ectopic** source. Such a situation may pertain following SAH: here denervation occurs in the presence of copious platelet-derived NE. Should a DSS subsequently pertain, then this would occur in the presence of large amounts of transmitter.

3.3.3 Membrane mechanisms for DSS

The development of non-specific DSS in VSMCs involves membrane depolarization in addition to increases in the *i*Ca pool[181]. There are also increases in intracellular IP_3 present, and the Ca-calmodulin system appears 'up-graded'. In some situations, there is increased cell-cell coupling present. Normally any agonist will only simultaneously affect a few cells due to the constraints of diffusion, but in some cases an enhanced synchrony of contraction is apparent due to the presence of increasingly efficient tight junctions. Following DSS, VSMCs characteristically become sensitive to many agonists indiscriminately[181]. As a result, the process of DSS appears attractive in explaining VSM; particularly with regard to its temporal delay (*see below*).

3.3.4 Time course of DSS

Whatever the case, DSS characteristically develops some days after the denervation[181]. A good example follows the chemical destruction of rat forebrain serotonergic neurones. Here, there is a **specific** DSS to 5HT within the **first week**, this coinciding with the disappearance of serotonergic histology. After this time a **non-specific increase in sensitivity** develops, eventually developing into a promiscuity to other agonists such as NE and γ-amino butyric acid (GABA)[181].

PART 4

REVIEW OF EXPERIMENTAL MODELS FOR THE STUDY OF SAH AND VSM

METHODOLOGY TO BE USED

Chapters 1-7

Chapter 1
Experimental models for the production of acute subarachnoid haemorrhage

1.1 Introduction

Apart from the controversial discovery of the calcium antagonist nimodipine, none of the results of current 'clot-VSM' studies have so far led to the development of efficacious treatment strategies in clinical DCI. Such a failure suggests that either DCI is primarily a species-specific phenomenon, or that such models are fundamentally flawed (in either design or application). Since most current 'SAH' models are in reality only models of VSM (i.e.'clot-VSM' models), such models overlook: 1) the central physiological disturbance (i.e. systemic and intracranial pressor effects) associated with the acute ictus, and 2) the properties of the ruptured vessel per se. The potential gravity of the acute ictal disturbance is reflected by the frequency of sudden death and coma clinically.

The purpose of this Part of the thesis is to critically review all experimental models that have been used to derive data for either SAH, VSM or DCI. The review will include both aspects of the thesis: i.e. the animal model used to create a representative SAH, as well as the small vessel study model used to subsequently assess cerebral vasoreactivity. The aim will be to delineate each model's assets and deficits: however, particular attention will be paid to identifying any potential flaws in design or application which may explain the model's failure to yield clinically-useful results. In light of this knowledge, a means of small vessel study of MCAs following a representative SAH will be conceived which, it is hoped, will overcome many—if not all—of the pitfalls thus identified. In consequence, the hypothesis will be formally tested that events associated with acute SAH elicit changes in MCA physio-pharmacology that are independent of any subsequent effects of chronic clot lysis—thus undermining the assumption implicit in 'clot-VSM' models that delayed VSM and DCI merely result from the chronic effects of clot lysis upon *normal* cerebral vessels.

1.2 Type I: Rupture of cerebral artery within a 'closed' skull

Vessel rupture can conceivably be achieved from within vessels (**endovascular**) or without (**extravascular**). The fact that not only is the former more physiological, but also an ever-present risk from such procedures as angiography, may make it seem somewhat surprising that no such model had hitherto been referred to[625]. Most efforts instead have involved some form of craniotomy or **craniectomy**. Whilst often only being carried out on larger animals such as primates and dogs, this does not necessarily have to be the case[30].

By far the most common route taken for such access is the **trans-orbital** route. By such means, the MCA, PCommA and the distal ICA have all been accessed. This procedure necessarily involves exenteration of the orbital contents and, therefore, adds in the further variables of surgical mutilation and immunological effects. Nevertheless, extremely useful models of SAH have been achieved and, where performed following healing around the rupture-device, within an essentially 'closed space'. All studies have usually documented well the acute changes in ICP, MAP, and CBF. Most have, additionally, studied some other area. For example, Nagai et al studied neuronal histopathological changes and ischaemic thresholds in monkeys following PCommA rupture[435], whilst Shigeno et al studied cerebral oedema formation following ICA rupture in the cat[546]. Kamiya et al documented changes in metabolic regulation and autoregulation in primates after PCommA rupture [306], whilst Clower et al directly assessed isolated cerebral vessel compliance following MCA rupture in monkeys[100].

Avoidance of orbital exenteration has been achieved by alternative approaches. For example, Asano and Sano achieved an extensive and detailed study of acute pathophysiological changes via **temporal craniectomy** and ICA rupture in dogs[18]: unfortunately, this was not accompanied by any vessel study. Toda et al, in contrast, did study vessel responses following ICA rupture in the dog[599]: however, they employed the helical strip method—a decidedly unphysiological model (*see below*). Others have utilized a **fronto-temporal craniectomy**. For example, Mendelow studied in vivo responses several days following MCA rupture in monkeys[408]: however, specific vessel responsiveness was not examined in this study (this being inferred instead from overall CBF effects). In an interesting alternative, Barry et al used a midline **clival craniectomy** anteriorly to directly study in vivo BA responses, in rats[30].

Although all of the above models have successfully reproduced the circumstances associated with the acute ictus, they have all the potential setbacks associated with craniectomy. Since most necessitate the prior placement of the rupture device, they impose the superadded variables of inflammatory reaction and infection over a period of several

days. Probably because of the belief that such models could only be performed in larger animals, their use has subsequently fallen into decline. However, Barry et al have clearly shown that small animals can be used[30].

Vessel rupture models closely represent the physiological 'ideal'. It is therefore surprising that most current models specifically avoid arterial rupture as the source of SAH. Although it may be the case that such avoidance relates to perceived cost and efficiency considerations in model requirements, it is probably more likely that most current researchers appear content to attribute DCI solely to VSM; and to attribute VSM to merely the prolonged surface contact of autologous blood with cerebral vessels. The current author regards this trend as a most disturbing one.

1.3 Type II: Artificial injection of autologous blood into the subarachnoid space

With the need to achieve increased efficiency in all aspects of SAH-VSM research, there has been a vastly increased tendency to use autologous blood injection to simulate SAH. After all, when reduced to first principles, this is exactly what SAH appears to represent: an injection of blood into the subarachnoid space (SAS).

1.3.1 Type IIa: with ICP-rise

In most cases, blood-injection models are achieved by injections **posteriorly**, via a cisterna magna (CM) catheter. A good example is that of Peterson and Cardoso who produced BBB disturbances by autologous blood injection into the CM. It is pertinent, however, that the latter authors specifically avoided steep δICP/δts in their study[488] (*see below*). In one sense, CM injection models feasibly represent posterior CoW SAH such as may occur, for example, with rupture from a posterior inferior cerebellar artery aneurysm. However, since 80% of aneurysmal SAH actually derives from the anterior CoW, posterior injection studies represent the considerably rarer clinical scenario.

In principle, the rapid injection of *any* fluid into the SAS ought to achieve hydraulic effects equivalent to those observed with autologous blood injection or SAH. Several experimenters have, indeed, confirmed analogous δICP/δts, δBP/δts, cardiac arrhythmias and pupil abnormalities to those seen with SAH clinically with CM saline injections[115, 258, 444]. Such studies, in fact, formed a considerable part of Cushing's original studies[115]. Nevertheless, equivalent 'hydraulic' effects between blood and saline are not invariably witnessed in experimental studies. For example, Lacey and Earle found that a rapid injection of blood into the CM of Sprague-Dawley rats elicited a much greater ΔICP than that observed with either saline or Dextran 40[343, 344]. In another study, blood was more

efficacious at reducing CBF for the same ΔICP as that seen with saline[558]. Moreover, CM saline injections do not result in VSM: blood is definitely required for this[441]. Indeed, saline injections may even elicit a vasodilation[148], possibly by diluting the $[K^+]_{CSF}$.

The recreation of more representative **anterior** SAHs have been made by some investigators. In one model, autologous blood injection was performed trans-hemispherically into the basal cisterns of rats via long plastic cannulae inserted through convexity burr holes[344, 345]. In an interesting variant, Yamashima and Yamamoto injected autologous blood via the optic canal into the chiasmatic cistern of dogs, along with various "test" agents such as epinephrine and NE[669]. In the latter study, injections were made *rapidly* and under *pressure* in order to mimic the clinical scenario. As a result, these authors produced vessel morphological change in addition to the expected acute hydraulic effects. In some cases the authors injected the "test" agent alone, this constituting a Type III VSM model (*see below*). The authors showed that injections of "test" agents alone could produce vessel change as well as a meningeal reaction[669].

One setback with all of the above models, however, is that autologous blood must be withdrawn from a peripheral vessel (or, even, in one case by direct cardiac puncture!) prior to its subsequent injection[344, 345]. Thus, **haemolysis** of the sample may introduce further variables, such as the injection of free haemoglobin and, potentially, high K^+ concentrations. Furthermore, systemic hypotension and **sympathetic activation** may also follow venesection[378, 481], particularly in a smaller laboratory animal. A final problem relates to the fact that most aliquots are **heparinized**. Since heparin can clearly interfere with vascular remodelling[469], this could be of crucial importance in a model that was promulgated to produce *chronic* VSM.

An ingenious way to overcome at least some of these setbacks was described by Steiner et al in 1975[561]. These authors used **direct shunts** of blood from extra-cranial vessels to the SAS. Usually blood was shunted, via a tap and tubing, from the femoral artery (FA); and the SAH produced terminated by its own tamponade within the SAS. By such means, blood was shunted to several sites; and not just into the CM. By droplet-flow measurement, it was shown that—more, or less—the same volume of blood (V_E: 20 mls) was passed irrespective of the site: although maxima and minima were obtained in the spinal canal and brain parenchyma respectively. Furthermore, the V_E was similar even when blood was shunted into a container; this implying that haemostasis was not dependent upon acute vasospasm in vivo (at least in dogs). Unfortunately, a considerable **column of 'dead-space' blood** is apparent in such models; this possibly proving critical in the smaller laboratory animal. Yet, in spite of this (and other) shortcomings; and in spite of

laying dormant for many years; this model has recently been revived—once more in dogs[402]. In this latter study, it was demonstrated that SAH lasted in excess of 4 mins: this emphasizes the occasionally prolonged duration of SAH[402].

1.3.2 Type IIb: without ICP-rise

The premise that the mere surface contact of autologous blood causes VSM (and ipso facto DCI) has reached its nadir in type II SAH models. At present, such studies represent the majority of SAH-VSM investigations. In these studies, sometimes great lengths are made to *avoid* steep δICP/δts. The more usual involve the slow injection of small quantities of blood over relatively prolonged periods[675]. However, many also remove an aliquot of CSF equivalent to the V_E immediately prior to blood injection[126, 268, 304, 318, 362, 635]. Some have also simultaneously measured the ICP in order to ensure that no ΔICP actually occurs![268]. Because most of these studies involve an unpressurized injection into the CM posteriorly, all of these animals are required to be tilted head-down in order to allow the blood to re-distribute anteriorly[304, 318]. Furthermore, as aforementioned, posterior blood injections denote the least common clinical analogue (*see* 1.2.1 above).

In order to allow for greater V_Es to be applied, some models have incorporated the use of in-dwelling catheters over a period of several days. Such models, however, superimpose the significant risk of **bacterial infection**: although this is claimed, naturally enough, to be "negligible"[126]. The possibility of super-added bacterial infection is extremely important to the study of VSM, because VSM angiographically and morphologically indistinguishable from SAH-VSM is frequently observed with bacterial meningitis (Part 1, 1.3.2.2). Nevertheless, and in spite of this risk, the "double haemorrhage" model (and, more recently the "triple haemorrhage" model) are both, currently, the most popular SAH model in existence[539].

In order to improve upon some of the aforementioned short-comings, other groups have recently returned to the production of **craniotomies**. However, such craniotomies have not been made to allow access for vessel rupture as in a type I model. Instead, they have been used merely to permit greater quantities of blood (V_E) to be placed, under direct vision, around particular vessels, unilaterally (the MCA being the most frequent)[174, 494]. Impressive V_Es can be applied by such means—yet all without steep δICP/δts[399]. Such models require especial mention because, by such means, the **contralateral vessel** is often considered the **paired 'control'**. The latter form of study is, usually, the biostatistical ideal; and usually permit a reduction in experimental numbers. However, with SAH-VSM paired-control studies are marred by the fact that clot-product diffusion may also affect the contralateral vessel. Such studies, therefore, may not truly create an isolated control.

Moreover, there are other reasons—discovered and emphasized in the current study—why 'paired-control' studies may not be appropriate with cerebral vessels (Part 6, Section II, 1.1).

1.4 Summary of subarachnoid haemorrhage models

The earlier models of acute SAH that produced SAH by arterial rupture were, ironically, the closer to the physiological ideal. Although they served largely to document acute physiological disturbances—systemic and intracranial—that accompanied SAH, at least some also attempted to study VSM-in-evolution. In contrast, more recent SAH models have increasingly focused only upon 'clot-VSM'. The premise in such studies is that VSM is caused by surface blood 'irritation' following chronic clot lysis; and that VSM, in turn, is the cause of DCI. However, since *VSM qua DCI* is not axiomatic, a clear dichotomy between the two processes should be maintained and emphasized in experimental studies—in the same way that has been proposed clinically[382] (Part 1, 1.5).

Chapter 2
Experimental models for the production of vasospasm

These models are required to be distinguished from SAH models. They are concerned only with the faithful reproduction of analogous vessel **morphological change**; and the production of **angiographic constriction**. They assume that VSM is the *conditio sine qua non* of DCI. Conceivably, then, there are three main types:

2.1 Type I: Activation of the cerebral sympathetic nervous system

In practice, probably no such model has successfully been developed. This is because most attempts to activate the cerebral SNS have, thus far, only activated the *ascending cervical* supply by way of the stellate ganglion. This, of course, supplies only a part of the total cerebral SNS (Part 2, 2.6). For example, D'Alecy and Feigl stimulated the stellate ganglion in ventilated dogs and noticed CBF reductions[119]. However, these authors also noticed that, rather incongruously, the ICP became elevated! A recent study by Pilati et al came closer to the ideal: the authors injected veratrine or citrate directly into the CM[493]. By such injections, these authors created a *systemic* sympathetic "storm" by an activation of the medullary vasomotor centre. Such a study would be clinically representative because the cerebral SNS is probably only influential at higher MAPs (Part 2, 2.6). Unfortunately, the authors in this study did not simultaneously study the cerebral vasculature (their aim being instead to create left ventricular dysfunction and ARDS). Because NE and 5HT both function as cerebrovascular neurotransmitters, the addition of these to the CM (Type III *below*) may prove representative[7, 670].

2.2 Type II: Application of adventitial autologous blood in vitro

Type II VSM studies are, in principle, equivalent to Type IIb SAH models (*see above*). A good example would be that of Tsuji and Cook (1988), who added blood directly to canine BAs in vitro[604].

2.3 Type III: Artificial injection of biochemical agents into the subarachnoid space

Such models concentrate upon the effects of blood constituents putatively responsible for VSM. In this regard, applications of NE, UTP and 5HT are all theoretically feasible. For example, NE was injected into the subarachnoid space by Yamashima and Yamamoto[669]; UTP by Urquilla[613, 614]; and 5HT by Cardoso et al[488]. Such models may also be used to enhance type II SAH models, such as in the studies of Wang et al[640] or Yamashima and Yamamoto[669] (*see above*). In another variant, Nagata et al[440] re-created a comparable VSM by the addition of talc to the CM of dogs. The latter underscores the "irritative" principle

of VSM actiology, and reaches its pinnacle in the next type of VSM model to be described (*see* 2.4 next).

2.4 Type IV: Direct vessel trauma

The main example here is that of Symon et al 1967[576]. In this study, the authors produced VSM in the MCA of baboons by repeated compression with non-toothed forceps. However, although this was successful at producing acute VSM, a prolonged VSM rarely resulted. It is interesting, however, that a recognized treatment for VSM (that of *local* intra-arterial PPV) is also efficacious at reversing traumatic VSM[622].

2.5 Type V: Artificial injection of cerebrospinal fluid from subarachnoid haemorrhage patients

CSF obtained from patients after SAH has often been used to supply the putative vasospastic agent directly to isolated vessels. For example, Sasaki et al utilized human SAH-CSF in an attempt to isolate the nature of the putative vasospastic substance[528]. Yamamoto et al provided indirect evidence that SAH-CSF produced cytokine-like activity toward cultured fibroblasts. [668] The problem with Type V VSM studies is that they include errors in both **temporal** and **spatial CSF sampling** (Part 2, Chapter 4): as a result, there is a great non-uniformity in results obtained.

2.6 Summary

- The majority of current models are of Type IIb autologous injection. Such studies *totally* betray the profound physiological disturbance associated with the acute ictus. The unphysiological nature of such studies is reflected in their low mortalities, and impossibly high survivals (>90%)—a finding clearly incompatible with the high incidence of 'sudden death' clinically.
- As well as being easier to perform, the Type IIa model is also more physiologically sound: yet is rarely performed. In addition to displaying all the faults associated with autologous blood injection (*see above*), Type IIa studies also betray acute vessel-rupture as the all-important source of the haemorrhage.
- In models employing acute vessel rupture (Type I) local craniectomies are invariably used. The latter may result in a wide array of additional variables being introduced, including inflammation and infection. Perhaps because of their complexity, few have proceeded to subsequently study isolated cerebral vessel reactivity.

Table 4. Summary of SAH and VSM models

SAH Model	Cerebral vessel rupture	ICP↑	V_E
I	✓	✓	variable
IIa	×	✓	variable
IIb	×	×	large

Key: **I** arterial rupture within a closed skull, **IIa** autologous blood injection into subarachnoid space with ICP rise, **IIb** autologous blood injection into subarachnoid space without ICP rise.

Chapter 3
Proposed model for the production of acute subarachnoid haemorrhage

3.1 The importance of vessel rupture

It is important to consider vessel rupture as this alone has been deemed the major differentiating factor between experimental SAH groups.

Most muscular arteries develop a functional spasm following haemorrhage through a breach of their walls. Some authors have hinted that such spasm results from a local neurovascular reflex[546], and Duckles et al have demonstrated alterations in both innervation and reactivity in monkey cerebral arteries following arterial rupture[140, 141]. Since SIT primarily follows **endothelial damage**[175], and since the latter must necessarily accompany rupture[178], this hints that vital reparative processes may be triggered by rupture that would not otherwise be triggered by the mere addition of surface blood. Certainly SIT appears intimately linked with **vessel trauma** per se[399, 584] (Part 1, 1.3.1.4). Further along these lines, an inhibitory heparan-like substance—derived from the endothelium[84]—has been shown to basally suppress VSMC proliferation, the production of which is increasingly released by a rejuvenating endothelium after injury[469]. Interestingly, Clower et al have demonstrated that rupture models tend, on the whole, to produce the most extensive morphological change of all SAH studies[100]. In addition, rupture models also more successfully recreate the decreased cerebral vessel compliance which is so much a feature of vessels in VSM[100].

In interpreting the above findings, some have proposed that rupture allows the infiltration of blood elements—under pressure—into the vessel wall[362]. By this means, blood and its contained agonists—such as platelet-derived growth factor (PDGF)—can, thus, be directly applied to target cells with minimal loss of effect. More recent studies have shown that vessel trauma results in the expression of *c-fos* and *c-myc* proto-oncogenes within VSMCs—the increase in *c-fos* being proportional to the injury sustained[282]. Thus, **VSMC rupture** elicits considerably **more SIT than** does mere **endothelial denudation**[75, 282]. Other groups have shown that the trigger towards SIT involves auto- and para- crine cytokine production, in addition to growth factor release[358]. A single transient stimulus can result in the considerable expression of local PDGF and FGF (fibroblast growth factor) by such mechanisms[89].

Whatever the underlying mechanisms, a quite overwhelming case for vessel rupture appears to be extant for precise SAH study.

3.2 Proposed experimental subarachnoid haemorrhage model for the current study

The main criteria for the production of a representative SAH were set out by **Shigeno et al** in **1982**[546]. Thus, for any putative model there should be:

- **Bleeding** from **acute vessel-rupture intracranially.**
- Acute pressure-loading **(acute ICP rise) within** a **closed skull.**
- A **sufficient amount clot** produced.

To these one should add:

- **Endovascular filament (EF) rupture *without* craniotomy.**

EF rupture without craniotomy was achieved in the current study by simply passing a length of fairly rigid 3/0 prolene intracranially within the lumen of the ICA, from a remote (extracranial) source in the neck. The thread was then made to rupture through the anterior CoW with minimal force before being withdrawn, thus allowing abrupt extravasation (Plates 5, 7 and 9). The model was essentially that developed in this laboratory[625] but with important modifications (*see below*). Other variations on this theme have subsequently been described[40, 41, 236, 302].

3.3 Details of the method used for the production of subarachnoid haemorrhage

All animals were anaesthetized with 25% urethane [with two exceptions*], injected intraperitoneally (1g/Kg), and then transferred to a hot plate which served as the work surface for the entire procedure. A rectal probe was inserted to record the core temperature throughout, this being kept at around 38°C. The animal breathed spontaneously on air (or an air-O_2 mixture, as required).

Following micro-dissection in the left groin, using small spring-loaded scissors and forceps, the left femoral artery (FA) was isolated from its vein and nerve. The distal FA was then tied, following which the femoral vein was coagulated: this was because the left leg was invariably became ischaemic. Through a small slit in the upper surface of the FA, a saline-pressurized cannula was inserted and advanced **into the lower abdominal aorta**. This was then double-tied, and the wound closed with 3/0 silk to obviate fluid loss. Through this cannula both MAP and arterial blood gases were continuously monitored, with frequent infusions of normal saline being given to maintain hydration. **No heparinization was used.** The technique was perfected so that no haemorrhage occurred whatsoever, thus obviating the risk of haemodynamic compromise throughout.

*One animal each from SAH Grp 2 and 3 were anaesthetized with **hypnorm** (0.5mg.ml^{-1}hr^{-1} fentanyl, 5mg.ml^{-1}hr^{-1} fluanisone)/**hypnovel** (midazolam)[625]—only *without* paralysis and ventilation.

After carefully turning the animal prone, a midline incision was made from the vertex to the spine of C1. Following careful microdissection of the suboccipital muscles, a small plastic cannula was inserted into the CM through a nick in the suboccipital membrane. This manoeuvre was carefully performed under maximum magnification, so as to ensure cannula positioning

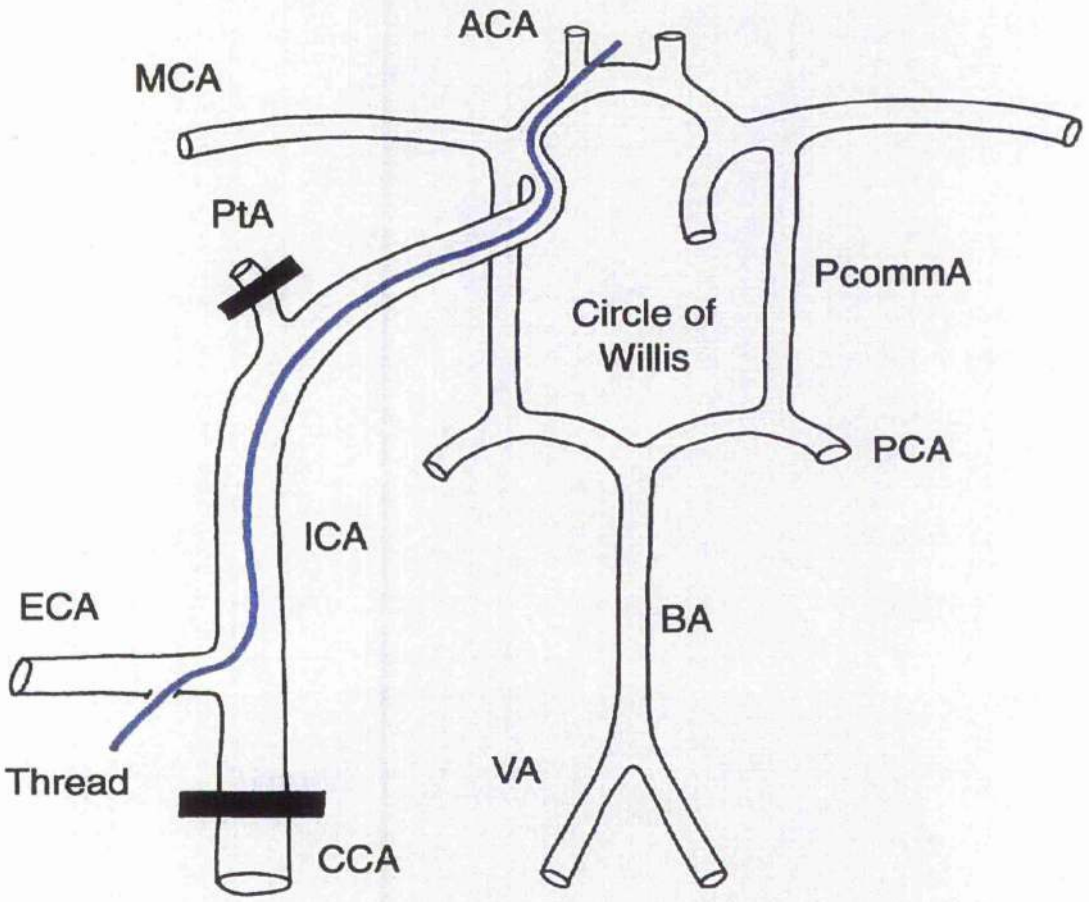


Fig 1. Schematic representation of SAH procedure. The thread is inserted into the temporarily clipped-off ICA segment, following which it is then advanced intracranially to rupture (most commonly) through the anterior communicating artery. *Key:* CCA common carotid artery, ECA external carotid artery, ICA internal carotid artery, VA vertebral artery, BA basilar artery, PCA posterior cerebral artery, PCommA posterior communicating artery, PtA ptergypopalatine artery, MCA middle cerebral artery, ACA anterior cerebral artery.

at an angle away from the spinal cord, into the lateral subarachnoid space. The cannula was also connected via a column of saline to the transducer of an oscilloscope to thus

record the intracranial pressure (ICP), a sinuous waveform indicating ideal positioning. It was "fixed" in position by pouring dental acrylate into the wound, and by tying-over the suboccipital muscles as this 'set' (Plate 4). Following this the animal was returned to the supine position.

A long midline incision was made from the sternal notch to the hyoid region. Following tracheostomy (achieved by inserting the cut-off port of a Swan-Ganz® catheter between the third and fourth tracheal rings anteriorly), the right common carotid artery (CCA) was dissected free from its vagus nerve, with the external carotid artery (ECA) being divided to a small stump. The pterygopalatine branch of the internal carotid artery (ICA) was temporarily clipped, as were both the distal ICA and proximal CCA (aneurysm clips), and within this isolated segment a small length of 3/0 prolene (about 3cm: wiped with 70% alcohol, and allowed to dry) was introduced through the ECA stump (Fig 1). The ICA clip was then removed to allow the thread to be advanced intracranially within the ICA lumen until, on passing through a small resistance, it ruptured through the wall of the anterior CoW, thus causing acute SAH (Fig 1). This was confirmed by a sudden elevation in ICP, which then fell to a lower plateau (Figs 8, 10 and 11). Frequently, respiratory irregularities occurred at this stage, but these were usually only transient: respiratory support was rarely required (but hence the prior tracheostomy). Sudden extensor posturing often also coincided here, sometimes resulting in the **loss of ICP tracing** (n = 7: Tables 17-19, Appendix: Fig 9 subscript). The thread was immediately withdrawn at this stage and, following coagulation of the ECA stump, reperfusion attempted by release of the CCA clip.

In most cases (**SAH Group 3**: n = 17) reperfusion was uneventful. However, in others, reperfusion resulted in a significant ICP re-rise. Because this was felt, as a result of previous studies[625], to risk catastrophic *secondary* SAH, the clip was repositioned in such cases so as to ensure adequate haemostasis from the primary SAH. **The rationale was to limit study to that of a single primary SAH.** In most cases where an ICP re-rise occurred (**SAH Group 2**: n = 13) CCA re-clamping spanned periods of less than 5 mins. In a few cases, however, CCA re-clamping was considered necessary for over 1 hour (this representing the entire study period in such cases—**SAH Group 1**, n = 4—because all of these animals died prematurely within the arbitrary three hours [Part 5, 1.4 & 1.5]). The possible superposition of unilateral carotid ischaemia in such cases—particularly the latter—upon SAH effects is fully discussed later (Part 6, section III). However, it is worth noting at this stage that, in a previous in vivo study, pial vasoreactivity remained remarkably unaffected by considerably longer periods (2-5 hours) of unilateral carotid ligation[513].

According to protocol (Part 4, Chapter 7), all animals were to be sacrificed by exsanguination after an arbitrary period of three hours following SAH. However, in a significant number of cases, a subacute cardiovascular collapse (SCC) ensued at some stage within this time scale (Part 5, 1.4; Part 6, Section I, 1.6; Part 6, Section III). If, in such cases, haemodynamic stability was not restored by simple oxygenation and repeated fluid-challenge, the animal was electively sacrificed by exsanguination at this point within the arbritary three hour period. **The rationale behind early sacrifice in these cases was to limit the confounding effects of a prolonged hypotensive state upon MCA reactivity**[335]. [Furthermore, it was decided not to restore systemic pressures with inotropic agents in these cases because of the risk of metabolic acidosis.] Although such methodology necessarily gave rise to **heterogeneous survival groups** (*see* Tables 20-22, Appendix), such methodology was considered to more closely reflect the clinical scenario, where sudden death is seen in up to 50%[477]. Indeed, unrepresentatively low mortalities reported in the vast majority of experimental SAH studies (in reality, type II VSM models) ought to be considered a distinct design weakness rather than—as is usually the case—a strength. Similar considerations of 'unrepresentativeness' also resulted in the abandonment, in the current study, of the sub-groups previously emphasized in the "Sheffield Model" of SAH (*see below*)[625].

Sham-operative group

8 animals underwent exactly the same procedure as for the SAH groups, but instead the thread was not allowed to rupture. In all cases, CCA cross-clamping was immediately curtailed after thread removal, and full reperfusion subsequently restored. Any deleterious effects of unilateral (i.e. right-sided) surgical manipulation, CCA cross-clamping, ECA obliteration and thread-insertion, were to be closely looked-for by examining for any right:left side differences in MCA in vitro responses (Part 5, Section II, Chapter 2). In all animals (from both sham and SAH groups) arterial blood gases were monitored intermittently. In the vast majority of cases, the animal breathed spontaneously on air without any further support of any kind: urethane anaesthesia proved remarkably efficacious in this study.

The procedure described above is essentially that of "The Sheffield model" of SAH[625]. However, **closed-chest pressure-ventilation** (with its deleterious effects upon cardiovascular function) was specifically **avoided** in the current model—the animal spontaneously breathing air throughout. Furthermore, **no attempt** was made to **create** the **"differing extremes" of SAH** emphasized in the former study, since in preliminary experiments in the current study, SAH appeared equally as extensive whichever method was employed; and because the 'lesser' SAH variety reported in the "Sheffield Model" [625]

(i.e. with thread left in situ and CCA permanently cross-clamped—causing unilateral MCA ischaemia) was considered wholly incompatible with 'pure' SAH study.

Chapter 4
Experimental methods for small vessel study

4.1 In vivo methods

There are several important setbacks with in vivo models for the study of VSM. Perhaps the most major is that of the **continuing effects of the anaesthetic agent** used, and its possible interaction. This is clearly not a problem with in vitro studies, excluding any permanent effects of the anaesthetic agent used. Nevertheless, the fact that vessels retain their natural systemic connexions, and that they have usually suffered a minimum of experimental manipulation, remain attractive features of in vivo models.

4.1.1 Angiography

Angiography is so frequently used in SAH studies that no list of examples is a realistic possibility. The study of Delgado et al in eighty seven rats represents a good example of its detailed use, albeit in a type IIb SAH model[126]. The major advantage of angiography is its relative ease of use, its **reproducibility over prolonged periods**, and the fact that it allows a **large area of vasculature** to be **studied**. Unfortunately, there is much room for **observer bias**, particularly at the stage of film examination where pencil is often used to highlight luminal borders[126]. There is also a problem over the agreed equation between extent of luminal narrowing and its significance. For example, Delgado et al considered a change of 8% from baseline to be significant, whilst Pluta et al[494] required 25%. Most, however, require this to be $\geq$ 40-50%. The better studies **rank** the **diameters** observed into categories. Thus, Liszczak et al[362] ranked a 10-20% decrease in diameter as 'mild', 20-30% as 'moderate', and 30-80% as 'severe', following a type IIb study in dogs. Nevertheless, in spite of these short-comings, angiography remains a frequently used device.

4.1.2 Cranial "window"

The technique involves the removal of a small piece of skull, usually over a convexity, and the subsequent isolation of a small network of pial vessels for video diameter study. The dura is reflected in such studies, and the exposed cortex bathed in a continuous flow of paraffin oil[378]. Various complexities of visual mensuration are utilized in order to assess vessel diameter change—these becoming increasingly sophisticated. The best examples have not, in fact, followed SAH. Barry et al have used an ingenious variant of this method to study VSM in the BA anteriorly (*see below*)[30]. The cranial window was, of course, elegantly used and described by Cushing in his classical study of 1901[115]. The latter literally was a window: a piece of glass screwed into the skull! Through this window, Cushing made careful drawings of the pial vasculature at various increments of ΔICP.

In criticising such models, one must consider the detrimental effects of the local craniotomy or surgical **trauma** upon the superficial region exposed[378]. Furthermore, by the very fact that the region under examination is being isolated (usually within an oil bath), such vessels must also remain as **isolated from** the **CSF** as they would be in vitro. Therefore, at least some of the theoretical advantage of the in vivo study may be lost. The effect of the bathing solution would also remain unknown in such studies.

4.1.3 Cisterna magna (CM) "window"

This is obviously a variant of the above cranial window: however, clearly no extensive craniotomy is required as the atlanto-occiptial membrane can itself act as the "window". This is a commonly used method, for both "SAH" and "VSM" models.

4.2 In vitro methods

These models have the distinct advantage in that they directly observe **isolated vessel characteristics** (Part 3, Chapter 2). Not only does this mean complete isolation from other important inputs, in particular those relating to sympathetic and metabolic regulation, but also the exclusion of other confounding variables such as continuing anaesthesia. Because of this it is possible, within reason, to limit many of the variables known to affect vessel function, such as pH, CO_2 and O_2 levels, temperature, agonist concentrations, and so on. The **control of confounding variables** is, of course, the over-riding aim of most scientific studies. The major criticism of in vitro studies is, however, that vasoreactivity is not being witnessed within its true physiological environs, and that vessel damage may necessarily have been incurred during handling[431].

4.2.1. Helical strips

This entails the cutting of a length of cerebral artery diagonally and continuously into a helical strip, and then its suspension vertically between hooks, one of which is attached to a force transducer. In such a manoeuvre, a concentric array of VSMCs is converted from a concentric one into a straight length in parallel. For example, Toda et al obtained MCAs from dogs following a Type I SAH model, helical strips being produced by cutting at an angle of 45°[599]. Sasaki et al similarly utilized dog BAs to carry out a Type V VSM model[528]. By the use of various antagonists, the latter authors ruled out 5HT, NE, HA and acetylcholine as mediators of VSM, concluding instead PGs or leukotrienes to be the culprit (Part 2, Chapter 4).

However, the problem with this technique is that a lot of manipulation is involved, and that the **normal architecture** of the vessel is completely **destroyed**[44]. Furthermore, the continuity of important layers such as the **endothelium** is similarly **deranged**, this risking

great departures from normal physiological function[44]. It is therefore a decidedly **unphysiological** form of small vessel in vitro study[639].

4.2.2. Ring preparations

The first to study VSM using ring preparations was probably Miller in 1971[417]. Although many experimenters have utilized either in vivo methods, or 'helical strip' in vitro methods, for cerebral vessel study in SAH models, a significantly large number have also utilized 'wire' (ring) myography[1, 2, 3, 9, 10, 14, 46-48, 57, 58, 63, 64, 108, 123, 124, 141-144, 152, 155, 190, 192, 254, 284, 308, 309, 326, 329, 337, 354, 368, 393, 417, 422, 437, 472, 473, 523, 524, 528, 529, 569, 574, 585, 598, 601, 604, 605, 623, 635, 637, 650, 654, 655, 661, 662, 667, 674, 682]. Such studies have, with the advent of Mulvany myography[429, 430], become the standard small vessel study research tool. Indeed, recent studies from influential centres[1-3, 14, 50, 605] have continued to use this method for SAH-VSM study despite some significant shortcomings (Part 4, 5.5). However, when myography has been used in SAH studies, it has invariably followed the use of a Type II SAH model. For example, Kim et al studied BA responses in the dog after a Type IIa SAH[326], whilst Vollmer et al studied BA responses in rabbits after a Type IIb SAH [635]. Ring myography will be discussed in more detail later (*see below*): it represented the method chosen in this study.

4.2.3 Pressure-cannulized studies

These, of course, represent the ideal type of in vitro small vessel study. Thus, **radial distending pressures** are experienced from fluid hydrodynamics, with agonist action being separated into **intra-** and **extra-luminal components**. Admittedly, some degree of manipulation is still apparent, this probably being comparable to that seen with wire myography. Nevertheless, this represents an altogether more physiological compromise. However, such models are very expensive and therefore in more limited supply. Because of this and other reasons, they are far less frequently used than wire myography. Indeed, even in units that possess both pressure and wire myographs, there appears to be a certain preference for the continued use of the latter. Furthermore, they are, at present, less comparable to the vast amount of studies being performed with ring myography. They have been rarely used thus far in SAH study, an example being that in the rabbit BA by Vollmer et al[635]. Although Tsuji and Cook used this model, they chose the BA for study and, worse still, only mimicked SAH by merely adding surface blood in vitro[604].

4.2.4 Brain slice microvessel studies

This technique involves the *en bloc* removal of sections of brain and the subsequent videomicroscopic mensuration of vessel diameter-change. However, an important setback is that the vessels are not subjected to any form of distension[521]. Furthermore, they tend to

be mounted in ambient **temperatures of 33°C**, which is clearly **unphysiological**[521]. One such model is worth especial mention because it was used to demonstrate a paradoxical vasoconstriction in pial vessels with 50 μM concentrations of PPV[293]. This subject will be returned to later on in the discussion.

Chapter 5
Proposed model for vessel study

5.1 Rationale

The purpose of this project was to study the **early responses** of **MCAs immediately following acute SAH** in a **model closely resembling** that of the **clinical scenario**. Thus, although both VSM and DCI represent processes essentially delayed in manifestation, it is difficult to concede that their seeds are not in some way critical sewn within the acute ictus. For example: vessel trauma, an acute SNS storm (*see below*), and dysautoregulation probably all relate directly to the acute impact—distinct from any further delayed effect consequent upon clot lysis. Furthermore, this **crucial period** of the **first few hours** has continued to remain relatively **under-explored**.

It was also necessary to have a model that balanced the needs of physiological representativeness with cost efficiency. As a result, the wire myograph was chosen:

1. Several aspects of the study required bilateral responses. This is difficult with most in vivo models.
2. It was considered important to confirm not only that SAH had occurred, but also to record its *extent*. The repeated addition of chemicals and saline (PSS) to the SAS throughout a study period could seriously interfere with such assessment.
3. The wire myograph is widely used and, in consequence, more comparable to the results obtained in other studies. It is also considerably cheaper than the pressure-perfusion version.
4. Apart from subtle differences in affinity with adrenergic responses, there have been no significant differences reported in response findings between wire and pressure-cannulized models[76, 167]. Furthermore, spontaneous myogenic tone—which is both characteristic of cerebral vessels and a testimony to the accuracy of the mounting procedure—can still be apparent with wire myography, depending upon the handling (*see below*).

5.2 Principles of wire myography

The essential principle is that of distending a fixed length of artery in order to simulate a luminal pressure closely resembling that experienced in vivo[44, 429, 430]. In consequence, changes in wall tension produced by agonist action will be more physiological and therefore representative. Since the transducer (*see below*) can only measure *force* (mN), this level of resting **pressure must** therefore **be extrapolated**. This is achieved by the assumption of the Laplace relationship (Part 2, 2.1), where $\mathbf{T} \propto \mathbf{P}$ [429, 430]. Thus, in the case of MCAs, the operating internal pressure will be around 90 mmHg. In order to achieve

this, the fixed MCA length is progressively distended by two intraluminal wires in small increments over a prolonged period, until the desired 'pressure' is achieved. A software programme then maintains this level, such that agonist-induced tension changes are proportional to diameter changes: i.e. $\mathbf{T} \propto \mathbf{D}$[429, 430].

5.2.1 Details of myography

The automated myograph (Fig 3) is able to alter vessel diameter according to the force recorded by the sensing jaw, through an electrically driven micrometer attached to a mobile jaw. Software control automatically pre-tensions vessels to simulate any required resting transmural pressure[429, 430]. The vessel **diameter** is **inferred from** the vessel **circumference at this** resting **pressure**: the 'normalised' lumen diameter (FD_{90}). Thus, for a cylinder, the Laplace relationship approximates to[663] :

$$T = P.a$$

Since vessel circumference is $C = 2\pi a$, we therefore obtain:

$$P = \frac{2\pi T}{C}$$

Now, where F is the force recorded by the transducer, and l is the segment length (i.e. the only viable length between the jaws, 2.3 mm: *see* Fig 4.3) [429, 430]:

$$T = \frac{F}{2l}$$

Furthermore, for a mounted vessel (Fig 5) the circumference, C', can be given by:

$$C' = (2+\pi)d + 2(gap)$$

where d is the diameter of the mounting wires (40 μm), and the **gap** is directly measured by the micrometer on the moving jaw as the **distance moved by the jaw away from the point when the wires on the respective jaws touch.**

The expressions for C and T can then be substituted into the Laplace equation, so that we can develop a relationship between the *gap*, *transducer force*, and equivalent *pressure*:

$$P = \frac{F\pi}{l[(2+\pi)d + 2(gap)]}$$

or:

$$T = \frac{2Pl}{\pi}.(gap) + \frac{Pld}{\pi}.(2+\pi)$$

$$\mathbf{T = c.gap + c'}$$

As a result, when the **pressure** is kept **constant** within a vessel, there is a **linear relationship** between **wall tension and** the **gap** between the jaws (Fig 2). Thus, a linear isobar may be plotted on a graph of force on the ordinate against gap on the abscissa, with the line having a **gradient 2Pl/π** and **y intercept Pld(2+π)/π**[429, 430]. (The value **l** remains constant as this is the segment length between the jaws that remains viable: i.e. this represents the active segment not crushed by the wire assembly against the jaws (*see* Fig 4.3).

This can be represented in the isobar for 90 mmHg in the graph in Fig 2 below:

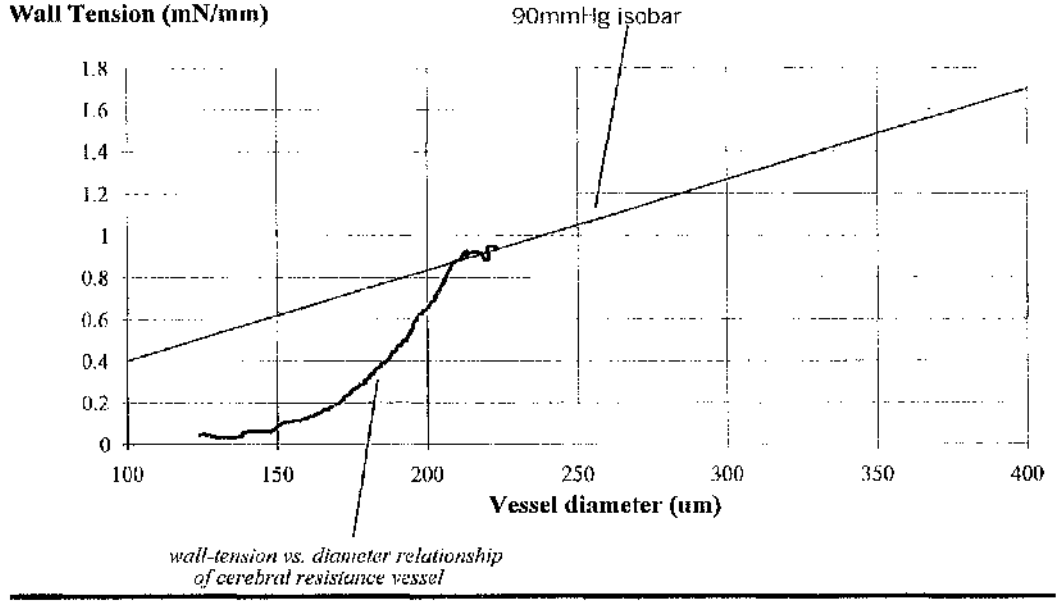


Fig 2. Diagrammatic representation of the normalisation routine. The isobar for the desired pressure is derived, in this case 90 mmHg. The myograph software then directs stretching of the vessel until the wall tension equilibrates at a point on the isobar. The wall tension is then equivalent to a transmural pressure of 90 mmHg.

The diameter, D, that the vessel would have had in its native cylindrical state may be derived from the equation:

$$C = \pi D$$

The myograph software plots the isobar for the required pressure on a graph of wall tension against gap. The software is then able to stretch the vessel until the approximately exponential relationship between gap and wall tension intersects with the isobar: at this point the tension in the vessel wall will be equivalent to the pressure of the isobar. Because all vessels show some degree of **plastic compliance** (Part 2, 3.1.1), the gap needs to be continually modulated by software control until equilibrium is reached: this takes about 20 minutes with force increments @ 0.70 mN/min. In the experiments reported here, once this loading procedure had been performed the vessels were studied **isometrically** (Part 2, 3.2): i.e. by keeping the **diameter constant** whilst measuring the changes in tension, ΔT, generated in the vessel wall[44, 429, 430].

5.2.2 Automated myograph structure

A major advantage of the automated myograph (Fig 3) is that it is automated by software control, thus allowing movement of a mobile jaw to be directed in response to tension recorded from the sensing jaw and gap. Thus **datum position** (the position of the jaws at which there is no wire gap), **zero tension, equivalent transmural pressure** and **normalised lumen diameter** are rapidly established or calculated by the command program, allowing uncomplicated, rapid and accurate pre-tensioning of vessels. Digital tension data from the entire experiment may be continuously recorded onto the hard disk, and the tension-time trace may be displayed on a visual display unit and/or be instantaneously printed out onto a hard copy. Instantaneous tension recordings may be frozen to aid their recording by hand, in a further manual log of the experiment[429, 430].

Each organ bath of the myograph accommodates 2 vessel segments, each one mounted on a pair of jaws as a ring preparation (Figs 4.3 & 4.4; Plate 3). The sensing jaw is connected to a novel feedback type force transducer, having a resolution of 0.05 mN and drift less than 0.05 mN/hour. The feedback design allows true isometric responses to be measured[429, 430] (Part 2, 3.2)

The myograph organ baths were fitted with a temperature sensor and heater. The myograph software maintained a constant temperature automatically by feedback control. All experiments reported in this thesis were performed at 37°C. The vessels were bathed in the organ bath in physiological salt solution (PSS) (Part 4, 6.1.1).

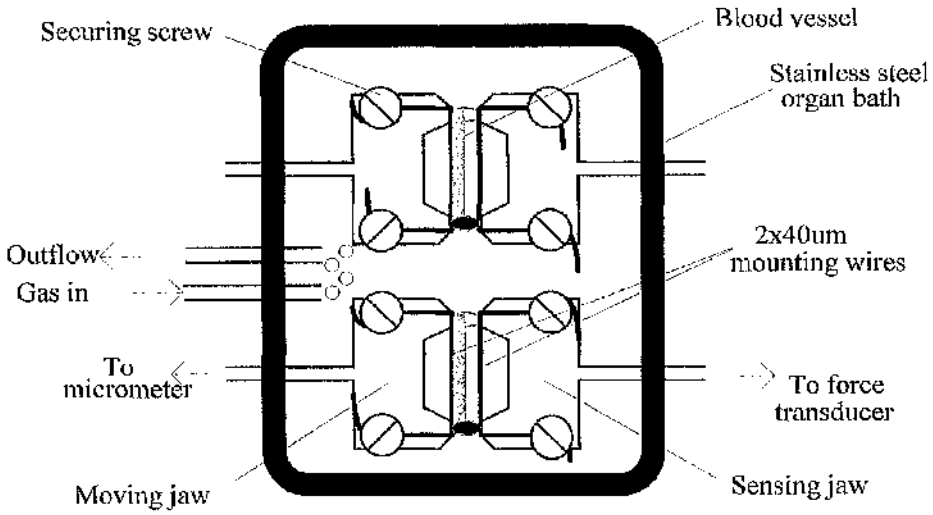


Fig 3. Bath of myograph. The left hand jaw can be moved by a motorised micrometer screw. The right hand jaw is connected to a force transducer. The bath is filled with physiological salt solution maintained at 37°C and bubbled with different gas mixtures. The situation is reversed in the upper jaws. The actual model used was the Mulvany 500A®, JP Trading, Aarhus, Denmark.

5.2.3 Calibration and up-keep

The force transducer was calibrated at intervals between once per day to once per week. This involves mounting a wire on to the transducer jaw, and placing a known weight (2g) on a beam and lever assembly to 'pull' on this. This produced a force of 9.81 mN on the transducer. Because of the use of Ca^{2+} in the PSS, the frequent build up of Ca deposits required the regular clean-out of the bath lining, this being achieved with 50% glacial acetic acid at intervals of once-weekly. At all times a sufficient amount of protective grease (applied in fresh weekly) was maintained around the force transducer armature, as water seepage into the micrometer housing is a potent cause of transducer decay.

5.3 Vessel harvesting and mounting procedure

All animals in all groups were sacrificed by exsanguination under urethane anaesthesia (*see below*). This not only satisfied schedule I (Home office) procedures, but also served to obviate any peri-mortem ooze which may hinder analysis of SAH. Following sacrifice, the rat was placed prone and a long midline incision made from the frontal region to the mid-cervical region. Skin and superficial fascia were swept laterally with large scissors, and the posterior cervical muscles cleared to reveal the C1-C3 vertebral spines. Using small bone forceps, C1 and C2 vertebral arches were removed, and the spinal cord then transected at this level through the clear meninges. By again using the bone forceps, the posterior cranium was successively removed, displaying the cerebrum and cerebellum. The brain was then lifted gently from the cut cord region, tilting gently anteriorly, with successive

excision of cranial nerves increasingly facilitating this. Extreme care was taken at the stage of excision of ICA and VA connexions, and with excision of the optic nerves: the latter representing the final resistance to the complete removal of the entire brain.

The brain was then placed basal surface upwards inside a petri dish, with continual PSS irrigation throughout (Plate 8). Under maximal magnification with the operating microscope (Carl Zeiss®), the proximal portion of each MCA was then removed by picking up the CoW on either side proximal to its division and by then cutting away the pia close to the vessel on each side. At all stages watchmakers forceps and scissors were used. Extreme care was taken to:

- **Not touch the MCA** in any part. The only part of the vessel touched was the CoW at either side (Fig 4.1) of the 'T'.
- **Not 'pull' on the MCA** segment from CoW hold as it was sequentially lifted out.

The removed MCAs were then immediately transferred to the myograph bath containing PSS. With a 40 μm wire already partially secured on the transducer side (one end curved onto the screw in clockwise fashion, the other free and straight in the PSS: Fig 4.2) one MCA was then sleeved over the latter, this being achieved through the V-shaped breach at the CoW:MCA T-junction (Fig 4.2; Plate 1). At no point was the wire-tip allowed to snag the endothelial lining. Once fully on, the free wire-tip was then finally secured onto the transducer jaw by again curving it onto the screw as the latter tightened in clockwise fashion (Plate 2). As the MCA length was longer than the vertical gap, it usually curved around the jaw at either end (Fig 4.3; Plate 2): this excess would later be removed. For the time being, however, another cut was made in the ante-jaw vessel-wall at one such curve, through which another straightened-and-smoothed 40 μm wire was inserted intraluminally, along the length of the segment, to thus appear through a similar breach at the other end (Fig 4.3). This was then secured onto the tensioning jaw as before. At all stages extreme care was taken:

- Not to 'pull' on the MCA during second wire insertion.
- Not to snag the second inserted wire-tip on the endothelium.

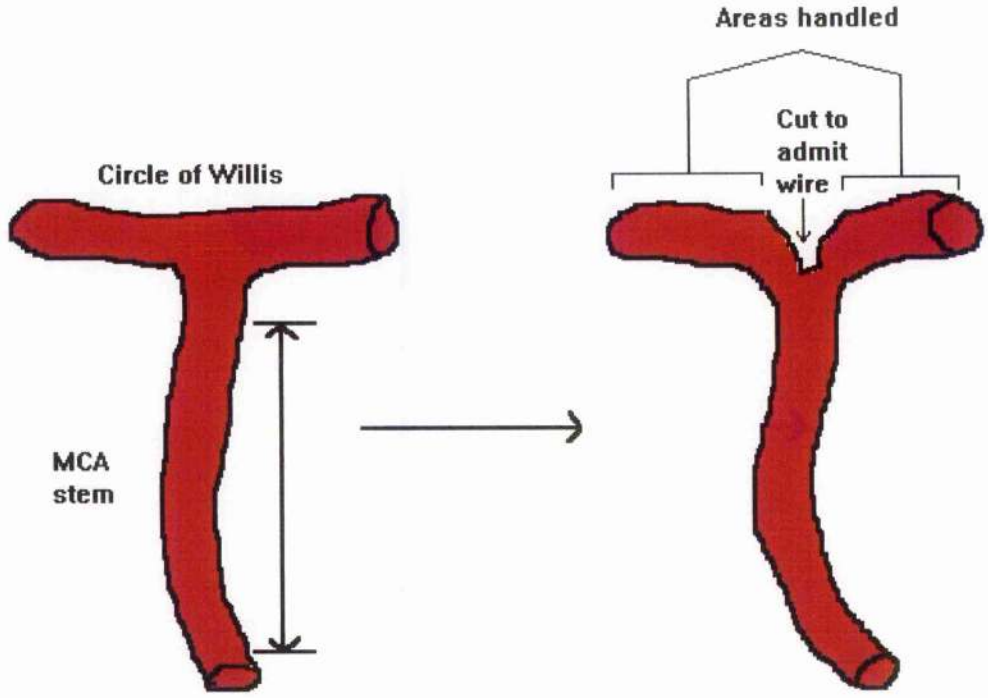


Fig 4.1. Manipulation of MCAs prior to mounting.

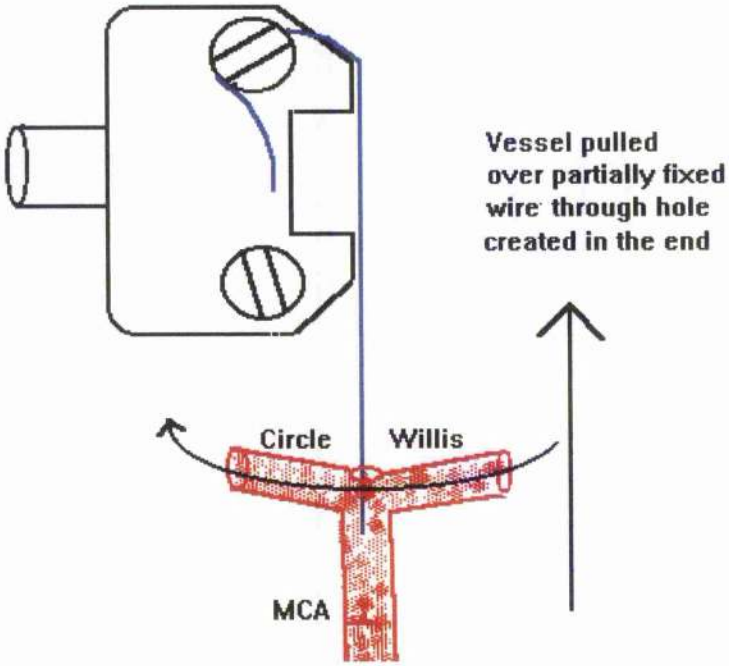


Fig 4.2. MCA being sleeved over mounting wire. The only parts touched are those of the bar of the 'T': i.e. the CoW. The MCA is not touched in any part.

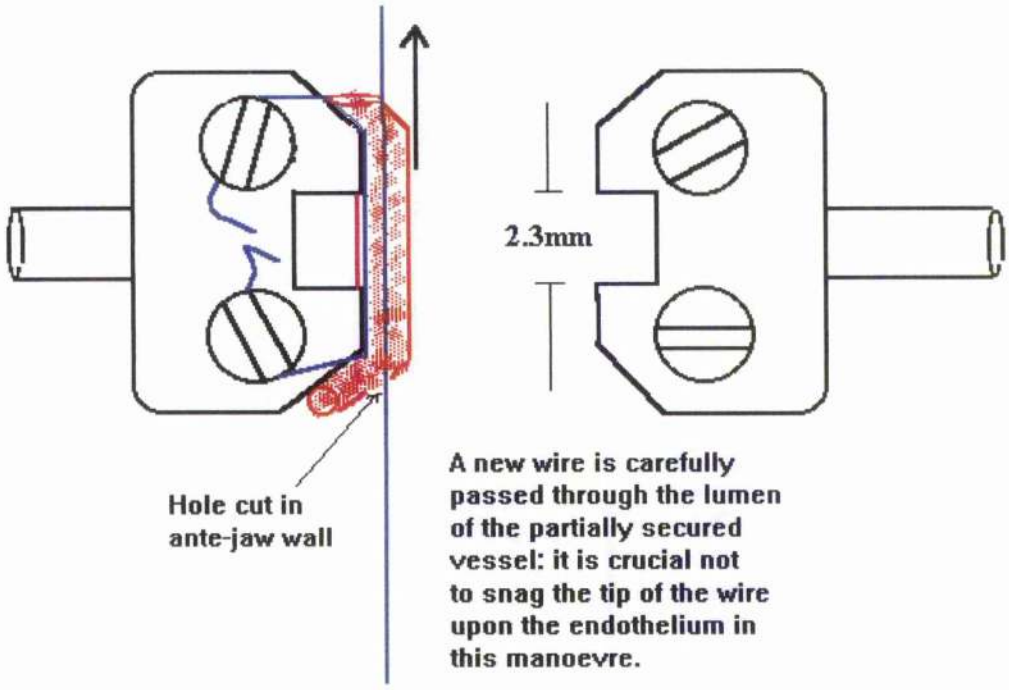


Fig 4.3. MCA secured onto transducer jaw of myograph.

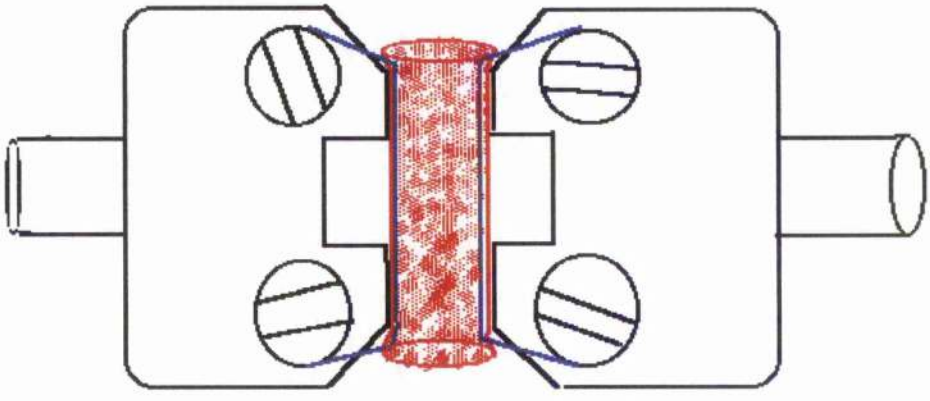


Fig 4.4. MCA fully mounted onto myograph jaws.

In fact, with practice, it was routinely possible to achieve *all* of these aims. At all stages during mounting maximal magnification was used with the operating microscope, and watchmakers forceps and scissors were used throughout.

After ensuring the two intraluminal wires to appear parallel, with adjustments being made as necessary, and following removal of excess tissue at either end (only the length between the jaws, i.e. the middle 2.3 mm, would be viable: Fig 4.3), the jaws were brought together so that the internal wires 'kissed' each other, this registering a *negative* reading on the force transducer. From this position, the movable jaw was then gradually moved away from the transducer jaw (in 1 μm increments) until the force recorded *stopped increasing dramatically in a positive direction*: this represented the **datum position** at which the **wires** were **just parted**, and could be confirmed visually. From this position the software programme could then begin its regime of sequential stretching at predetermined increments (usually 0.70 mN), so producing an exponential curve of *Tension v gap* (Fig 2). From this the desired internal pressure (90 mmHg) could then be extrapolated, and the diameter set, by the software programme (Fig 4.4; Plate 3); the diameter thereafter being referred to as the FD_{90}. At all stages during this procedure the PSS bath level was kept at 10 mls, and bubbled continuously with 95% O_2 and 5% CO_2, at 37°C.

The stretch routine was deliberately performed slowly in the current study because cerebral vessels develop myogenic tone to distension (Part 2, 2.2). The average length of time taken was around 25 mins. The vessels were then left to equilibrate for at least 45 minutes before any attempt at constriction was made. The final tensions and FD_{90} values were obtained before attempting agonist study. In some vessels, the distending pressure was repeated at different levels of normalization. Thus functional diameters were obtained at extrapolated luminal pressures of 70, 90, 110, 130, and 150 mmHg (Fig 31). In these cases, the diameter recorded would obviously be FD_{70}, FD_{90}, $FD_{110}$and so on (Part 5, Section II, 3.1).

5.4 Rejected vessels: bifid middle cerebral arteries

There is a lower limit to the vessel size compatible with wire myography. This is not just because of technical problems in manipulating and inserting 40 μm diameter wires into the lumena: as will subsequently be demonstrated, agonist contractile responses are proportional in magnitude to the size of the vessel obtained (Part 6, section II, 1.1). As a result, the small responses obtained by tiny vessels were generally incompatible with the detection of statistical significance. The most common cause for such vessels was the presence of bifid MCAs: here the sum of each FD_{90} branch obtained approached normal dimensions, but each itself was small. Such vessels were, in general, rejected from further study. Vessels in which KCl constrictions were poor were also rejected from the study.

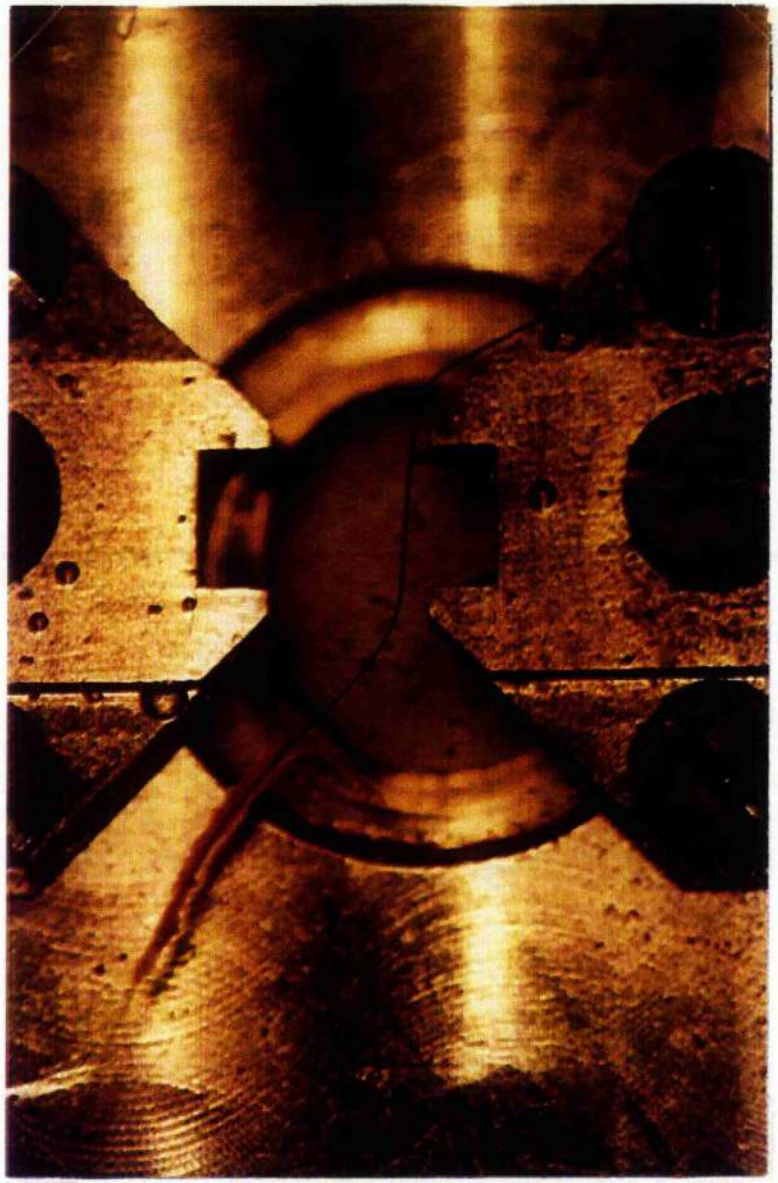


Plate 1. MCA sleeved over stainless steel wire

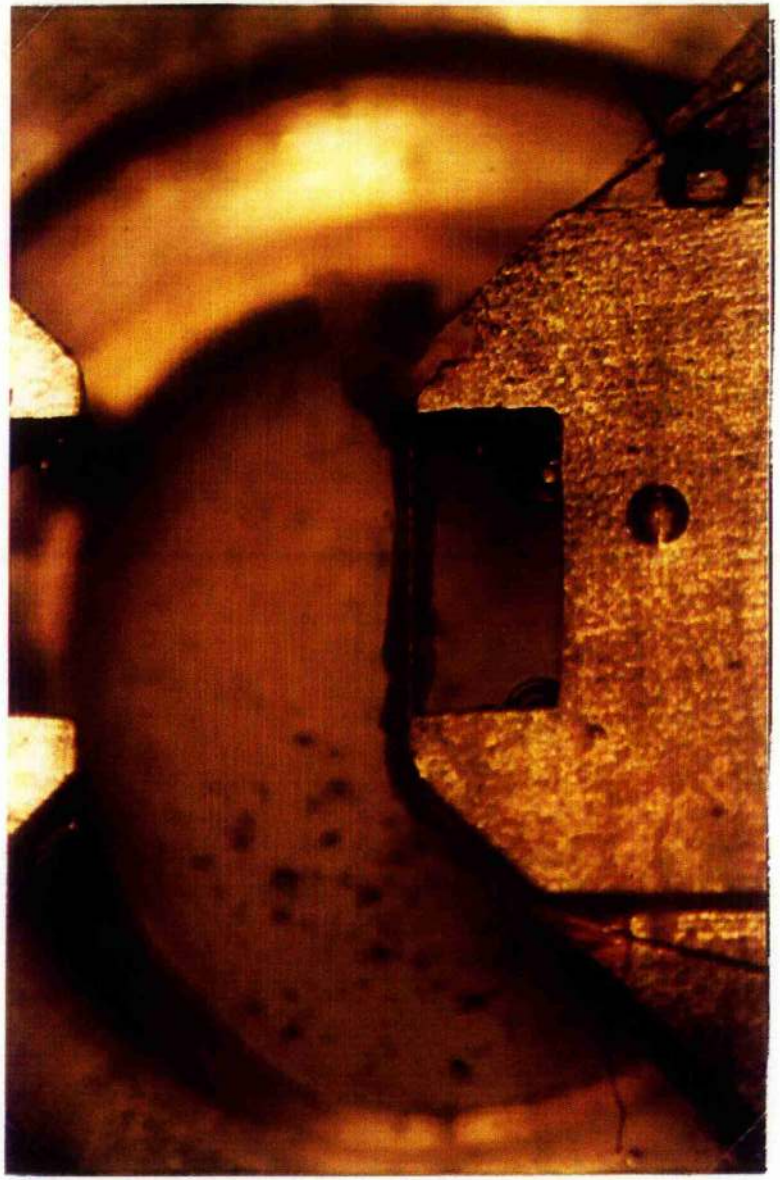


Plate 2. MCA fully mounted onto transducer jaw

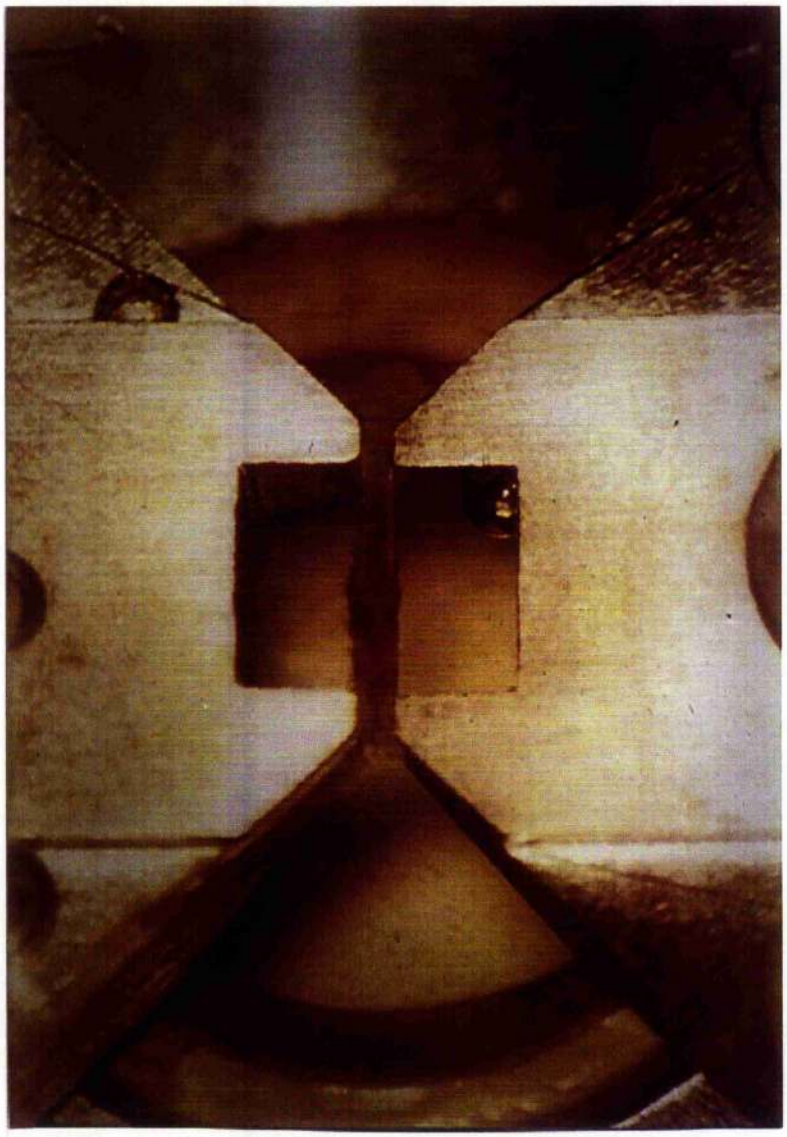


Plate 3. MCA fully mounted on myograph and stretched to an internal 'pressure' of 90 mmHg. The diameter obtained is therefore the FD_{90}. The myograph model used throughout was the Mulvany 500A®—the most current model. Endothelial function tests (L-ARG, L-NAME and hypoxia) confirm that no endothelial damage has been incurred.

5.5 Faults with the wire myograph

Apart from the fact that considerable dexterity is required to remove and mount the vessels without complication, several important criticisms distinguish wire myography from pressure-cannulized preparations:

1. The distending pressure is not truly radial (Fig 5).
2. The distending pressure is from 40 μm diameter wires which may damage the endothelium during both mounting and stretching.
3. It is not possible to separate agonist action into intraluminal and extraluminal effects.
4. The distending pressure is inferred, and not directly measured.

Nevertheless, wire myography continues to represent the 'gold standard' small vessel study model. It is also widely used for the study of cerebral vessels—including that of VSM after SAH (*see* Part 4, 4.2.2 for sixty-one non-exhaustive references). In spite of technical difficulties, with practice the vessels mounted produced myogenic tone (MT) and EDRF production in all cases—this indicating minimal endothelial and VSMC damage.

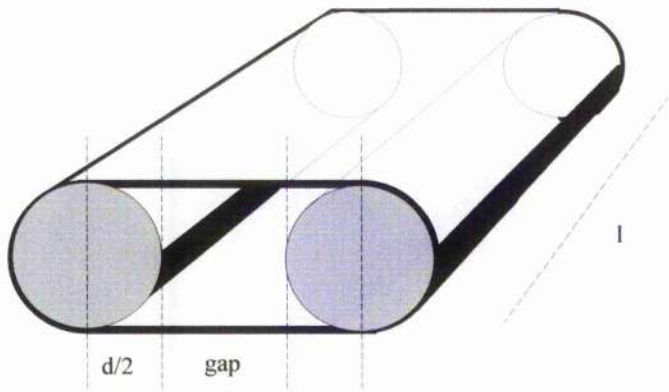


Fig 5. Diagram of vessel mounted on wires, showing gap, segment length and derivation of vessel circumference. Note how the distension produced differs from the radial distension achieved in vivo. Any length of vessel >**l** will be crushed against the jaws, and will not be viable for study. The circumference is $\pi/2.d + 2[d/2 + d/2] + 2gap + \pi/2.d$, which is $(2 + \pi)d + 2$ gap.

Chapter 6
Measurement of in vitro agonist activity

6.1 Solutions and drugs

6.1.1 Physiological saline solution (PSS)

One litre of PSS contained, in mM: NaCl 119, NaH_2CO_3 25, KCl 4.7, $CaCl_2.2H_2O$ 2.5, $MgSO_4.7H_2O$ 1.17, $K1I_2PO_4$ 1.18, Na_2EDTA 0.026 and D-Glucose 5.5. The $CaCl_2.2H_2O$ was only added to the solution following continuous bubbling with 95%O_2 and 5%CO_2 for > 30mins: this was to ensure its complete dissolution, a constant problem with myography. This was kept bubbling at 37°C in a separate container in an immersion bath: failure to do this often resulted in Ca^{2+} precipitation.

At all times the myograph bath level itself was kept at 10 mls. This was crucial because all in vitro agonist concentrations were calculated assuming this as the final bath dilution. This level was ensured by using a 10 ml syringe for PSS additions, and by keeping the perspex roof to the myograph bath in place throughout the entire procedure, so preventing evaporation.

Following each agonist study, the myograph bath contents were suctioned out (maintaining the perspex roof throughout), and the bath irrigated with fresh PSS at least three of four times. Following this, a suitable period of equilibration was again allowed. However, with repeated study, the vessels frequently became 'trained', and soon regained their prior baseline values for tension and diameter.

6.1.2 Drugs

KCl solution was made up as a 1M stock solution of 1 litre. PGF2α was obtained from the Royal Hallamshire Hospital pharmacy department as prostin®. All other drugs and chemicals were obtained from Sigma® Chemicals Ltd, Parkway, Sheffield. PPV and HA were obtained as the hydrochloride salts. Each agonist was made up as 1M stock solutions (in distilled water) from which 10^{-1}, 10^{-2}, 10^{-3}.....10^{-8} M solutions were then obtained by serial dilution (with distilled water). All solutions were kept in light-proof containers, and were made up fresh weekly. However NE, for example, was also made freshly each day. Each was kept refrigerated until the time of use, at which point they were equilibrated to 37°C.

6.2 Measurement of agonist action

For contractile agonists responses were obtained as **increases** in either tension or **force** generated in the vessel wall following agonist addition. In some cases this occurred

quickly (KCl, Fig 15), whilst in others this took several minutes (PGF2α, Fig 19). In all cases the progressive **results** obtained were **expressed** only as **ΔT or ΔF**: absolute values not being further considered. Usually the ΔF (mN) was only considered as tension was constantly proportional to this: this was because the active vessel length itself was constant at 2.3 mm (Fig 4.3).

For vasodilators, responses were measured as the progressive **decrease of force** (or tension) following each dose increment given. For most this involved the **progressive dilation of pre-constricted agonist tone**, this being achieved by vessel pre-constriction with **30 μM PGF2α**. This is standard myograph procedure. However, with some (hypoxia) this was achieved by progressive dilation of only baseline tone: thus, no PGF2α pre-constriction was used here. In all former cases, however, the dilation achieved was expressed the **percentage (%) relaxation of the preconstricted tonic value achieved**, for that agonist in that vessel. Thus, if a MCA was initially pre-constricted from a baseline of F1 to F2, and then progressively dilated from F2 to a minimum of F3 (where F3<F2), the maximum dilation then achieved would be:

$$\frac{(F2-F3)}{(F2-F1)} \times 100\% \text{ of pre-constricted tone (i.e. F2-F1) achieved.}$$

In most cases F1>F3<F2: i.e. E_{max} vasodilation achieved was <100%. In these cases each agonist dilated only a proportion of the PGF2α-induced tone that was additional to baseline value. However, in some cases F3<F1 with E_{max}>100%: in these cases the agonist had additionally dilated baseline myogenic tone in addition to that of the superadded PGF2α.

Obviously this could not be the form of expression used for hypoxic baseline dilation. Here I chose to express this as the decrease of force in mN: i.e. the exact reverse for agonist constriction. Others, however, express this as the precentage of myogenic tone achieved.

6.3 KCl responses

Contractile responses to KCl were obtained as abrupt changes to addition of maximal agonist concentration. No attempt at progressive constriction was made here. This was primarily because KCl was to be used as a marker of vessel viability; both at initiation and termination of vessel study. However, it was also because one wanted to obviate any endothelial damage resulting from more prolonged exposure (Part 3, 1.2). Thus, only E_{max} values were expressed: i.e. the **efficacy** of response.

6.4 Hypoxic responses

Again, only maximal responses were obtained to a 'fixed-dose' as with KCl above. That is, no attempt was made to achieve progressive dilations to incremental 'doses' (i.e.

intermediate pO_2 values) as in a formal dose-response curve. Thus only $\mathbf{E_{max}}$ values were expressed: i.e. the **efficacy** of response. Hypoxic responses were only recorded in controls.

6.5 Concentration-response (C-R) curves

In all other cases, each agonist was added to the bath in ever-increasing dose increments, in order to obtain progressive increases in response up to the maximal value obtainable, this being achieved at maximal agonist concentrations (but *see* PGF2α, Fig 33). Such concentration-response curves provide much better characterization of agonist responses, this being standardized by the $\mathbf{EC_{50}}$ **value**: the effective concentration at which 50% of the maximal response ($\mathbf{E_{max}}$) would be achieved (Fig 6). This describes the **affinity** of agonist response, a theoretical concept of the degree of

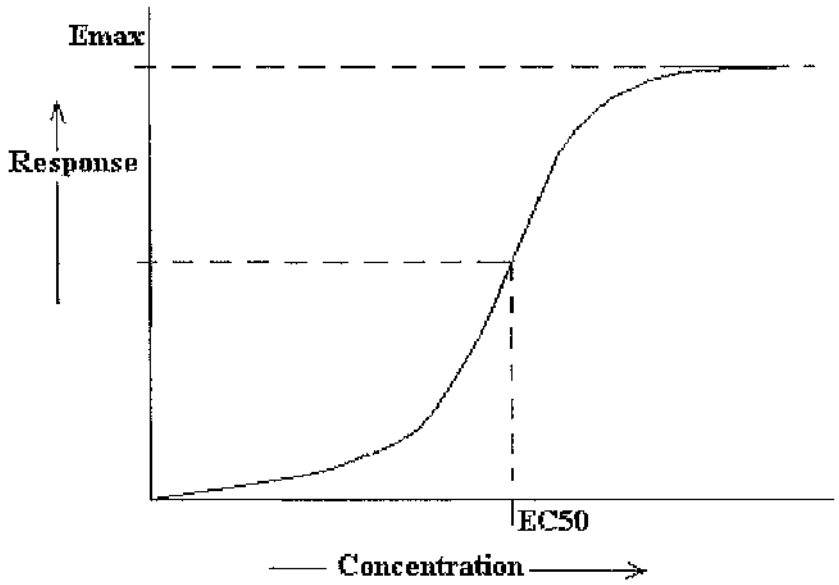


Fig 6. Hypothetical Concentration-response (C-R) curve. E_{max} and EC_{50} values.

agonist-receptor interaction. It is a necessarily inferred value, as the C-R curve must itself be plotted to determine the dose, or concentration (abscissa), at which 50%E_{max} occurs. The concentration range for each agonist was worked out individually: thus the range for 5HT, for example, was several orders of magnitude lower than with other contractile agonists (Part 5, Results). In occasional cases, in order to save time, complete concentration-response curves were not performed: only maximal responses (E_{max} values) were obtained instead. A good example would be L-NAME responses. EC_{50} values were therefore obviated in such cases and, in these MCAs, ***n* values for $\mathbf{EC_{50}}$< $\mathbf{E_{max}}$.** Furthermore, in sporadic MCAs, PGF2α pre-constriction was temporarily inadequate enough for some agonists to allow an accurate C-R curve to be determined. NE responses

were singular in that, for some MCAs, no response at all could be reliably detected: in these cases, both E_{max} and EC_{50} n values were lower than with other agonists.

6.6 Group comparisons and statistical methods used

Inter-group agonist response analysis was performed by comparing **continuous data** of E_{max} values and, where appropriate, comparison of EC_{50} values. This was performed by using a **one-way analysis of variance (ANOVA)**. Values of $p<0.05$ were considered statistically significant.

However, in some cases the study of **proportions** was required. For example, with HA responses (Table 6: Part 5, Section II, 3.7) it was required to consider proportions of sub-responders and responders between each group. In this case **2 x *k* tables** were constructed (in this case $k = 3$) with evaluation of χ^2 being obtained. By consideration of the χ^2 **distribution** for the number degrees of freedom permitted ([n - 1] = 2, in this case), values of $p<0.05$ were again considered statistically significant.

All data of continuous variables are stated as mean±SE except FD_{90} values which were expressed as mean±SD.

6.7 Histopathological study

The brain and vessels were occasionally studied at the end of the experiment. The heart was rarely considered for study as the time period after SAH was too short for histological changes to be incurred[331].

6.8 Choice of vessel for study

Most SAH studies choose the BA. However, there are several setbacks with this. Firstly, although the BA may develop VSM, DIDs are rare in its territory[267]. Secondly, its study does not permit the analysis of lateralization with DCI, as the BA is a single midline vessel. Thirdly, the BA has decidedly different physiological and pharmacological properties in comparison to anterior circulation vessels[290, 620].

It is also important to analyze a **particular length** of the branch considered, as properties differ markedly between proximal and distal segments[237]. Thus NE activity is greater in the proximal MCA[237], this according with the greater adrenergic innervation centred on the CoW[620]. Responses may also differ with age, hence the importance in studying a small age range[620].

Chapter 7
Central hypothesis and Experimental protocols

7.1 Central Hypothesis and Aim of Study

The principle hypothesis of the current study is that events associated with acute SAH elicit changes in MCA physio-pharmacology that are independent of any subsequent effects of chronic clot lysis. In consequence, such changes might potentially explain why currently favoured 'clot-VSM' fail to adequately explain delayed VSM and DCI—because, by demonstrating post-ictal alterations, the assumption of 'clot-VSM' models that delayed VSM merely results from chronic clot lysis upon *normal* cerebral vessels is therefore undermined. The principle aim of this thesis is therefore to study the physio-pharmacological responsiveness of isolated MCAs to an array of conceivably-relevant agonists in the acute period (i.e. within three hours) of a truly representative SAH (i.e. EF-SAH), and to compare these responses with those of sham operative and non-operative controls. To this end, it is intended that both MCAs from all animals medial to the olfactory tract are subjected to in vitro myography using all of the physiological and pharmacological agonists listed in Part 3. *No attempt will be made to study the delayed effects of chronic clot lysis in this thesis.*

In each in vitro experiment in the current study, the sequence of agonist responses obtained will be relatively fixed as: KCl x2, PGF2α, PPV, UTP, HA, L-ARG, 5HT, SNP, NE, KCl (final). It is important to assess the KCl (final) as this confirms vessel viability even after 10 hours or so of in vitro study. In addition, vessel viability will also be sporadically checked throughout the study by analyzing KCl constrictions: poor responses will result in premature termination of in vitro myography.

If, as a result of this analysis, no differences are found between SAH vessels and controls, the central hypothesis of the current study would therefore have to be rejected. Such a finding would, instead, be supportive of the alternative hypothesis: i.e. that the current failure of SAH models to elicit clinically useful information most likely results from the fact that DCI is a species-specific pathophysiological process; and that results obtained from one species are not readily translatable to those of another.

A secondary hypothesis of the current thesis is that EF-SAH fundamentally introduce a 'side bias' upon post-SAH vasoreactivity that potentially confounds their use for studying delayed VSM. A secondary aim of the thesis, then, is to formally assess for such bias by comparing constrictory and dilatory MCA responses ipsilateral and contralateral to EF insertion. If, as a result, any significant side differences were found, then this would be

supportive of the fact that the combination: *1)* unilateral carotid manipulation, *2)* unilateral EF insertion or *3)* potentially ipsilateral intracranial rupture introduces a 'side bias' on MCA reactivity that potentially confounds their continued use in analysing the delayed effects of chronic clot lysis in VSM or DCI.

7.2 Experimental protocols and practice

7.2.1 Non-operative controls

A total of 20 animals were used. Animals were anaesthetized with 25% urethane intraperitoneally (1g/Kg) and sacrificed by aortic exsanguination. Vessels were immediately transferred for myography: **no MCAs were fridge-kept for use on subsequent days**.

7.2.2 Sham operative controls

A total of 8 animals was used. Animals were anaesthetized with 25% urethane intraperitoneally (1g/Kg) and sacrificed by aortic exsanguination. Operating time varied between 1.5-3.0 hours. All animals underwent the same procedure as SAH animals **except that the thread was not allowed to perforate the CoW**. After thread withdrawal, all animals were kept alive for 3.0 hours. Total anaesthetic time therefore spanned up to 6.0 hours. Vessels were immediately transferred for myography: **no MCAs were fridge-kept for use on subsequent days**.

7.2.3 Subarachnoid haemorrhage groups

A total of 34 animals were studied. Animals were anaesthetized with 25% urethane intraperitoneally (1g/Kg) [except two anaesthetized with hypnorm and hypnovel], and then sacrificed by aortic exsanguination. In vivo operating time varied between 1.5-3.0 hours, following which SAH was induced. All animals were then to be kept alive for up to 3.0 hours. However, in those cases in which a subacute cardiovascular collapse (SCC) occurred—analogous to cases of 'sudden death' clinically—such animals were electively sacrificed within three hours (see **Results**). Total anaesthetic time thus spanned up to 6.0 hours. MCAs were immediately transferred for myography: **no MCAs were fridge-kept for use on subsequent days**.

7.2.4 Myography

Wire myography was to be used in all MCAs in all three groups as described in Part 4, Ch 5. Adequate KCl constrictions were a condition of vessel viability: repeated poor contractile responses resulted in the abandonment of the vessel study at whichever stage this occurred. [**NB in most cases, poor contractile responses were only a temporary phenomena: a brief period of vessel relaxation usually resulted in a full return of**

normal activity.] The target for vessel study was the proximal 3 mm of the MCA, proximal to the olfactory tract. Mounting time was approximately 1 hour. In vitro analysis took up to 11 hours (approximately one hour for each agonist). Therefore total duration for myography was up to 12 hours. Vessels were immediately transferred for myography: **no MCAs were fridge-kept for use on subsequent days**. Myography was performed in all animals obtained from shams and controls, and in 30/34 (88%) of animals after SAH. Notwithstanding, not *all* agonists were used in *all* MCAs in *all* experimental groups—time frequently proved to be of the essence for one researcher in this study, where total experimental times frequently exceeded 15 hours in one day!

PART 5

RESULTS

SECTION I Chapters 1-2
SECTION II Chapters 1-3

SECTION I
Whole animal responses

SECTION II
In vitro vessel responses

SECTION I
Whole animal responses
Chapter 1
Cardiovascular and intracranial pressor responses

1.1 Recording bias with multi-channel flat bed pen recorders

Both arterial and ICP pressure transducers used in this study were doubly connected to both a patient monitor (for direct visual and digital display), as well as to a multi-channel flat bed pen recorder (Rikadenki®) for permanent **graphical** print-out of **qualitative trends**. Correlations between sphygmomanometer values, and those displayed on the patient monitor, were regularly checked (**validation** of transducer precision and accuracy). **Direct callibration** of *static* sphygmomanometer pressures onto the multi-channel flat bed recorder were intermittently performed: however, frequent checks were made (re-validation) on these values by reference to those displayed on the patient monitor.

Throughout the period of the study, and despite re-validation, it was noted that pen recorder BP values were frequently at variance either with directly calibrated pen recorder values, or with those displayed simultaneously on the patient monitor. In particular, systolic-diastolic BP fluctuations—at times—appeared grossly uncorrelated with calibrated values. Mean BP values (MAP), however, appeared to correlate with both pen recorder and patient monitor values, as did ICP values (systolic, diastolic or mean). Re-checking static pen recorder values against sphygmomanometer values failed to reveal that any "drift" had occurred when these anomalies were noted. The problem encountered, therefore, did not appear to relate to calibration errors.

Because the patient monitor (validation of arterial transducer) had faithfully recorded absolute pressures, it was initially presumed that possible systolic-diastolic pen recorder aberrations could be adequately compensated for. This resulted from the original intention merely to use pen printouts to demonstrate qualitative trends in pressure fluctuations. However, the (unexpected) acute pressor changes ultimately found clearly revealed a need for their accurate quantification (*see below*). The clue to the solution of the problem, it later transpired, lay in the fact that only BP systolic-diastolic fluctuations appeared affected: MAP values or [systolic, diastolic and mean] ICP values appeared unaffected.

BP systolic-diastolic fluctuations differ from ICP fluctuations on the pen recorder in one important respect: they are considerably *wider* (one can, of course, make them equal by changing the gain on the signal). Thus, whilst ICP systolic-diastolic fluctuations usually extend for less than 3 or 4 mmHg, BP fluctuations frequently exceed 30 or 40 mmHg. Such fluctuations

are represented on flat bed recorders by pen excursions between systolic and diastolic extremes. It is not difficult to envisage that a pen, already moving rapidly to and fro against resistance in a vertical direction, might become progressively restricted in its aims by decreases in the time available for this excursion. Thus, where the pen is required to move a set distance with increased rapidity (i.e. with **increasing heart rate**), there may arise a situation, in the limit, where it simply cannot achieve its full excursion in the diminished time available. A rapidly supervening diastolic signal could, for example, abruptly coerce a pen into its reversed direction *before* it had reached its systolic height in its original direction. Ultimately, one can surmise, the **systolic-diastolic bandwidth** will become **increasingly constricted** with increased tachycardia, the bandwidth becoming **increasingly uncorrelated** with its true width as extrapolated from both static pen recorder calibrations and those displayed on the patient monitor.

This hypothesis was directly tested in the present thesis by sending two fixed electrical signals to a pen recorder, at variable speeds, in order to create pen excursions mimicking systolic-diastolic pressure fluctuations. These ("pressure") **signals were fixed** and **did not vary**: only the rate of signal alternation was allowed to vary. The results of the experiment are shown in Figs 7a and b. In both Figs, the systolic-diastolic signals were fixed at 30 mmHg throughout all sequences: only the 'heart-rate' (HR) was allowed to vary. Increasing the HR from 60 b.p.m. on the left hand side, to 300 b.p.m. on the right, in Fig 7a, clearly caused the systolic-diastolic pen excursion ('bandwidth') to contract: exactly as hypothesized. As is shown, the contraction was uniform (approximately 50%) about the MAP (defined as the mid-point of the systolic-diastolic excursion) which remained invariant. Thus, both **systolic** and **diastolic values contracted equally about the MAP** at higher pen speeds.

In Fig 7b, increasing speeds through 450, 600 and 750 b.p.m produced corresponding decreases in bandwidth dimension. Again, the contraction approximated to 50% at each level. Because of this approximation, greater magnitude effects are to be expected early on in the sequence: i.e. bandwidth contraction probably exhibits exponential decay (Fig 47, Appendix). Importantly, significant bandwidth contraction is apparent in the HR range 300-450 b.p.m.: precisely that of the normal rat[343, 344]. A possible example from the thesis is witnessed in Fig 11, where bandwidth contraction occurred with tachycardia during a CHR: (however, a genuinely decreased pulse pressure could also have occurred here).

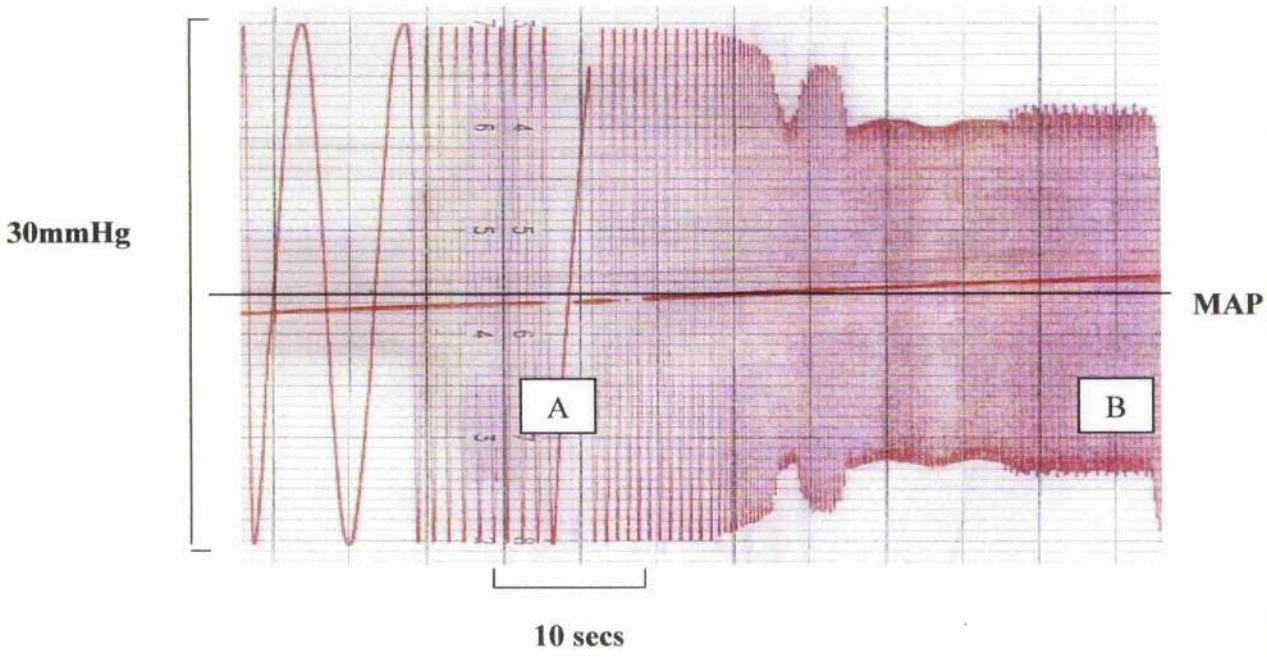


Fig 7a. Pen recorder speed-inertia artefact.
Electrical signals are set to mimic a **constant** systolic-diastolic fluctuation of **30mmHg**. On the left-hand side (A), the speed ('heart rate' [HR]) is set at 60 b.p.m. On the right hand side (B), HR is increased to 300 b.p.m. Clearly, the bandwidth has contracted from A to B. The '**MAP**' (the mid-point), however, remains **invariant** under the transformation.

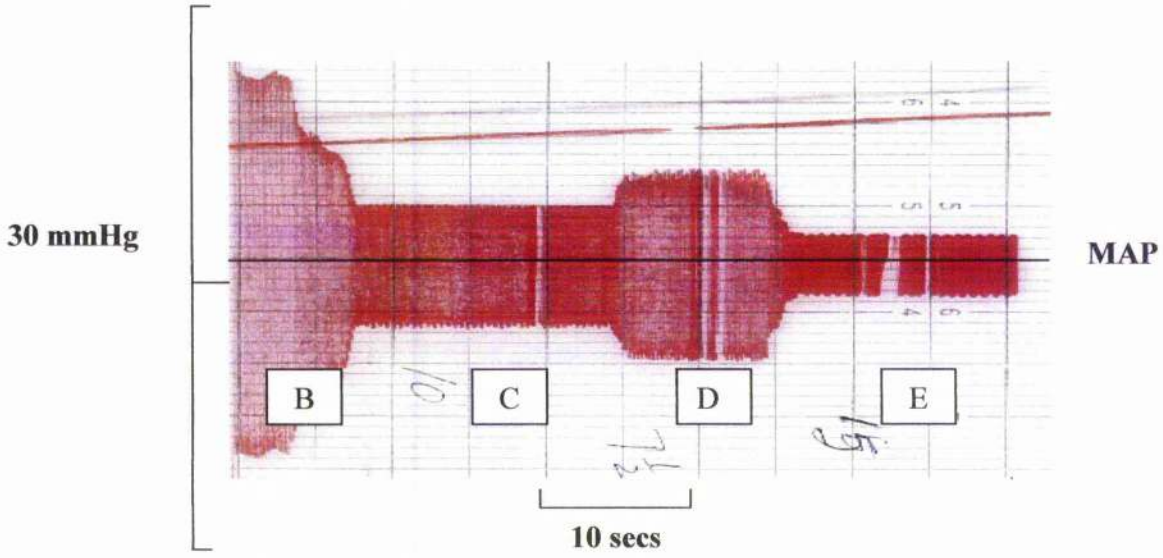


Fig 7b. Pen recorder speed-inertia artefact.
This tracing is continued from Fig 7a, right hand side (moving L to R). The HR is varied through 300 (B), 600, (C), 450 (D) and 750 (E) b.p.m. The bandwidth in the normal rat HR range (300-450 b.p.m: B-D) is clearly a considerable fraction of its true 'static' value (left hand scale): the systolic BP has 'decreased', the diastolic 'increased'; the MAP is invariant. **Dynamic-static correlations** are therefore **only** valid with **MAP**s: **systolic** and **diastolic** BPs become increasingly **uncorrelated** at higher pen speeds (i.e. faster HRs).

The current findings can be summarized:

- **Dynamic** systolic and diastolic BP values become uncorrelated with **static** pen recorder calibrations at higher pen speeds (i.e. faster HRs). ICP values—because of smaller pen excursions—remain unaffected.
- **True dynamic** systolic and diastolic BP **values** can only be reliably documented from a **patient monitor**

An example of the error that can be incurred by using systolic and diastolic values to estimate pressure changes is demonstrated in Fig 8. In this tracing, the baseline BP *appears* to be 125/102 (MAP 114) mmHg. In fact, the patient monitor shows that it is actually 146/84 (MAP 115) mmHg. Thus, the bandwidth has contracted—by reference to static calibrations—by approximately 21 mmHg systolic, and 18 mmHg diastolic. This is a symmetric contraction, within the error of the approximations; the difference in the MAPs being negligible. Using the contracted bandwidth, 23mm on the graph paper represents 62 mmHg (on static calibrations). Since the measured $-\Delta MAP$ is 31mm on the paper, this translates to a $-\Delta MAP$ of 84 mmHg and a MAP_2 of only 31 mmHg (on static calibrations). In fact, the actual $-\Delta MAP$ is merely 34 mmHg, the MAP_2 much greater at 81 mmHg. The pen recorder speed artefact tends, therefore, to exaggerate the magnitude of ΔBPs.

Figs 7a & b—as well as Fig 8—amply demonstrate, however, that the value that can be quantified throughout any HR transformation is the MAP: effectively the mid-point of the pen excursion. This is important because the **MAP correlates** directly with **static pen recorder calibrations** as well as to **patient monitor displays**. It also directly correlates with the animal's aortic MAP, since the MAP here is half the pulse pressure plus the diastolic value (i.e. the arithmetic mean of the pulse pressure)[663]. **Quantification of all pressure changes in this thesis is therefore based upon MAP values.**

A problem still remains, however, in how to display pressor fluctuations. Since more modern patient monitors are able to store and print out graphical representations of pressor values, every encouragement should now be given to their full employment in experimental studies, since they obviate the error incurred by a rapidly moving pen. However, where these remain unavailable, and where the use of a pen recorder is mandatory, three methods are available to display pressor fluctuations—each with their advantages and disadvantages:

1. **Display pen recorder print-out with *y*-axis respecting MAP values**. *Advantage*: the in situ experimental recording is demonstrated, producing 'hard' evidence of authenticity. *Disadvantage*: systolic values are artefactually decreased, diastolic values

artefactually increased, with increasing HR. This leads to inconsistencies between patient monitor (or sphygmomanometer) values and those inferred from the graphical display.

2. **Display MAP values as a 'straight-line' graph**. *Advantage*: this allows a true representation of the only reliable pressor value, with avoidance of contradictory systolic-diastolic values on the same graph. *Disadvantage*: the 'straight-line' graph is *derived*, therefore losing the 'hard' evidence of authenticity supplied by the in situ tracing. (However, one could 'shrink' the systolic-diastolic fluctuations by changing the gain on the recorder channel).
3. **Display pen recorder print-out with two *y*-axis scales**: one respecting MAP values, the other respecting systolic and diastolic BPs. *Advantage*: this is more informative than a single-axis scale. *Disadvantage*: two *y*-axes are unconventional and therefore potentially confusing.

1.2. Sham operative control MAP and ICP values

Satisfactory MAP and ICP values were obtained in 8/8 rats (Table 16, Appendix). The group mean MAP was 112±6 mmHg, the group mean ICP value 7±1 mmHg. However, the MAP at the start of the experiment—i.e. that immediately following arterial cannula insertion—was usually much lower (97 mmHg). The cause of this elevation was not related to inadequate anaesthesia as the animal did not exhibit any response to painful stimuli (respiration remained regular, there were no contralateral response to hind-limb tweaking, and there were no further MAP increase etc). However, in one or two cases where this was performed, concurrent section of the ipsilateral femoral nerve appeared to reverse the hypertension.

Mean arterial blood gases are displayed in Table 15, Appendix. At no point was any blood observed in the ICP cannula, nor was any found within the subarachnoid space at post mortem (Plates 4, 6 and 8).

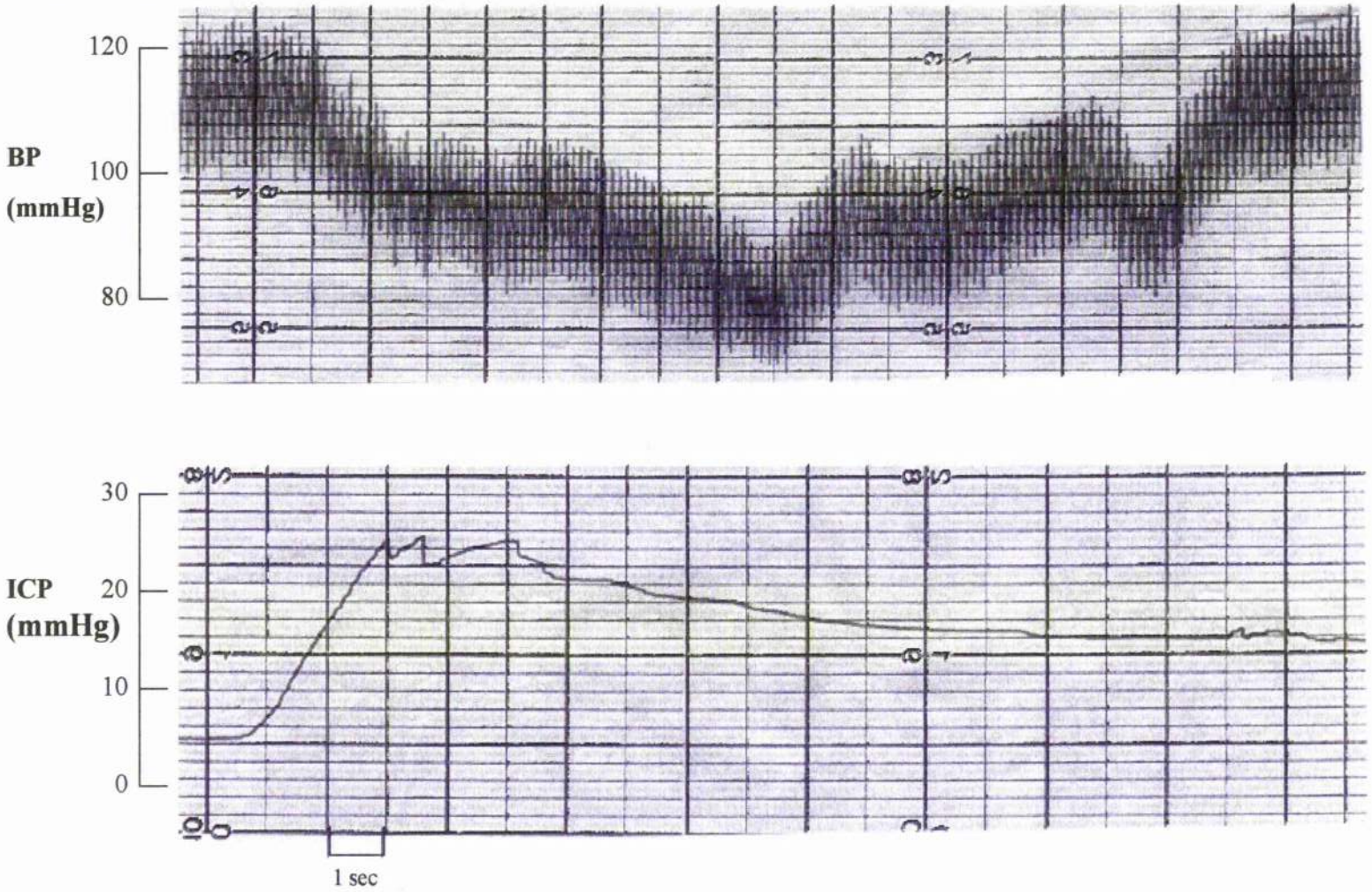
1.3 Subarachnoid haemorrhage groups MAP and ICP values

Mean arterial blood gases are displayed in Table 15, Appendix: these did not significantly differ from those in sham operative controls. Satisfactory pen-recorder BP tracings were obtained in 33/34 rats (patient monitor and arterial blood gas values confirmed adequate perfusion in one animal without a tracing); satisfactory ICP tracings in only 26/34 [*see* Part 4, 3.3; also Tables 17-19, Appendix]. Nevertheless, continuous digital read-outs from the patient monitor confirmed adequate BPs as well as ΔICPs upon arterial rupture. Mean baseline MAP was 123±5 mmHg: this value did not significantly differ from that in sham operative controls ($p>>0.2$). However, once again the initial MAP had been lower, a return to this level possible by sectioning the femoral nerve. The mean baseline ICP was 8±1 mmHg: after acute SAH, this rose sixfold over 1.8±0.4 secs to 49±4 mmHg, gradually falling thereafter to near-baseline values. Respiratory irregularities and transient falls in pO_2 occurred at this juncture, but only one animal required respiratory support. There were three clearly defined pressor responses *concurrent* with SAH (Figs 8, 10, 11):-

1.3.1 Type I acute pressor response (acute hypotension)

Acute hypotension was concurrent with SAH in 21/33 cases (64%), forming a distinct and large sub-group of animals. It resulted in a significant fall of MAP (**–ΔMAP**) of **30±4 mmHg** over 2.4±0.3 secs, from 128±6 to 98±7 mmHg ($p = 0.03$); following which the MAP gradually recovered pari passu with ICP resolution (Table 17). [This drop is similar in magnitude to that seen following sympatholysis (Part 2, 2.6)].

Fig 8. Type I pressor response (Hypotension) after SAH



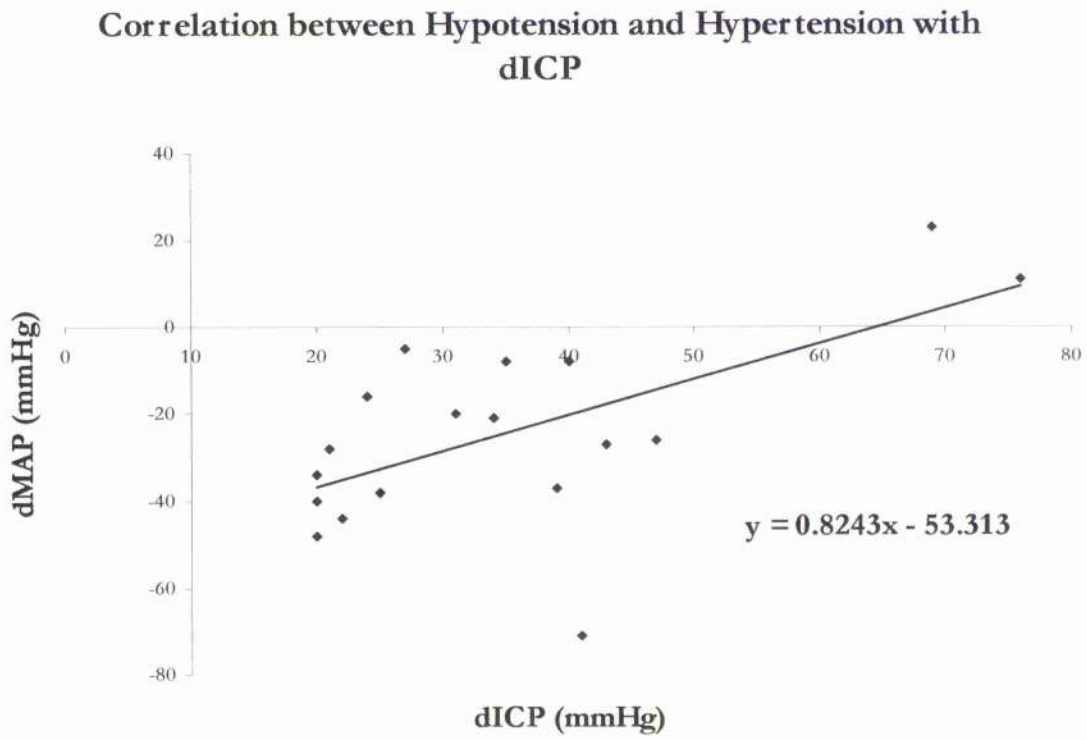


Fig 9. Correlation between hypotension and hypertension with ΔICP after SAH. Acute hypotension correlates with smaller ΔICPs; acute hypertension with higher ΔICPs (i.e. ΔICP>65 mmHg) [$r = 0.6$, $p<0.01$]. The correlation coefficient (r) becomes 0.77 ($p<0.001$) by exclusion of the gross outlier [dICP 41mmHg, dMAP –71mmHg]: this is highly significant, and suggests that 60% of the variance in acute pressor response *directly relates* to SAH-induced ΔICP.

NOTE: there are only n = 18 pts on the graph. This is because in 4/7 hypotensive-group animals, the ICP tracing was lost (Part 4, 3.3; Table 17-19, Appendix). In one other animal the pen-recorder tracing for both BP and ICP values was subsequently lost in transit—experimental notes at the time, however, confirmed that an acute hypotension had occurred. The total combined number of animals in hypo- and hypertensive groups is, of course, 23.

MAP recovery was sometimes prolonged slightly beyond ICP recovery (Fig 8). The **ICP_{max}** attained in this group (**38±2 mmHg**) was significantly **lower than** that obtained in either **pressor type II** (60±6 mmHg) **or III** (83±5 mmHg) [$p<0.001$]. Furthermore, the change in ICP (**ΔICP:** 31±2 mmHg) was also **significantly smaller** in this group relative to that in **pressor type II** (50±6 mmHg) **and III** (73±4 mmHg) [$p<0.001$]. In fact, a significant **correlation** existed **between** the type and magnitude of the acute **pressor response** and the **ΔICP** (**$r = 0.6$, $p < 0.01$: Fig 9**). Fig 9 shows that –ΔMAP was closely linked to smaller ΔICPs: +ΔMAP to higher ΔICPs (i.e. ΔICP>65 mmHg). [By excluding the statistically gross outlier in Fig 9, the significance of the correlation is increased by an order of magnitude ($r = 0.77$, $p<0.001$)].

Fig 10. **Type II pressor response (invariant) after SAH.**

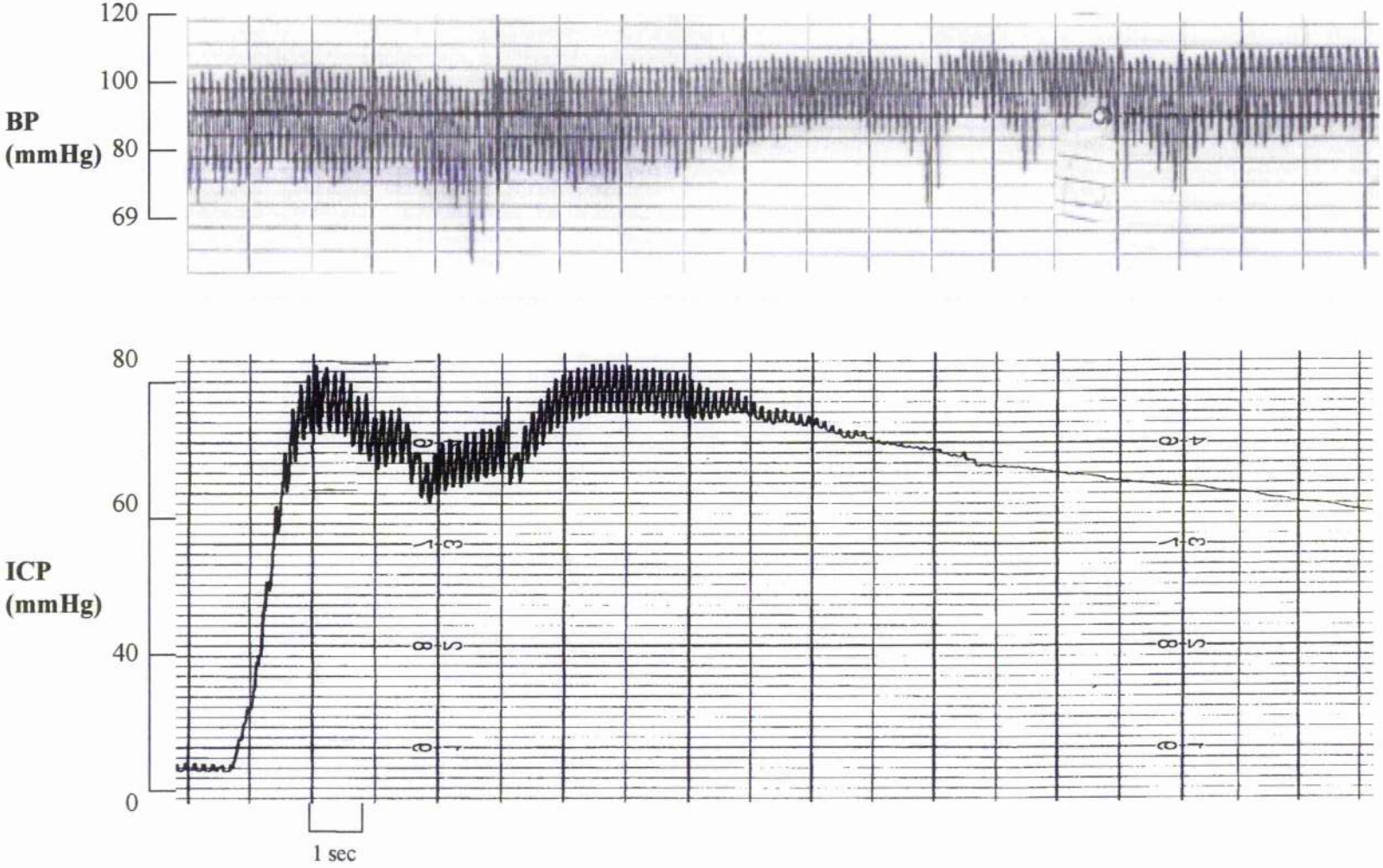
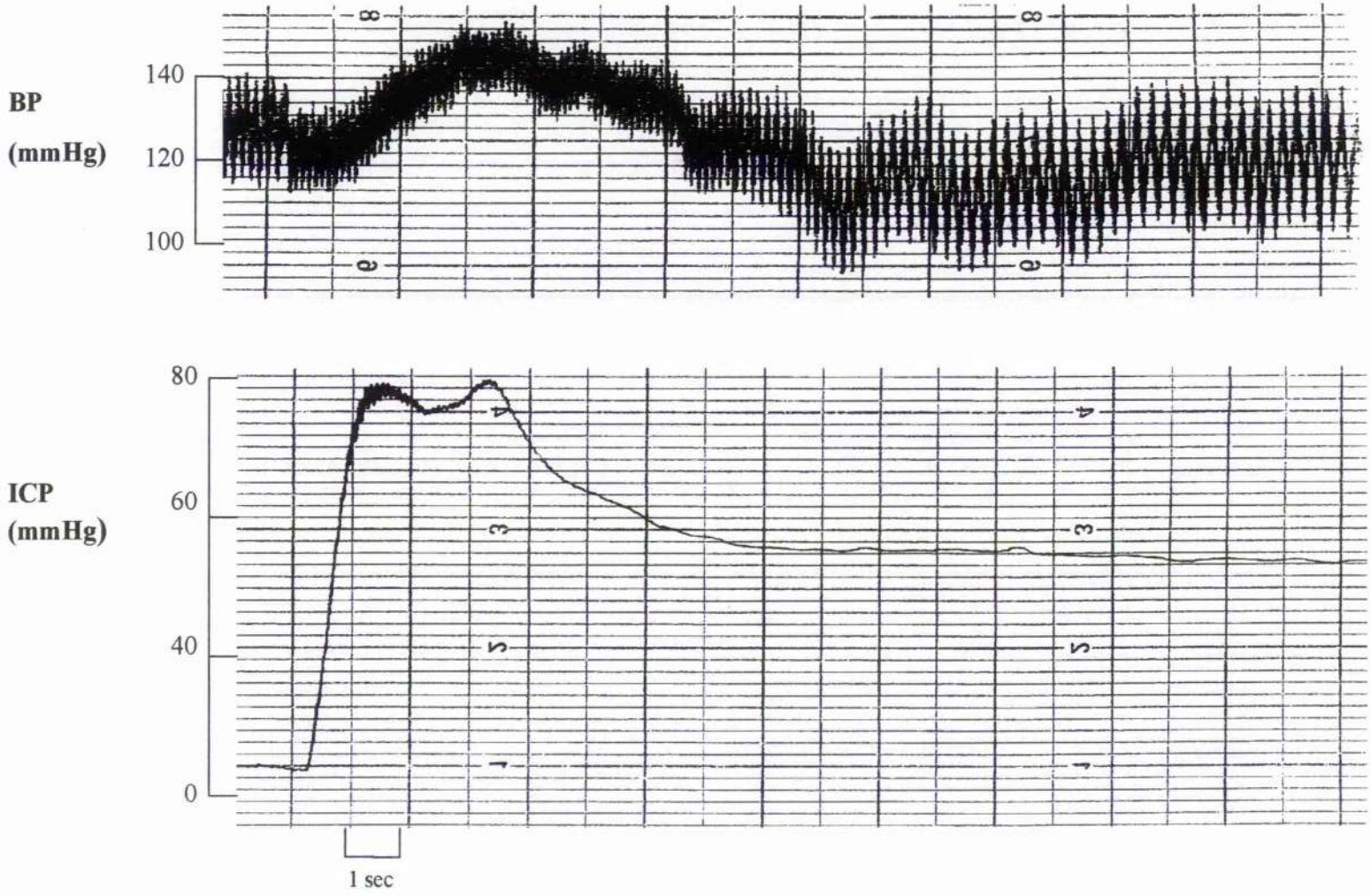


Fig 11. Type III pressor response (Cushing Hypertensive) after SAH



Hypotension associated with modest ΔICP and ICP_{max} values after SAH may be more than mere coincidence. Hypotension effectively increased the ICP_{max}:MAP ratio closer to the range observed in the other two groups. Thus, the ICP_{max}:MAP ratio that would have been seen *had –ΔMAP not occurred* (31±3%) was significantly increased to 41±3% as a result of –ΔMAP ($p = 0.02$). Importantly, whilst the former ratio (31±3%) differed highly significantly from that of either acute pressor response type II (53±8%) or III (57±4%) [$p = 0.001$], the latter value after –ΔMAP (41±3%) was only just significant [$p = 0.046$]. This suggests that relatively modest ICP_{max}:MAP ratios—perhaps only in the range 40-60%—are required to stanch SAH. The rate of ICP_{max} attainment (ΔICP/Δt) also appeared more prolonged (2.4±0.3 secs) in Type I compared to that in groups II and III (compare Figs 8, 10 & 11).

1.3.2 Type II acute pressor response (invariant) and Type III pressor response (Cushing hypertensive)

In n = 10 (30%) the MAP level was **invariant** after SAH (**Type II**, Fig 10); whilst in n = 2 (6%) the MAP demonstrated a classic **Cushing-type** response (**Type III "CHR"**, Fig 11). Baseline pre-SAH MAPs did not significantly differ across the three pressor groups (128±6 mmHg, 113±7 mmHg and 128±3 mmHg, $p = 0.38$). As stated in 1.3.1 above, both the **ICP_{max}** and **ΔICP** were **significantly greater** in these two groups relative to Type I responses ($p<0.001$: Tables 17-19, Appendix). Furthermore, the δICP/δt appeared much steeper (compare Figs 8, 10 & 11). In pressor groups II and III, the ICP_{max} represented 53±8% and 65±2% that of the pre-SAH baseline MAP respectively: however, the ratio in group III (CHR response) effectively *decreased to* 57±4% *as a result of the hypertension* (+ΔMAP) *that supervened.* In this respect the CHR appears paradoxical, offsetting SAH-tamponade and courting re-haemorrhage. The progressively higher ICP_{max} values and ICP_{max}:MAP ratios **across the three pressor groups** suggest that **pressure-venting was increasingly less successful** across the series: type I—type II—type III acute pressor responses. Fig 9 suggests that the CHR is likely to be triggered when ΔICP exceeds ~65 mmHg, as a result of inefficient venting.

1.4 Subacute Cardiovascular Collapse (SCC) and 'Survival'

In n = 14 (42%) animals (Table 20, Appendix), a hypotensive state ensued at some point within the arbritary three hours which proved refractory to intravenous fluid challenge and 100% O_2 administration. Its occurrence was **temporally distinct** and **unrelated** to the **type I pressor response** (which was always transient and spontaneously reversible). The decision was made early on in the study to **sacrifice** any such animal **within** the arbitrary **three hour period**, the rationale being to limit the known confounding effects of a prolonged hypotensive state upon subsequent in vitro MCA reactivity[335]. [Furthermore, it

was decided not to restore systemic pressures with inotropic agents in these cases because of the risk of metabolic acidosis.] Although this methodology necessarily gave rise to **heterogeneous survival groups**, this scenario was considered to be more representative of the clinical one (Part 4, 3.3; Part 6, Section III).

Animals developing subacute cardiovascular collapse (SCC) did so in the 1st and 2nd hours of SAH: animals surviving into the 3rd hour did not develop SCC (SCC is equivalent to mortality in this thesis). SCC ensued in 9/21 (43%) animals exhibiting **acute pressor Type I responses**, 4/10 (40%) exhibiting **Type II**, and 1/2 (50%) exhibiting **Type III responses**. Thus, to a first approximation, it would seem that SCC was equally likely irrespective of the type of pressor response—intracranial or systemic—that preceded it. This result was not as expected, since SCC is thought to relate a SNS "storm" after SAH (and ipso facto to a CHR: Part 1, 1.3.2.1). Therefore, the possibility that SCC *could* relate to the acute pressor response was investigated further, with rigour. The results and calculations are shown in (Table 20, Appendix): as is apparent, no statistical significance was demonstrated ($\chi^2 = 1.06$, $p \gg 0.2$). In consequence, any particular **acute pressor response is not predictive of SCC**: SCC is equally as likely whichever acute pressor change occurs. (Note that the total number of animals in Table 20 is 33 and not 34: this is because in one animal the *type* of acute pressor response was not recorded on a pen tracing; nor could it be stored onto the hard disk of the patient monitor).

1.5 CCA re-clamping and SAH Groups 1-3

As described in Part 4, 3.3, the aim of the study was to produce SAH by endovascular filament rupture and, then, to immediately restore normal perfusion by CCA clamp release. This occurred successfully in n = 17 animals (**SAH Group 3**). However, in n = 13 an ICP re-rise occurred following CCA clamp release. Because an ICP re-rise was felt—as a result of previous findings[625]—to represent a re-bleed and, therefore, possibly premature mortality; it was decided to re-clamp the CCA in such cases for a few minutes more in order to ensure haemostasis (the precise period in all cases being less than five minutes: **SAH Group 2**). The over-riding aim, therefore, was to limit study to that of a single primary SAH with full reperfusion. Unfortunately, in n = 4 animals, a further ICP re-rise followed reperfusion—even after 5 minutes of CCA re-clamping. In such cases, the CCA clamp was re-positioned for periods of over 1 hour (**SAH Group 1**): this, effectively, represented *permanent* CCA re-clamping in such cases, because of this group's premature mortality (Table 21, Appendix).

As Table 21, Appendix shows, SAH Group 1 suffered significantly greater mortality

(100%) than either SAH Groups 2 (39%) or 3 (29%) [$\chi^2 = 6.76$, $p<0.05$]. Importantly, the trend clearly suggested that survival improved in the direction Group1 → Group2 → Group3. This is interesting because it is exactly the opposite trend to that implied in the former study from this laboratory[625]. [Note that all animals examined in this thesis could be incorporated into Table 21, Appendix].

The distribution of acute pressor response with CCA re-clamping is shown in Table 22, Appendix. The fact that no statistical difference is demonstrated suggests that the type of acute pressor response observed did not significantly influence the occurrence—or repeated occurrence—of an ICP re-rise upon reperfusion, or vice versa. **NOTE:** the total group number in Table 22, Appendix is 29 and not 34. This is because Table 22 was used only to provide some explanation for PGF2α and NE EC_{50} values as discussed in Part 6, Section II, 3.2 & 3.5. In consequence, only 'acute pressor/CCA re-clamp' values for which myographic data were available were required in this Table. Myography, of course, was only performed in n = 30 animals (Protocols: Part 4, 7.4). However, in one animal, myography was performed without knowledge of the acute *qualitative* pressor response (because the pen recorder tracing was subsequently lost, and because the 'patient monitor' values could not be retrieved from a hard disk). Therefore, the myographic denominator must be reduced from 30 to 29. Because, however, BP values from the 'patient monitor'—as well as intermittent arterial blood gases—confirmed that the latter animal was adequately perfused, its myographic data could still be used elsewhere in the thesis. The χ^2 value was similarly insignificant when n = 33 animals were considered for Table 22 ($p>0.2$).

1.6 Conclusions: implications of 'whole animal response' sub-groups for subsequent small vessel study.

Different sub-groups arose as a result of the acute physiological ictus in this study. Some of these related directly to the ictus itself: for example, animals demonstrating a type I arterial pressor response had significantly lower ICP_{max} values than those demonstrating type II or III responses; whilst approximately 40% animals dying within the arbitary three hour period were distributed across all three 'arterial presssor' groups equally. Other sub-groups arose by virtue of the methodology used: thus, SAH groups 1, 2 and 3 arose solely by virtue of the (somewhat arbitary) CCA-clamp duration. The creation of such sub-groups is important to note because MCA responses may differ significantly between them; and, thus, individually account for overall differences in 'SAH-vessels' from controls. Moreover, in cases where no overall differences are noted in 'SAH-vessels' from controls, isolated sub-group differences (that have thus become 'submerged' when considering

'SAH-vessels' overall) might still prove significantly different from controls, with separate implications for later study with chronic clot lysis and delayed VSM.

SECTION I
Whole animal responses
Chapter 2
Post mortem evidence and extent of subarachnoid haemorrhage

2.1 Confirmation and extent of subarachnoid haemorrhage

The first confirmation of SAH was obtained by the finding of **blood** issuing along the **ICP cannula** from the CM posteriorly (Plate 5). This was never seen in any control animal, nor in any animal prior to SAH induction (Plate 4). Its extent indicated a priori a free communication between anterior and posterior subarachnoid space (SAS) cisterns. The next indication of SAH was the finding of extensive **blood** in the cisterna magna **(CM)** (Plate 7) at the commencement of brain removal. This was also found to extend caudally along the **spinal SAS**. Again, such findings were entirely absent in control animals (Plate 6). Following removal of the brain extensive SAH was often noticed over **convexities** frontally and basally: however, much of this became separated during removal. As a result, the final picture remaining in the **basal cisterns** following complete removal and petri-dish placement, was diminished. Nevertheless, a considerable quantity still remained (Plate 9). This contrasts markedly to the rather anaemic picture obtained in control II brains (Plate 8). (NB all animals in all groups were **exsanguinated** at sacrifice).

It soon became apparent that no meaningful categorization of SAH V_E at post mortem could be realizable in this study. This was because in almost every case the aforementioned findings pertained: i.e. all cases appeared 'severe'. Moreover, in contrast to Veelken et al[625]—but in agreement with Harada et al[236]—**SAH** was **not** found to **lateralize** to **either side**. Most variability was found in the 'final' picture obtained following complete brain removal: however, this was merely because of pial tearing during removal, and subsequent dispersion of V_E as a result. In consequence, it is strongly recommended to not base assessment of V_E upon the '**final**' **picture**, as this may be **under-representative**.

A final assessment of SAH was the thick coating of clot present around the major arteries themselves. However, with saline irrigation and increased confidence with operative technique, MCAs could be as reliably removed with a 'no touch' technique as in sham operative and non-operative controls.

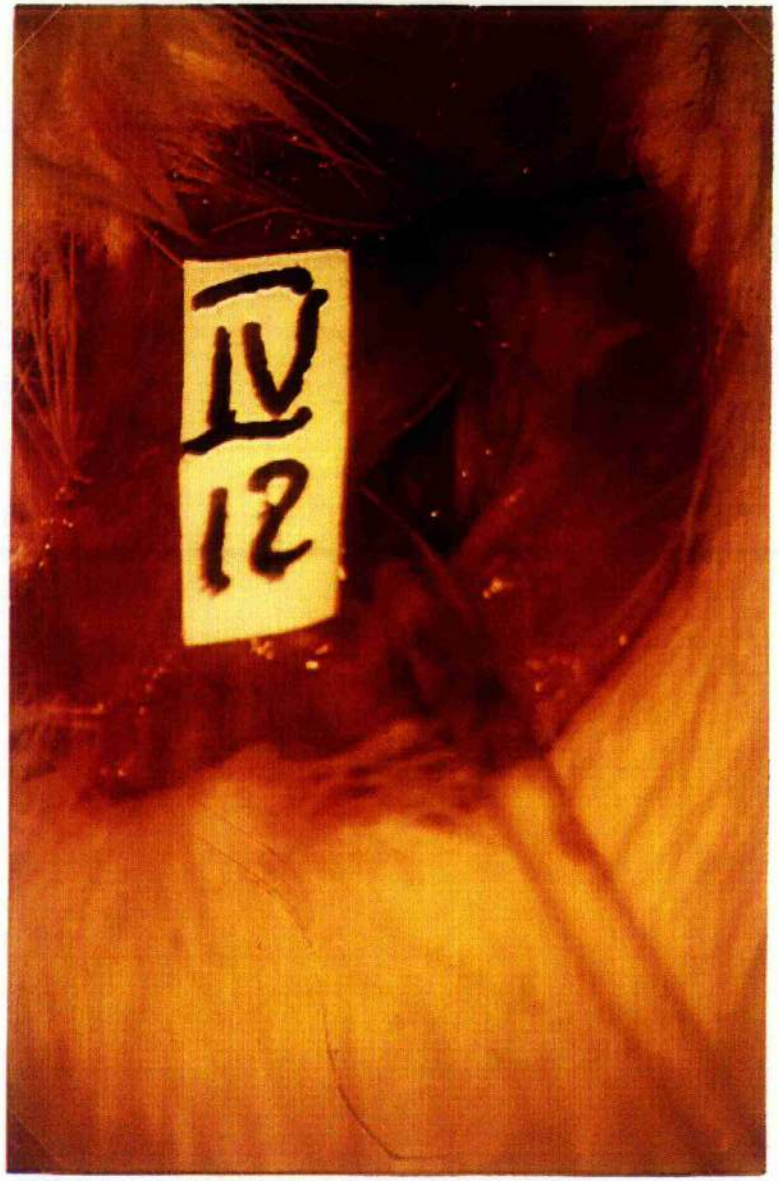


Plate 4. ICP cannula in cisterna magna with clear saline column (sham operative control)

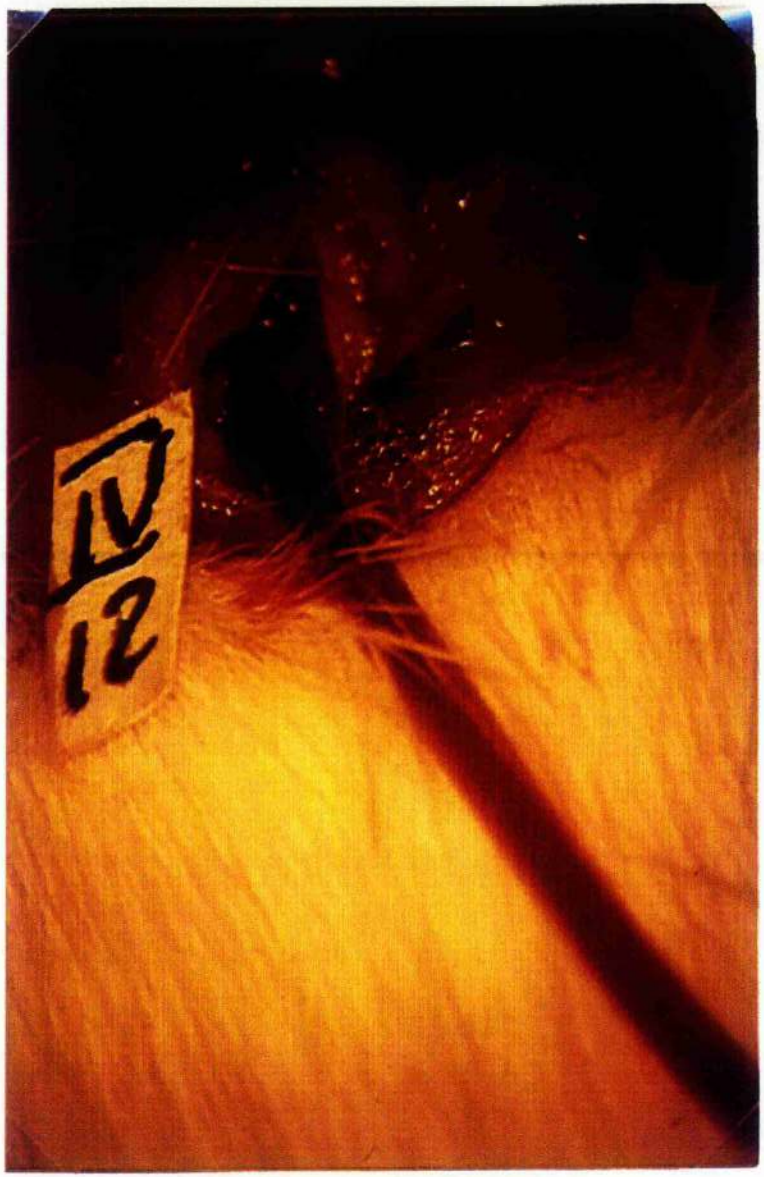


Plate 5. Blood in ICP cannula after SAH

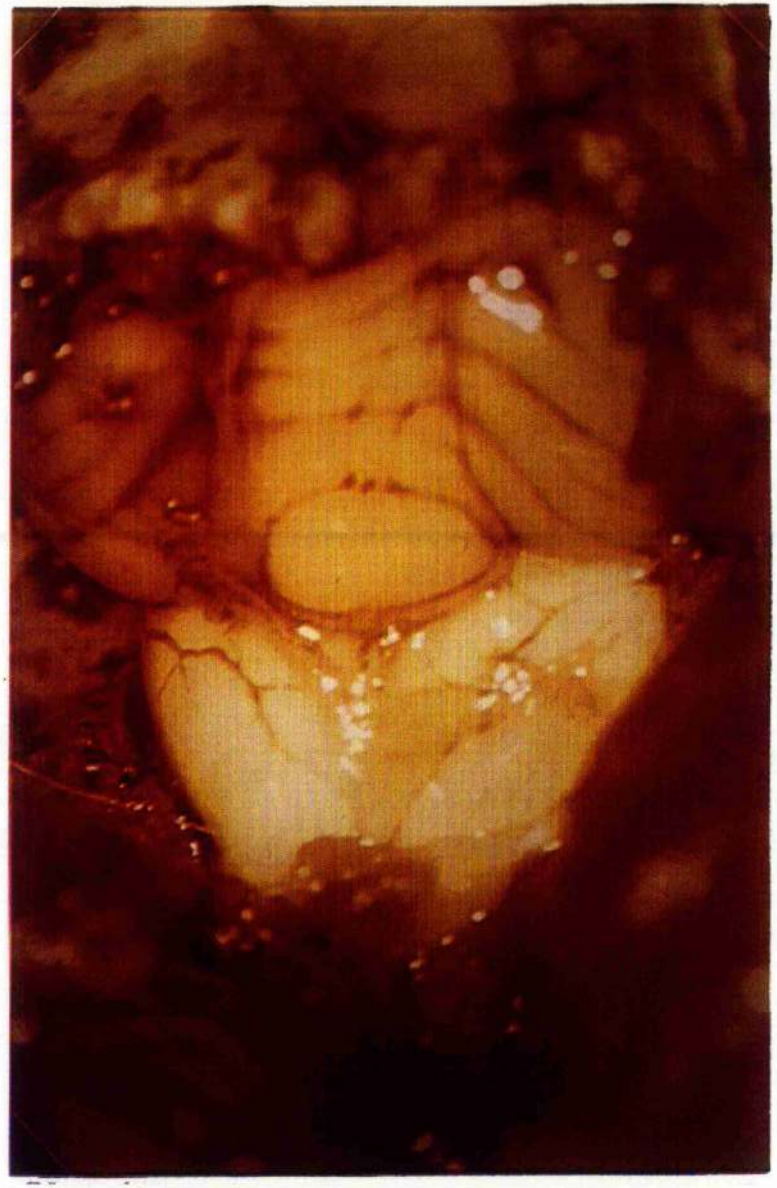


Plate 6. Cisterna magna in sham operative control

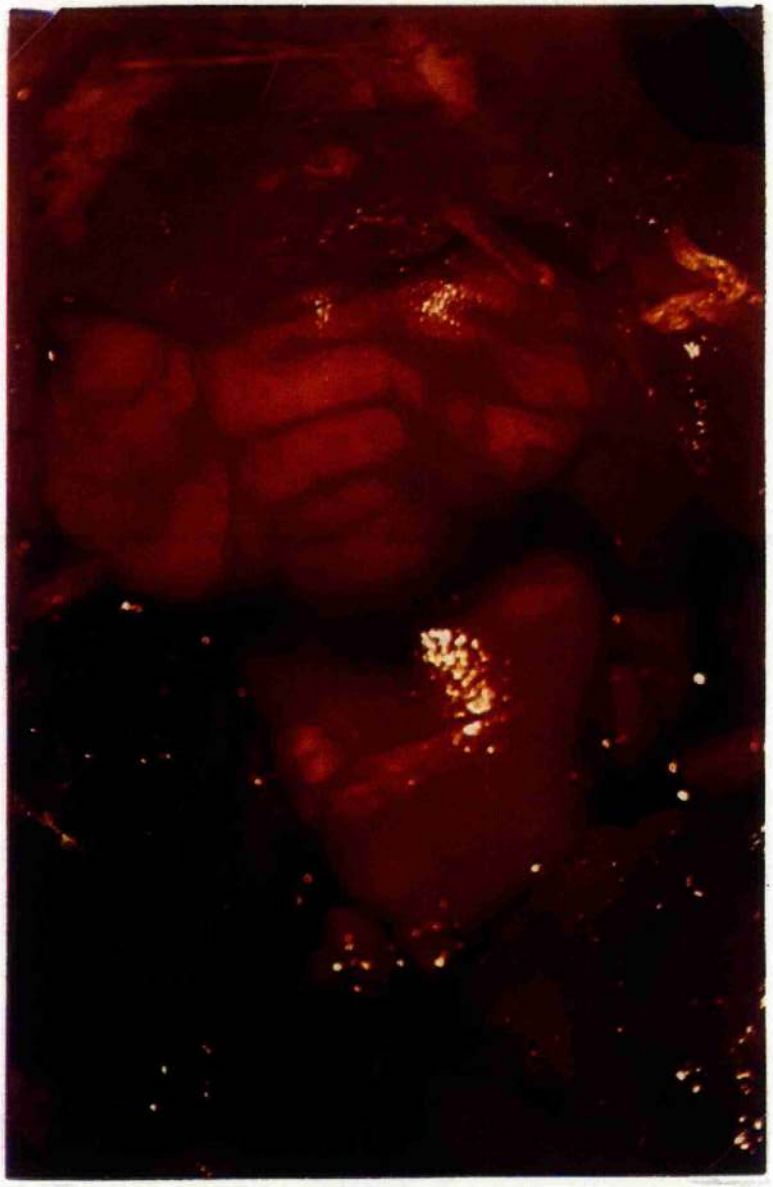


Plate 7. Blood in cisterna magna after SAH

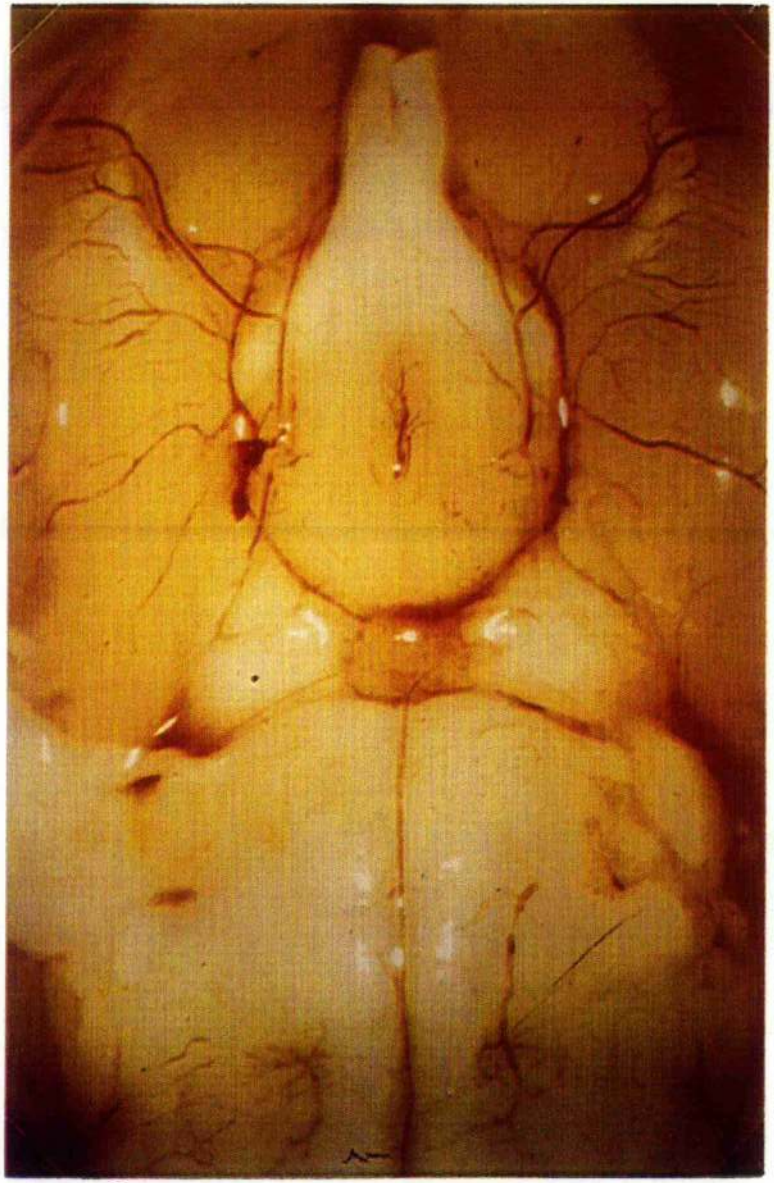


Plate 8. Basal cisterns in sham operative control

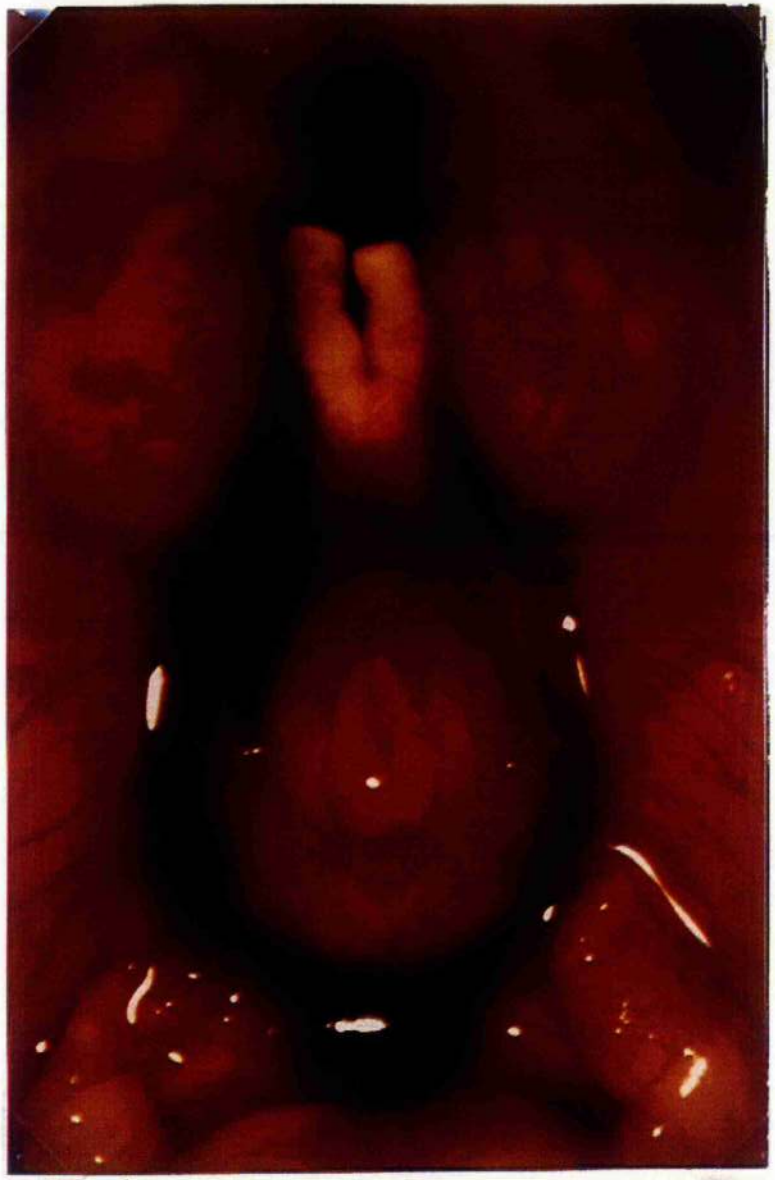


Plate 9. Basal cisterns after SAH

2.2 Position of rupture

The point of rupture can only be determined by leaving the thread in situ until sacrifice, thus directly noting its point of exit from the CoW, as in Veelken et al[625]. This, however, was clearly incompatible with the proposed form of study here: (in the previous study, such a manoeuvre formed part of the study protocol[625]). As stated in Part 4, 3.3, this aspect of the "Sheffield Model" (i.e. with thread left in situ) was abandoned in the present study, partly because of its unphysiological implications; but also because prior studies suggested that it did not minimize either V_E or mortality. From prior studies, however, rupture was confirmed to occur from the ACommA anteriorly. Although the site of rupture could still be approximately ascertained following brain removal by noting a breach in the CoW, extensive clot in the basal cisterns often rendered this difficult. As a result, the precise site of rupture must remain conjectural in the large majority of cases.

2.3 Histopathological examination

Histological analysis confirmed widespread SAH with otherwise normal brain anatomy (not shown). There was sometimes blood found within the ventricular system: although this may have been due to thread-tracking, no evidence for this was found at histology. Pathological analysis of the heart revealed no abnormality, even in those cases suffering a cardiovascular collapse (not shown). The lungs in these cases, however, often showed evidence of pulmonary oedema (not shown).

MCAs appeared architecturally unchanged by either sham operation, acute SAH or prolonged myography. Plates 10 and 11 overleaf demonstrate this with light microscopic power at x100. Thus plate 10 denotes MCAs in control animals that have recently been sacrificed but which have not yet undergone myography. Note the thick IEL. Plate 11, in turn, demonstrates MCAs following SAH and following 12 hours of myography. The apparent discontinuity in the IEL at 6°clock can be shown to be an artefact upon racking the microscope up and down. Results obtained with EM again revealed no abnormality following acute SAH and up to 12 hours myography (not shown).

2.4 Conclusions: implications of SAH extent for subsequent small vessel study

Before this study was undertaken, it was asssumed that differing degrees of SAH severity would be reflected in differing amounts of V_E: clearly, however, the current results refute this beyond doubt. As a result, similar degrees of V_E appeared to result in all animals studied. Furthermore, no lateralization of clot was apparent either. In consequence, 'blood coating' of MCAs was probably equal amongst all animals. This, coupled with the even more important fact that study was restricted to 3 hours post-SAH, implies that MCA

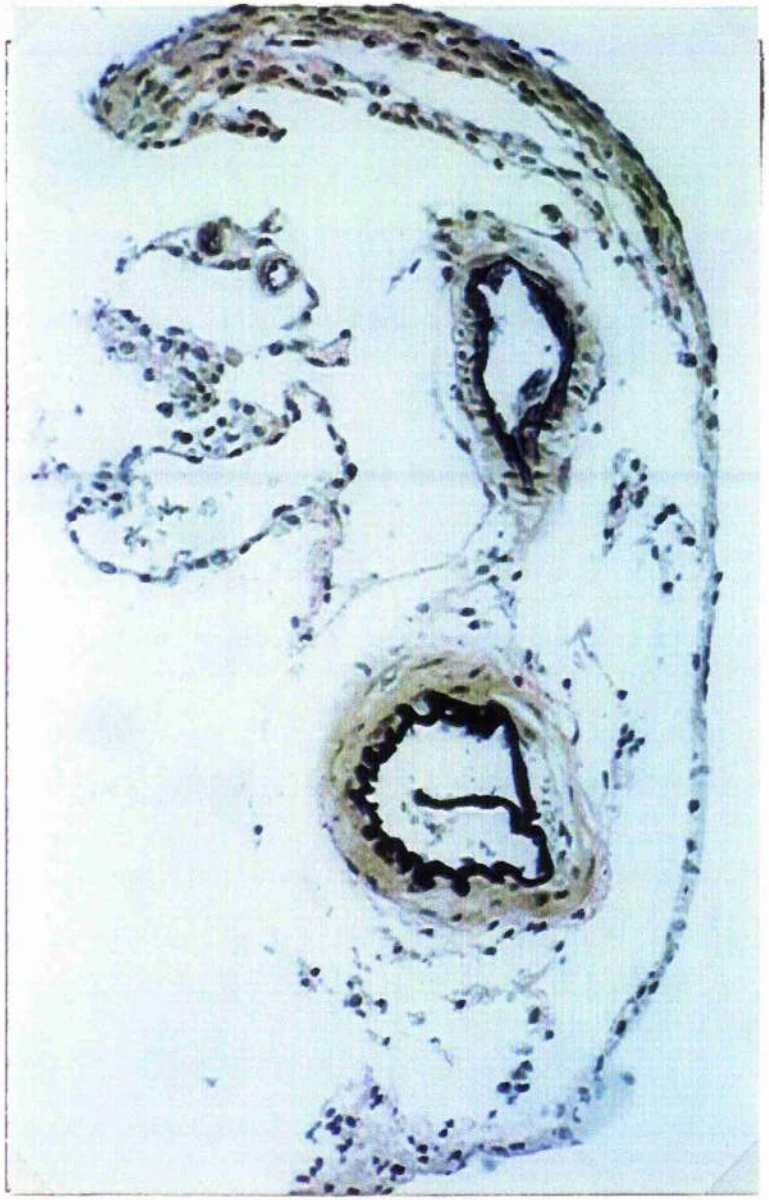


Plate 10. Control MCAs immediately following sacrifice

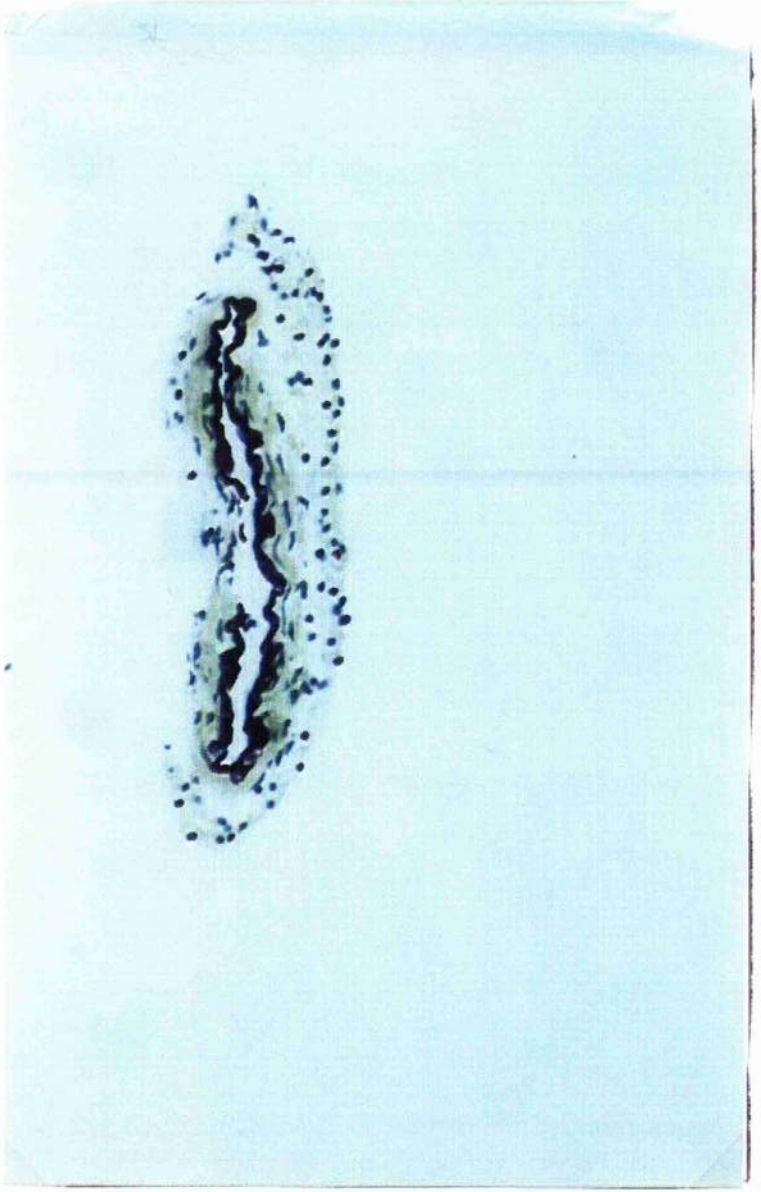


Plate 11. MCAs after SAH and Myography

reactivity study was subsequently restricted overwhelmingly to the effects of the acute ictus (rather than to any significant effects of clot lysis); or that any such 'clot-effects' were equally distributed across all animals.

SECTION II
In vitro vessel responses
Chapter 1
Non-operative controls

1.1 Myogenic tone

Spontaneous myogenic tone (MT) was evidenced in all vessels tested (Fig 12) and in all study groups. It was indicated by **continuous vasomotion** with intraluminal distension that remained throughout the period of myography. It occurred at rates of between 3/min up to 8/min (Fig 12) and is compatible with previous findings[22] (Part 2, 2.2). It strongly suggests that MCAs, at least in rats, behave much as pre-capillary resistance vessels (Part 2, 2.2). However, MT was also indicated by other features found in this study (*see* relevant sections):

- Dilation of pre-constricted tone past baseline (i.e. E_{max} >100%: Part 5, Section II, 1.8 & 1.9; Part 6, Section II, 1.4).
- Direct dilation of baseline tone by hypoxia (Part 5, Section II, 1.13).
- The invariance of the internal diameter to increasing luminal distension (Fig 14).

Thus, increasing distension in MCAs throughout the working range of MAPs (70-150 mmHg) invariably resulted in a corresponding increase of MT to counteract it, rendering functional diameters more or less invariant (Fig 14). This contrasts markedly with the response obtained from pulmonary arteries in their normal pressure range (15-35 mmHg) where MT is absent, and where elastic distension is apparent with increased luminal pressure (Fig 13, n = 13).

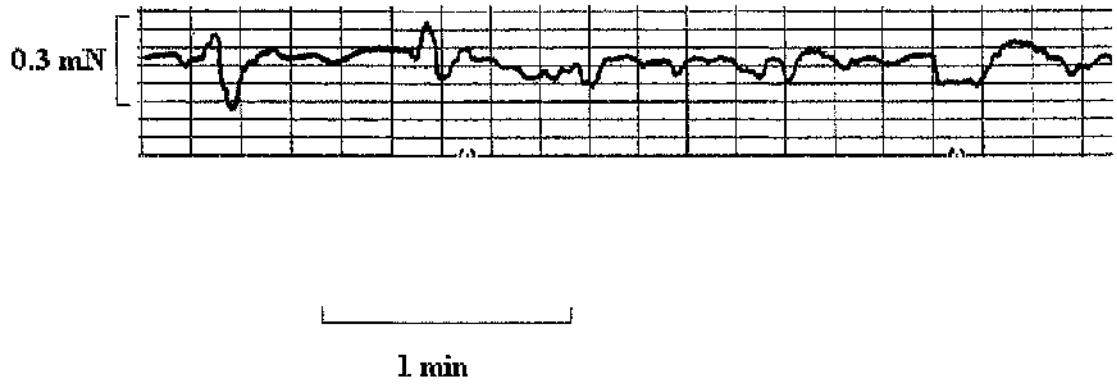


Fig 12. Spontaneous MT in MCA. This may result from pacemaker activity spreading throughout a syncitium of VSMCs (Part 2, 2.2). The rate varies from 3-8/min[22].

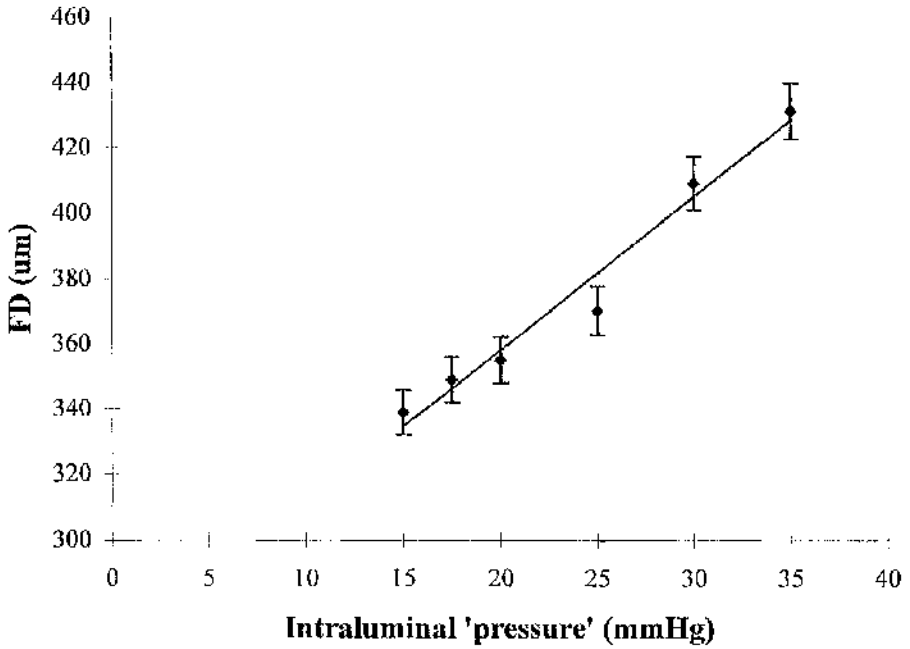


Fig 13. The effect of intraluminal pressure on pulmonary arteries. (n = 13). Clearly, pulmonary arteries distend passively to luminal pressure, behaving as 'Laplace' elastic cylinders. The correlation is highly significant ($r = 0.98$, $p \ll 0.001$). *Results reprinted by permission of Dr J.S. Thompson see Acknowledgements, p 8.*

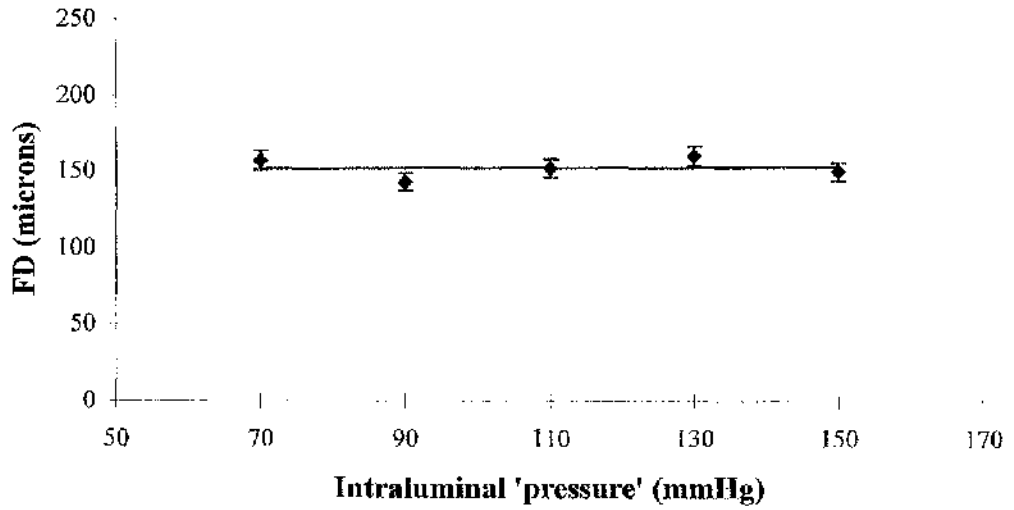


Fig 14. Invariance of functional diameter with increasing intraluminal pressure. MCAs resist distension with active MT (SAH vessels). No correlation is demonstrated between FD and intraluminal pressure. ($n = 27$, $r = 0.072$, $p = 0.79$).

1.2 Functional diameters (FD_{90})

Values were recorded in 39 vessels. The normalized diameters achieved by the programme were referred to as FD_{90} values ('functional diameter at 90 mmHg'). The mean right MCA FD_{90} was 160±31 μm, the mean left 152±51 μm. The overall control group mean FD_{90} was thus 156±42 μm. The range of diameters (71-237 μm) was large and approximated to 50% either side of the mean. However, in n = 10 rats (53%) one vessel was larger than its fellow by at least one standard deviation (SD). Thus, significant **asymmetry** is present in *individual rat* MCAs.

1.3. KCl responses

KCl depolarizing constrictions produced typical phasic and tonic components (Fig 15). As the tonic component proved quite variable, only phasic response was considered. Responses were recorded in n = 34 vessels. The KCl responses (Fig 16) exhibited wide variance with an E_{max} of 5.5±0.3 mN. Thus, the frequency histogram only approximately reflects that of a normal distribution (Fig 16), this being due to significant numbers deviating from the group mean E_{max}.

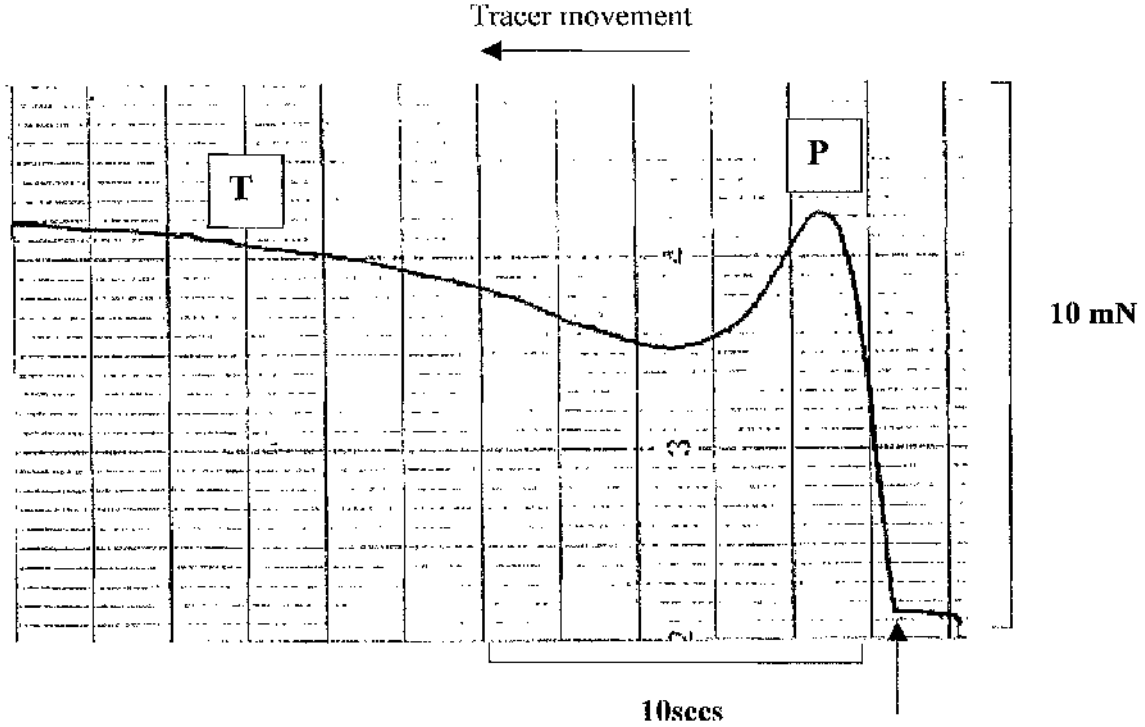


Fig 15. Phasic (P) and tonic (T) KCl constrictions. NOTE: tracing moves from right to left. Vertical arrow defines when KCl was added to the bath.

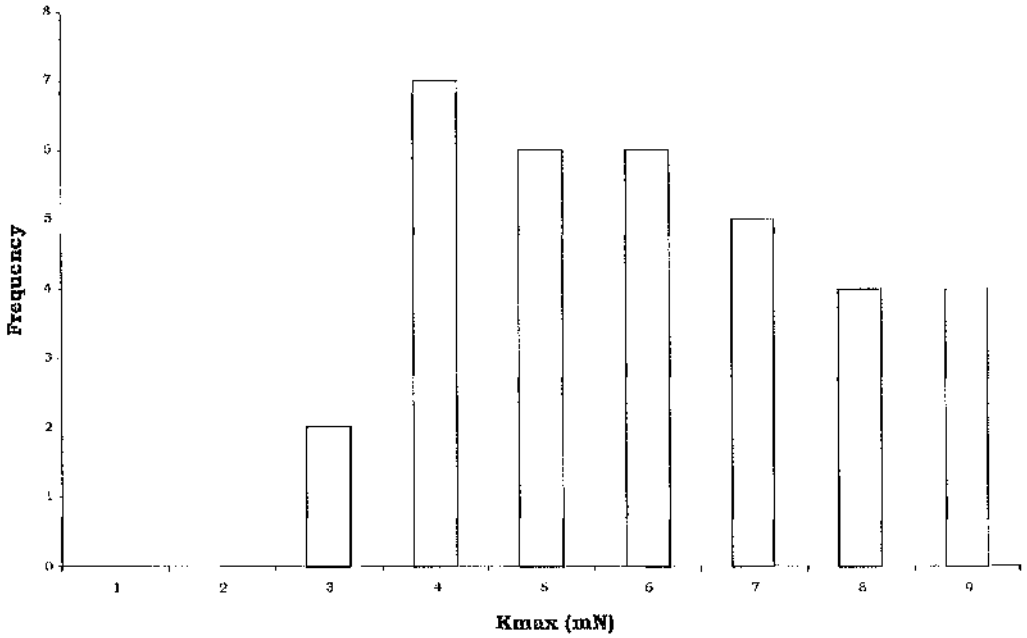


Fig 16. Control K_{max} frequency histogram (Non-operative controls). This only vaguely corresponds to a normal distribution. However, it is symmetrical, with similar mean (5.5 mN) and median (5.4 mN) values.

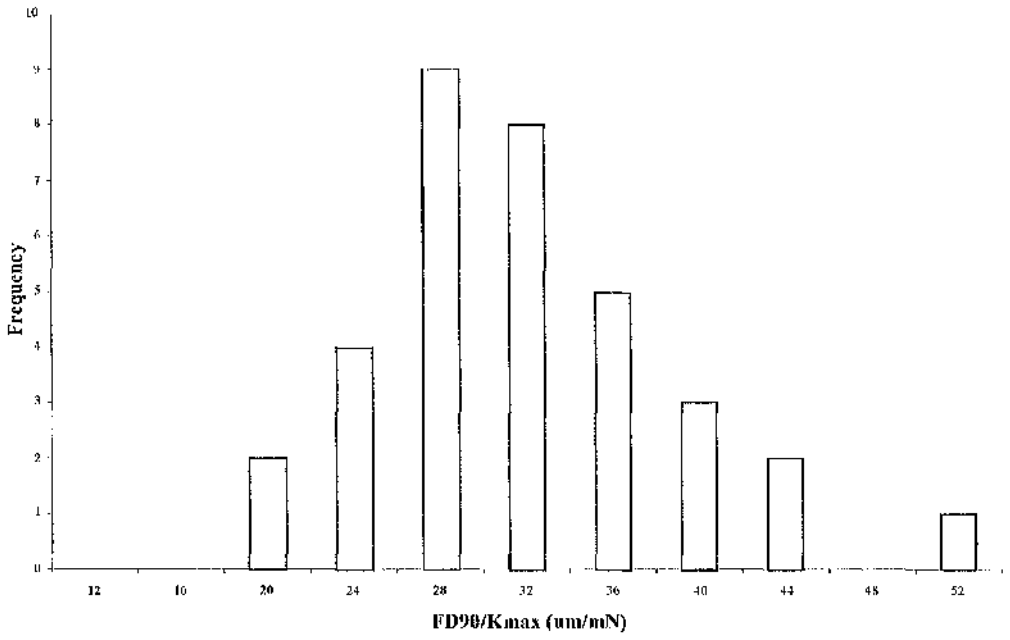
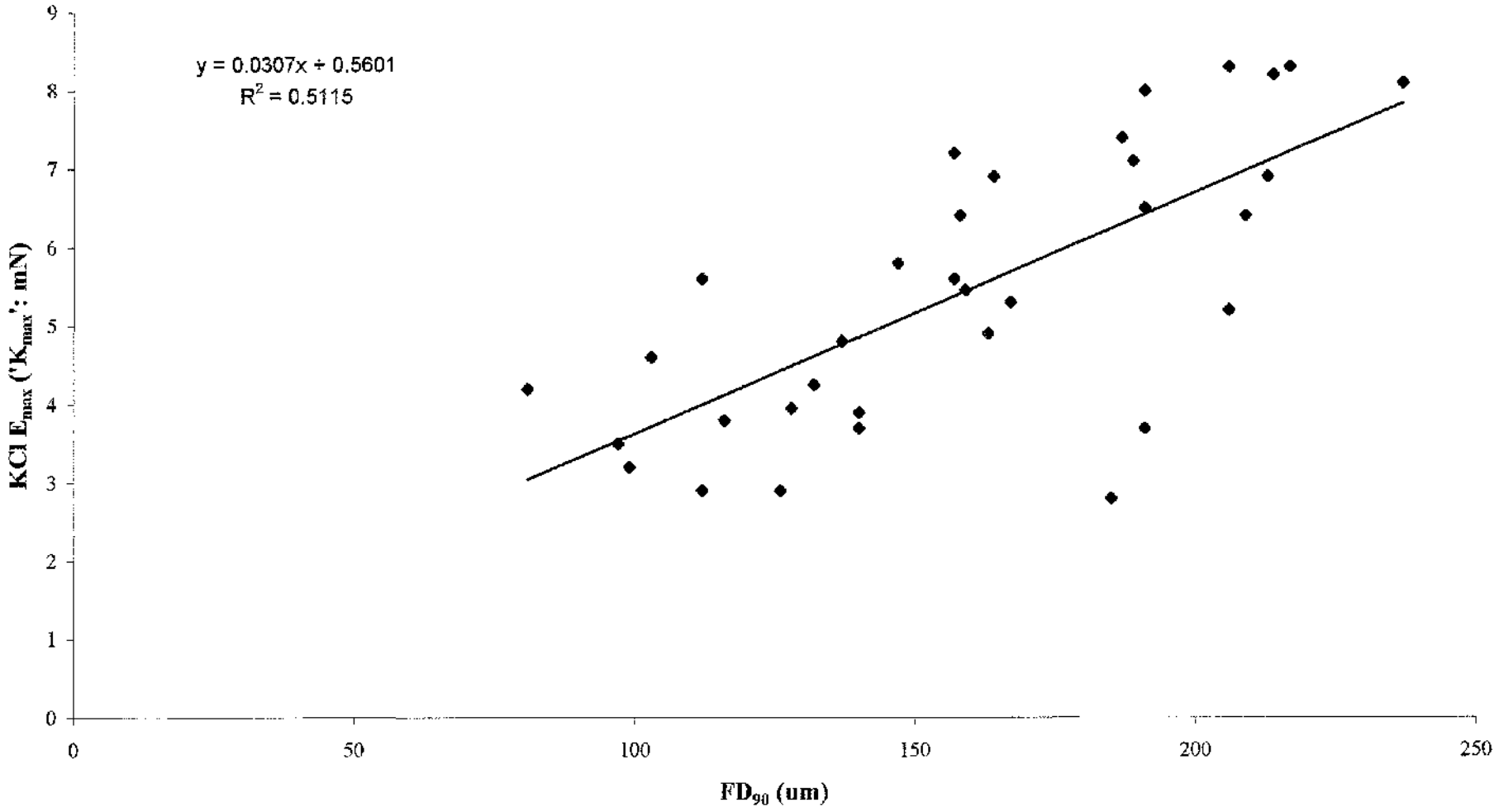


Fig 17. Transformed Control K_{max} frequency histogram (Non-operative controls). The transformation FD_{90} / K_{max} enhances the normalization. This suggests that FD_{90} significantly influences K_{max}.

Fig 18. **K_{max} v FD_{90}.** More than half the variance in K_{max} can be attributable to variance in FD_{90}



It was felt, however, that a large factor involved here was the *size* of the MCA concerned. This was borne out by relating the K_{max} to the individual FD_{90} (Fig 17). Thus, the transformed FD_{90}/K_{max} more closely resembles a normal distribution. The diameter influence was further confirmed by the regression plot of K_{max} v FD_{90} (Fig 18): here the correlation was highly significant with $r = 0.75$ ($p<0.001$: Table 31, Appendix). In fact, a linear E_{max} v FD_{90} pattern pertained, to greater or lesser degrees, with all other contractile agonists tested in this thesis (*see below*).

1.4 PGF2α responses

The PGF2α-induced constriction was distinct from the KCl response. Thus, the slope of tension-rise was more gradual, and an acute phasic response was conspicuously absent. Furthermore, the contraction displayed **continuous vasomotion** throughout its ascent (Fig 19). Responses were recorded in n = 22 MCAs. The E_{max} was 5.1±0.4 mN and the EC_{50} 6.4±1.0 μM. As with KCl responses, variance in PGF2α E_{max} appeared to reflect variance in FD_{90}. This was subsequently confirmed by a linear $PGF2\alpha_{max}$ v FD_{90} regression plot (Fig 20: $r = 0.45$, $p<0.05$: Table 31, Appendix).

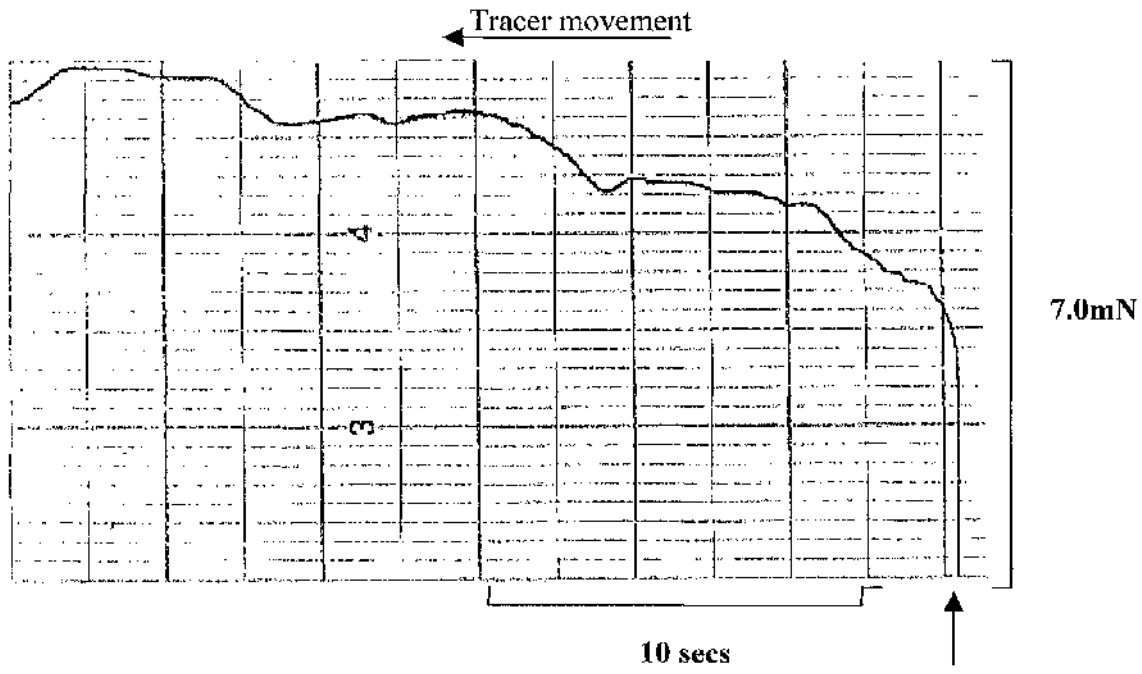
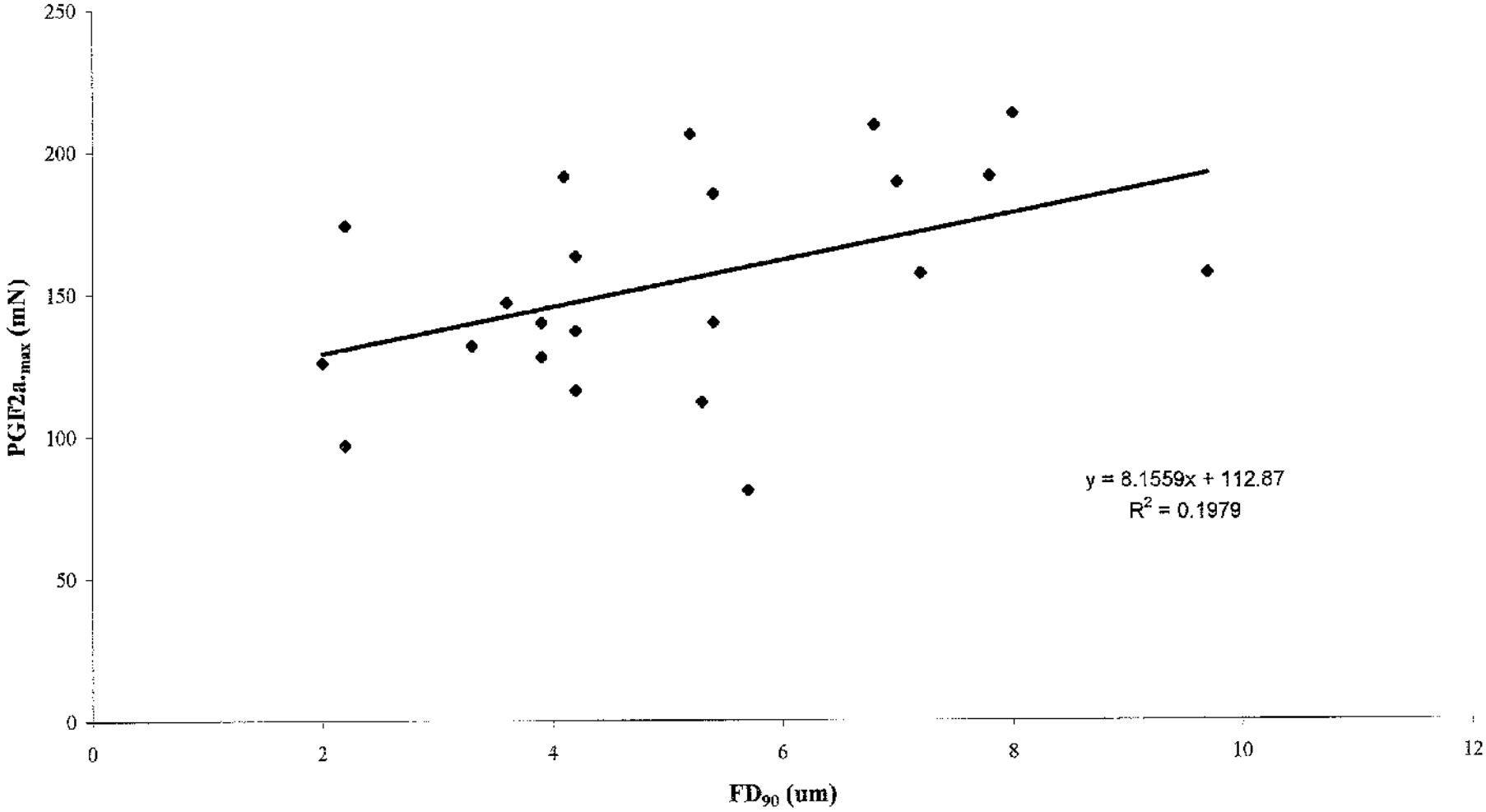


Fig 19. The PGF2α-induced constriction. Note the absence of the phasic component and continued vasomotion. Vertical arrow indicates when PGF2α was administered.

Fig 20. $PGF2a._{max}$ v FD_{90}.



1.5 5HT responses

Responses were recorded in n = 24 vessels. The contractile response demonstrated phasic and tonic components that more resembled KCl than PGF2α. However, the tension-rise was slower, and the E_{max} (4.4±0.3 mN) less efficacious than KCl (p = 0.019). Again, a linear relationship between E_{max} and FD_{90} was apparent (Fig 21): however, the correlation was less close than with KCl (r = 0.523, p<0.02: Table 31, Appendix). Thus, less than 30% of the E_{max} variance could be attributable to diameter variance with 5HT. This suggested a degree of **heterogeneity** in receptor/second-messenger activity also found with PGF2α for which the wide diversity of receptors mediating the effects of one agent (Part 3, 1.4) could have possibly accounted for this. The EC_{50} was 0.09±0.02 μM: this value is several orders of magnitude lower than that of any other agonist used. 5HT responses are therefore especially **potent** in rat MCAs.

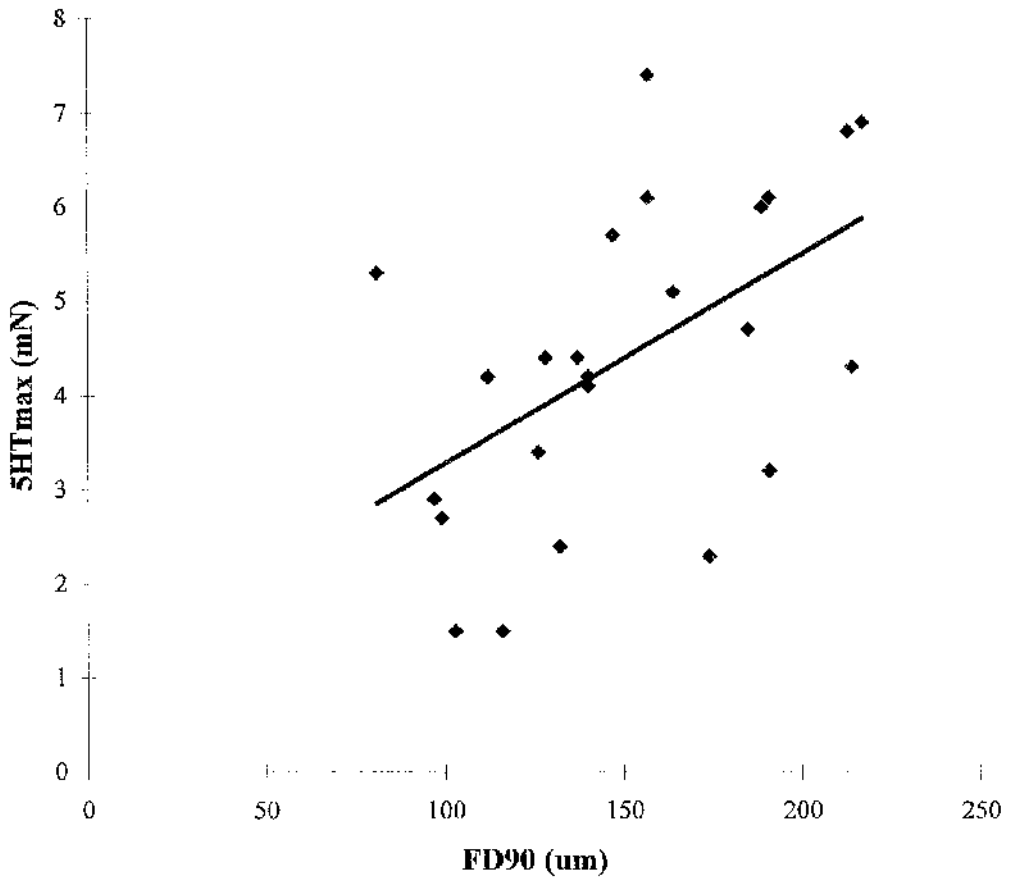


Fig 21. Non-operative controls 5HT E_{max} v FD_{90}. The linear association is much less exact than with KCl, although the correlation remains significant (r = 0.523, p<0.02). The regression equation obeys E_{max} = (0.022). FD_{90} + 1.06. The r^2 value of 0.27 indicates that only 27% of the variance in E_{max} can be accounted for by variance in FD_{90}.

1.6. UTP responses

Responses were obtained in n = 17 MCAs. The contractile response demonstrated phasic and tonic components that more closely resembled KCl than PGF2α. The UTP E_{max} was 3.4±0.4 mN; this was significantly weaker than the control K_{max} (p = 0.0001). A considerably poorer linear relationship between E_{max} and FD_{90} was also apparent with UTP (Fig 22: $r = 0.31$, p>0.10). The poor linearity for UTP responses was distinctly at variance with all other contractile agonists used; as well as from UTP responses in shams and after SAH (Table 31, Appendix). The EC_{50} was 16.4±2.8 μM: this value was an order of magnitude greater than that of any other agonist used. UTP responses are therefore much **less potent** than other agonists in rat MCAs.

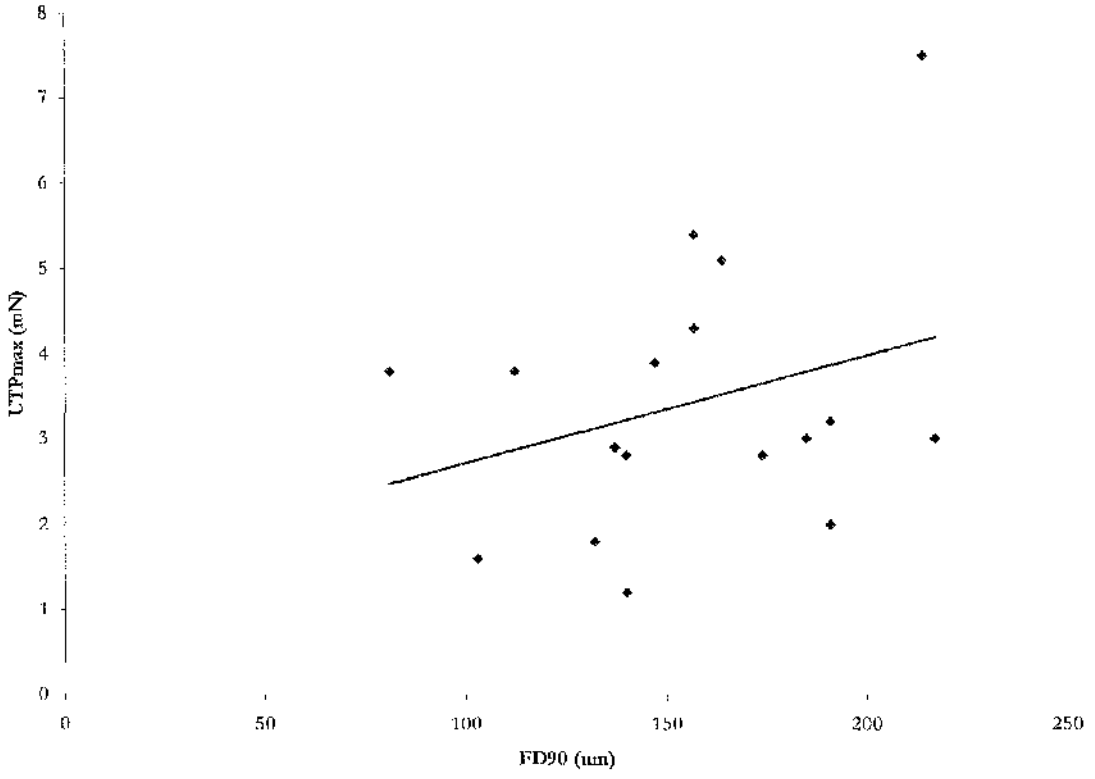


Fig 22. Non-operative controls UTP_{max} v FD_{90}. The linear relationship and correlation is poor ($r = 0.31$), and not statistically significant (p>0.10). This was the only example in the thesis where a contractile E_{max} failed to attain a significant correlation with FD_{90}; other than NE (uncorrected for 'subresponders': *see* below).

1.7 NE responses

NE responses were *attempted* in n = 34 MCAs. The greater scatter apparent with 5HT and UTP—in contrast to that with KCl or PGF2α—was greater still with NE. In fact, no clear linear relationship was initially apparent on the NE_{max} v FD_{90} plot, with only 10% of the NE_{max} variance being possibly attributable to diameter variance. Clearly, receptor-mediated factors played a greater role here than 'muscle-bulk' effects. However, a closer look at the plot revealed that many responses clustered in the range 0-1 mN: implying a discontinuous distribution (Fig 23). Such values were, in reality, too close to baseline vasomotion to be reliably quantifiable in any case (Fig 12). Furthermore, corresponding contractions to other agonists were invariably satisfactory (e.g. Fig 24): thus, **NE responses** were **specifically poor** in these MCAs.

It was therefore decided to separately classify **NE_{max} values ≤1.0 mN** as NE-**'subresponders'** and, thus, to distinguish them from NE-**'responders'** (whose values were distributed normally and continuously). 'Subresponders' were therefore excluded from analysis of continuous data, and instead analyzed as **categorical data.** Upon exclusion of 'subresponders', a linear relationship once again pertained between 'responder' NE_{max} and FD_{90} (r = 0.62, p<0.01: Figs 23; Table 31, Appendix). The NE_{max} in 'responders' was 3.2±0.3 mN (n = 26): this was significantly weaker than either the KCl E_{max} (p = 0.0001: Fig 24) or the PGF2α E_{max} (p = 0.0006). The EC_{50} in 'responders' was 2.4±1.0 μM.

1.7.1 NE-'responders' (Table 10, Appendix)

These were defined as NE_{max} ≥1.1 mN. A total of 26/34 (77%) MCAs showed an adequate NE response. Because in n = 2 animals, one MCA was *generally* unresponsive to all agonists contralateral to a specifically NE-subresponsive MCA (and so excluded from study: *see* Part 4, 7.4), the denominator over which responses are considered must be reduced from 18 to 16. Thus, 77% MCAs showed a response in 14/16 (88%) animals. NE responses were obtained bilaterally in 12/16 (75%) animals. NE responses were therefore recordably asymmetrical (i.e. a 'subresponder' contralateral to a 'responder') in 2/16 (12.5%) animals.

1.7.2 NE-'subresponders' (Table 10, Appendix)

These were defined as NE_{max} <1.1 mN. A total of 8/34 (24 %) MCAs were subresponsive to NE in 6/18 (33%) animals. (The denominator is restored to its 'normal' value here because the two animals with a generally unresponsive MCA contained a *specifically*

Fig 23. NE_{max} **v** FD_{90}. NE_{max}<1.1mN ('subresponders') are distinguished from NE_{max}>1.1mN ('responders')

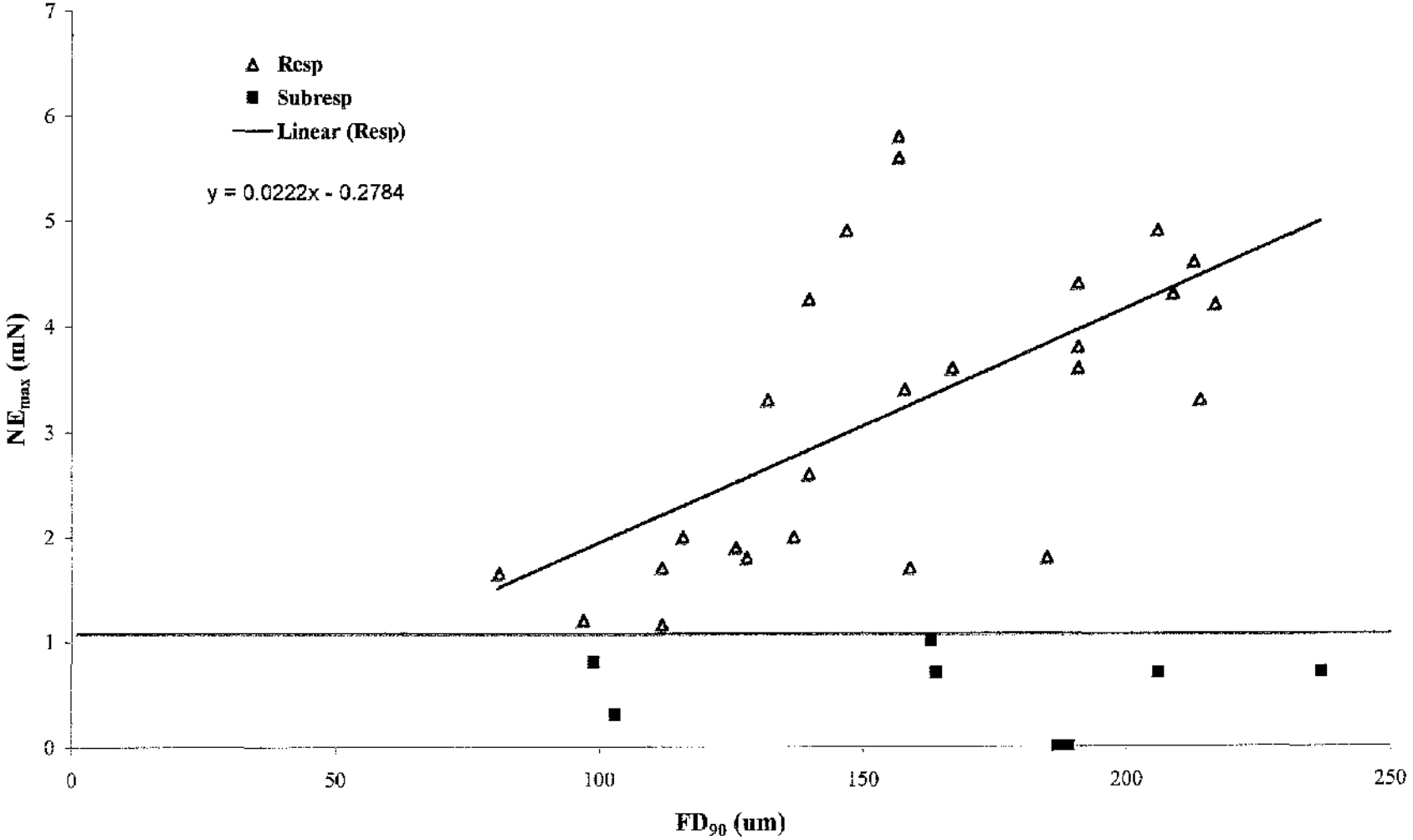
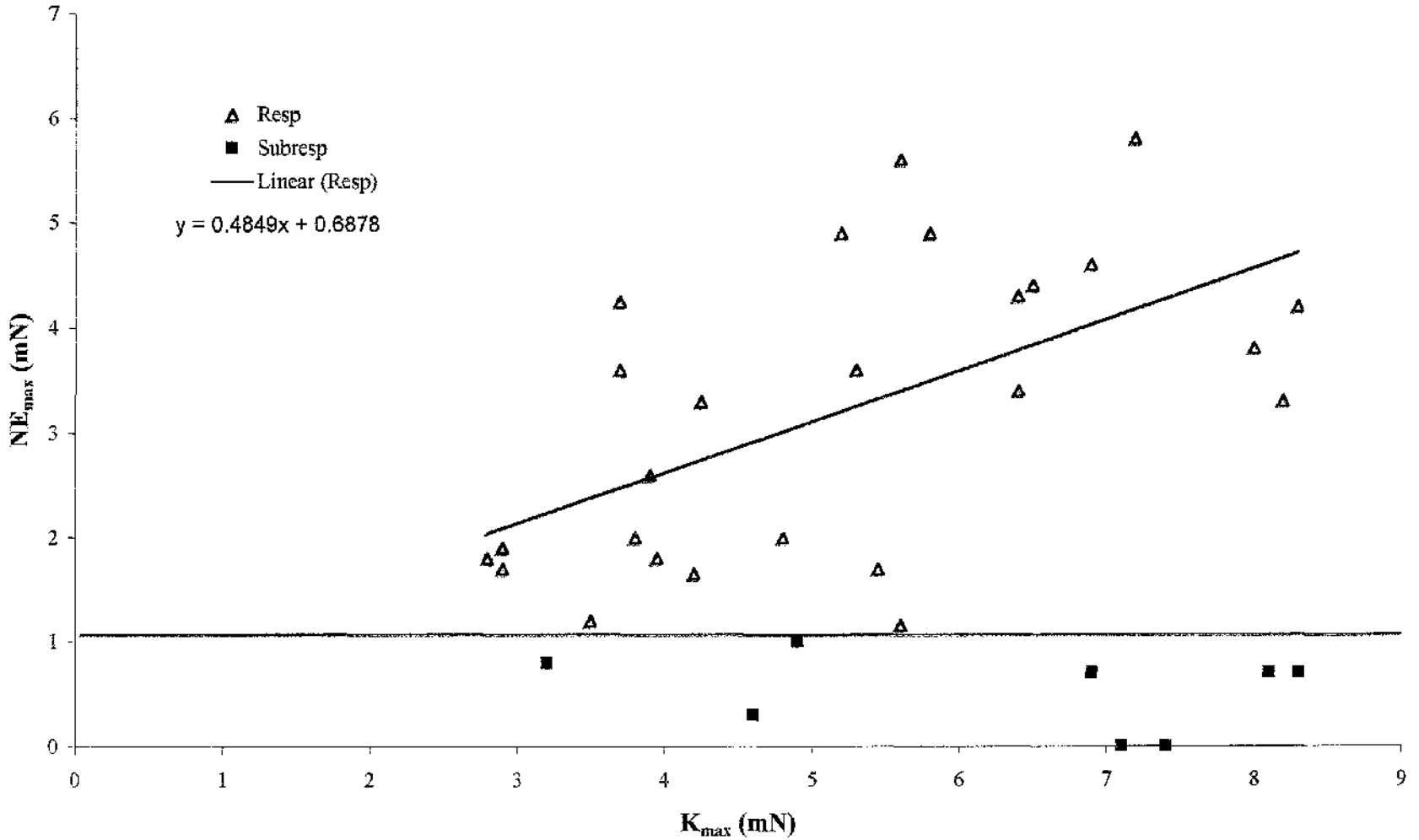


Fig 24. **NE_{max} v K_{max}.** NE 'subresponders' (NE_{max}<1.1 mN) show correspondingly adequate K_{max} values (K_{max} >3.0 mN)



subresponsive MCA to NE). NE-'subresponders' were obtained bilaterally in 2/16 (12.5%) animals. NE responses were recordably asymmetrical (i.e. one 'subresponder' contralateral to a 'responder') in 2/16 (12.5%) animals, because in n = 2 animals, one vessel from each was excluded from analysis (Part 4, 6.5 & 7.4; Table 10, Appendix).

1.8 HA responses

Responses were recorded in n = 23 MCAs. HA was a potent and efficacious dilator of MCAs with an E_{max} of 102±5 % and an EC_{50} of 2.3±0.6 μM. The vasodilation achieved often exceeded baseline values (i.e. E_{max} >100%, n = 12, 52%): this can be appreciated in the right hand tail of the frequency distribution shown in Fig 25. Thus, HA must frequently dilate MT in addition to AT because it relaxes more vessel tone than the applied agonist had been responsible for.

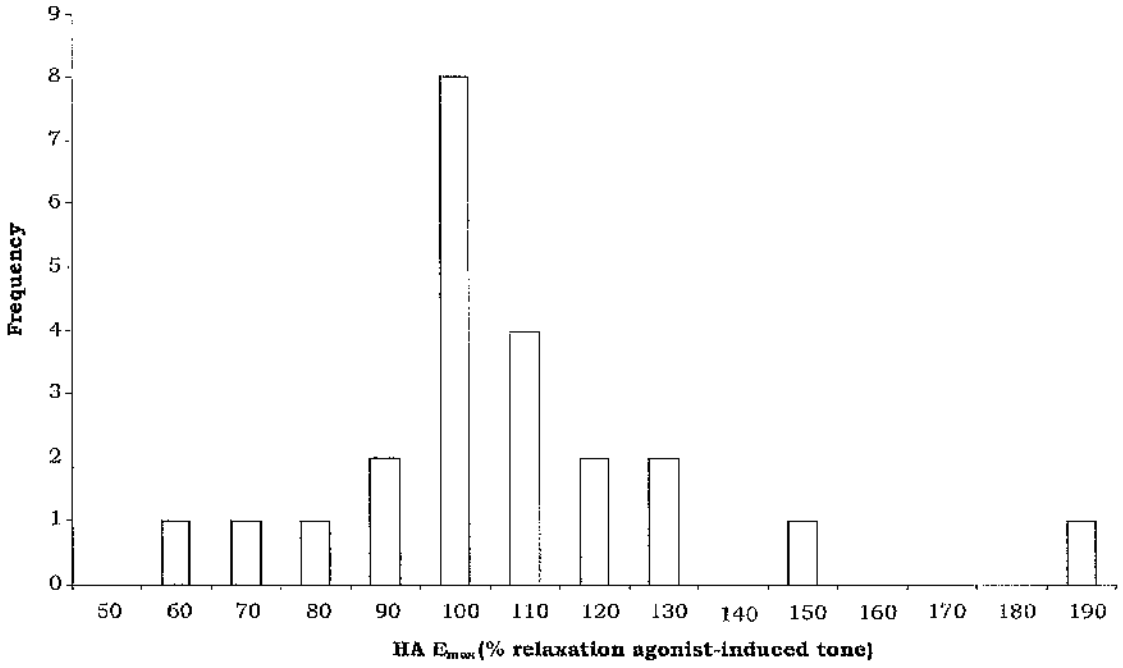


Fig 25. Non-operative controls HA E_{max} Frequency histogram. Because the mean is centred on HA E_{max} = 100% (i.e. complete relaxation of AT), the right hand tail must reflect additional dilation of background MT. In two extreme cases, the MT dilated was equivalent to 50 and 90% that of the AT induced—i.e. such MCAs were effectively paralyzed.

As more than one receptor may be involved (Part 3, 1.11), it was important to identify the major receptor of action. Following pre-constriction with PGF2α, 200 μM ranitidine was added to the bath. The resultant HA C-R curve (n = 18 vessels) shows that HA relaxation of AT was severely obtunded (Fig 26). Thus, the E_{max} obtained following HA2 blockade was 18±9%: the HA2 receptor therefore mediates approximately 82% of the HA response.

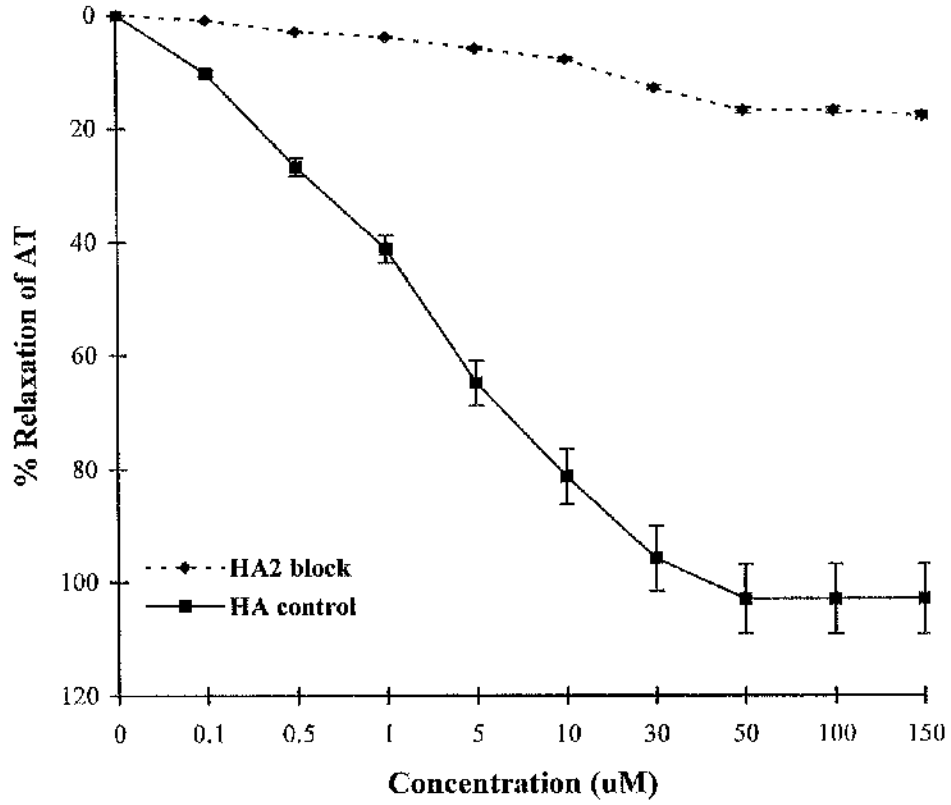


Fig 26. The effect of HA2 blockade (200 μM ranitidine) on MCA HA response in n = 18 vessels (Non-operative controls). The HA2 receptor appears to mediate approximately 82% of the HA response in rat MCAs.

1.9 PPV responses

Responses were recorded in n = 22 vessels. PPV, like HA, was a potent and efficacious dilator of MCAs. Thus, the E_{max} was 106±9%, and the EC_{50} 2.1±0.6 μM. As with HA, PPV frequently dilated MCAs past baseline tone (i.e. E_{max} >100%), indicating dilation of MT (Fig 40; Part 5, Section II). The C-R curve is presented on the combined group graph in Fig 39. Frequent abnormalities in the C-R curve were observed in individual MCAs, these occurring at the 50 μM level and consisting of a transient loss of relaxation (Fig 41).

1.10 L-ARG and L-NAME responses

Responses were recorded in n = 23 (L-ARG) and n = 8 (L-NAME) MCAs respectively. The L-ARG E_{max} was 72±4 % and the EC_{50} 5.0±1.3 μM. The C-R curve can be visualized in the combined graph of Fig 44 (Part 5, Section II). As the combined control frequency distribution shows in Fig 43, L-ARG rarely dilated baseline MT (i.e. invariably $E_{max} \leq 100\%$). The L-NAME E_{max} was 1.3±0.4 mN: no EC_{50} value was attempted. Thus 'tonic release' of NO with PGF2α-induced AT has 'basally' reduced AT from 6.4 mN (i.e. 5.1 + 1.3) to 5.1 mN [*see* PGF2α E_{max} and L-NAME E_{max} values, Table 28, Appendix]. From this we can derive the % maximal NO release that 'basal NO release' represents. Thus, L-ARG maximally relaxes AT from 5.1 by 3.67 mN to 1.4 mN (i.e. 72% relaxation—the mean L-ARG E_{max}). But we know that 'basal release' had already reduced AT by 1.3 mN *prior* to any L-ARG. Therefore, basal release represents $\frac{1.3}{(1.3+3.67)} \times 100\%$ or 26 %. That is, **basal NO release** with AT represents **26%** of the **total NO release possible.**

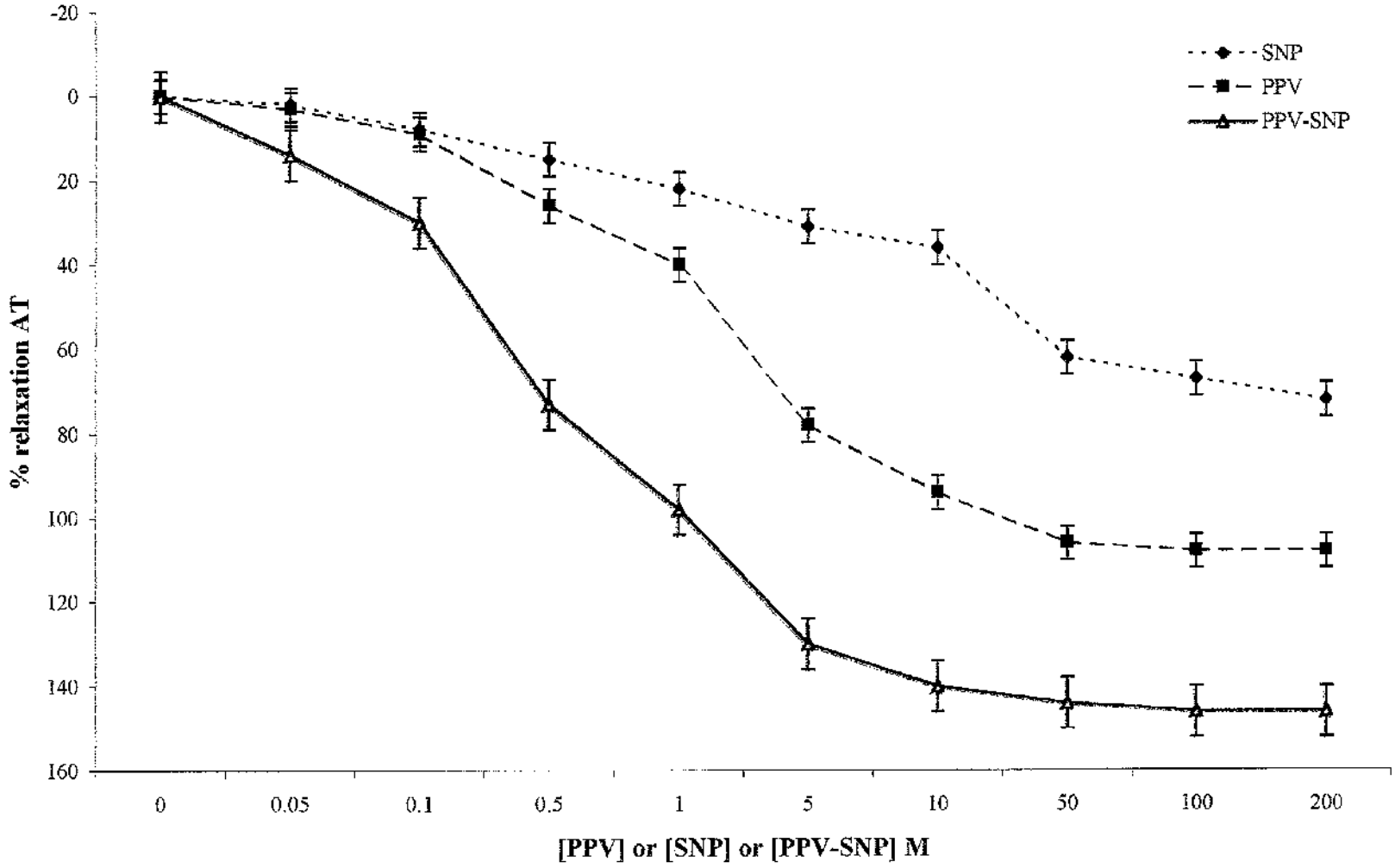
1.11 SNP responses

Responses were recorded in n = 24 vessels. The E_{max} was 72±4% and the EC_{50} 10.7±4.3 μ M. The C-R curve is presented on the combined group graph in Fig 46. SNP, like L-ARG, clearly acts mainly upon AT: i.e. SNP rarely dilates >100% AT.

1.12 PPV-SNP interaction

Because of the theoretical possibility of an interaction between PPV and SNP, and because of their occasional co-administration at aneurysm surgery, the combination of PPV and SNP was performed in n = 17 MCAs. As a result, the C-R curve (Fig 27) was significantly left-shifted with an E_{max} of 146±12 % and an EC_{50} of 0.48±0.06 μM. Both values differed significantly from either PPV or SNP values alone ($p = 0.01$ and $p = 0.014$ respectively). Therefore, the significant degree of MT dilation (i.e. E_{max} >100%) that PPV achieves alone is significantly enhanced by its co-administration with SNP, thus supporting a possible interaction between the two agents.

Fig 27. Non-operative controls PPV-SNP interaction



1.13 Hypoxia

Hypoxic responses were only recorded in non-operative control MCAs. Here dilation was achieved by replacing the 95%O_2/5%CO_2 mixture in the myograph bath by 95%N_2-5%CO_2. This was performed for 15-20 mins at the end of the study period in order to obviate metabolic exhaustion of the MCA concerned. Since there had been no prior addition of PGF2α, these relaxations were achieved against a background only of MT. Responses were obtained in n = 14 MCAs: the mean relaxation produced was 3.6±0.6 mN. Thus, the total tone possible in normal MCAs is 3.6 + 5.5 mN (i.e.[hypoxic dilation]mN + [KCl E_{max}]mN), which is 9.1 mN. As a result, **MT** therefore **represents** (3.6 x 100)/(9.1) = **40%** of the **total tone possible**.

SECTION II
In vitro vessel responses
Chapter 2
Sham operative controls

2.1 Functional diameters (FD_{90})

Values were recorded in n = 16 MCAs. The mean sham MCA FD_{90} was 157±41(SD) μm which strikingly resembled the control value (156±42 μm). As with non-operative controls, a significant degree of asymmetry was apparent in MCAs from individual animals (not shown). Thus, in 4/8 (50%) of animals the right MCA FD_{90} differed from the left by at least one overall group SD (i.e. approximately 40 μm): this proportion did not differ significantly from that in non-operative controls. Nevertheless, no significant overall group mean right:left side difference in FD_{90} was apparent (Table 23, Appendix), suggesting that unilateral CCA manipulation and thread insertion were without ipsilateral effect.

2.2 KCl responses

Responses were recorded in n = 16 vessels. The mean KCl E_{max} was 5.9±0.5 mN which did not differ significantly from non-operative controls. As with non-operative controls, a strong linear relationship was apparent between E_{max} and FD_{90} (r = 0.78, $p<0.001$: Fig 28; Table 31, Appendix). No significant right:left side difference was apparent in either E_{max} or EC_{50}, suggesting that unilateral CCA manipulation and thread insertion were without ipsilateral effect (Table 23, Appendix).

2.3 PGF2α responses

Responses were recorded in n = 16 vessels. The PGF2α E_{max} was 5.9±0.6 mN and the EC_{50} 6.2±1.1 μM. Neither value significantly differed from that in non-operative controls. A significant linear relationship was again apparent between E_{max} and FD_{90} (r = 0.87, $p<0.001$: Table 31, Appendix). No significant right:left side difference was apparent in either E_{max} or EC_{50} value, suggesting that unilateral CCA manipulation and thread insertion were without ipsilateral effect (Table 23, Appendix). The C-R curve for PGF2α is presented in the combination graph of Fig 33.

2.4 5HT responses

Responses were recorded in n = 16 vessels. The E_{max} was 5.4±0.7 mN and the EC_{50} 0.08±0.02 μM. Neither value was significantly different from that obtained in non-operative controls.

As with non-operative controls, a linear relationship was apparent between E_{max} and FD_{90}, this being less strong than with PGF2α or KCl ($r = 0.64$, $p<0.01$: Table 31, Appendix). The C-R curve is presented in the combination graph of Fig 35. No significant right:left side difference was apparent in either E_{max} or EC_{50} value, suggesting that unilateral CCA manipulation and thread insertion were without ipsilateral effect (Table 23, Appendix).

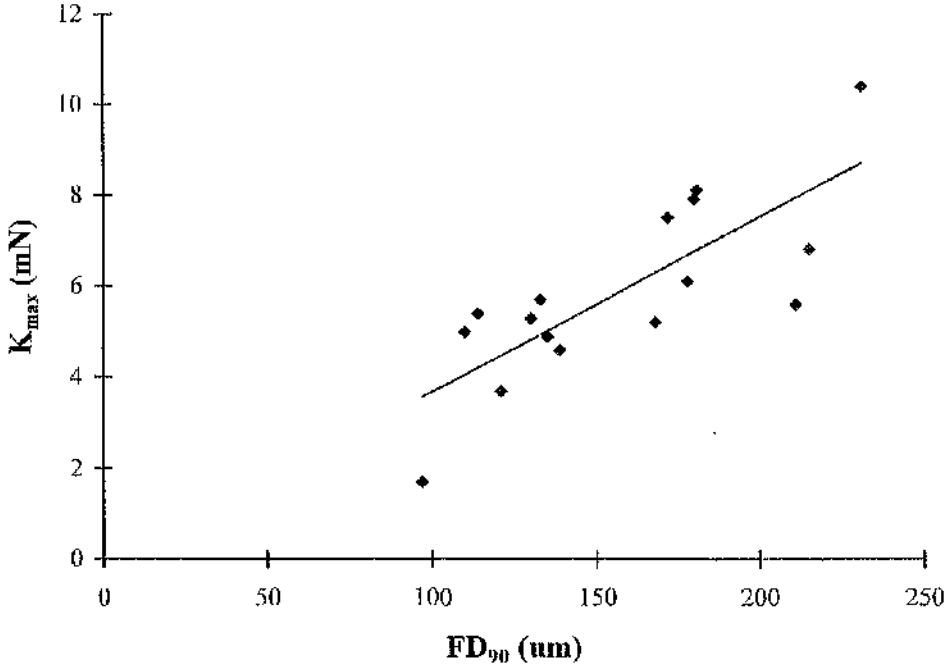


Fig 28. Sham operative control K_{max} v FD_{90}. Once again the correlation is extremely close with $r = 0.78$; n = 16 ($p<0.001$). The r^2 value suggests that more than 60% of the KCl E_{max} can be attributable simply to the variance in FD_{90}.

2.5 UTP responses

Responses were recorded in n = 16 vessels. The E_{max} was 4.7±0.5 mN and the EC_{50} 20.1±1.4 μM: neither value differed significantly with non-operative controls. Unlike control UTP responses, a linear E_{max} v FD_{90} relationship was apparent ($r = 0.74$, $p<0.01$: Table 31, Appendix). No significant right:left side difference was apparent in either E_{max} or EC_{50} value, suggesting that unilateral CCA manipulation and thread insertion were without ipsilateral effect (Table 23, Appendix). The combined C-R curve is shown in Fig 37.

2.6 NE responses

NE responses were *attempted* in n = 16 MCAs: however, as with non-operative controls, a sub group of NE-'subresponders' was apparent in which effectively no contractions were observed (NE_{max} ≤1.0 mN: *see below*). The mean NE_{max} in responders was 3.3±0.4 mN, the mean EC_{50} 3.1±0.9 μM: neither value differed significantly from non-operative controls. No significant right:left side difference was apparent in either E_{max} or EC_{50} value, suggesting that unilateral CCA manipulation and thread insertion were without ipsilateral effect. As with non-operative controls, a linear relationship was apparent between E_{max} and FD_{90} in 'responders' ($r = 0.72$, $p<0.001$: Table 31, Appendix), therefore justifying the exclusion of discontinuous 'subresponders'.

2.6.1 NE-'responders'

These referred to NE_{max} >1.0 mN. A total of 14/16 (88%) of MCAs showed a response in 8/8 (100%) animals (Table 11, Appendix). NE responses were obtained bilaterally in 6/8 (75%). NE responses were therefore asymmetrical (i.e. one 'responder' contralateral to a 'subresponder') in 2/8 (25%) animals.

2.6.2 NE-'subresponders'

These referred to NE_{max}≤1.0 mN. A total of 2/16 (12.5%) of MCAs were subresponsive to NE in 2/8 (25%) animals (Table 11, Appendix). No animal contained bilaterally subresponsive MCAs. NE responses were therefore asymmetrical (i.e. one 'responder' contralateral to a 'subresponder') in 2/8 (25%) animals.

2.7 HA responses

Responses were recorded in n = 16 vessels. The E_{max} was 82±5 % and the EC_{50} 6.8±1.6 μM. Both of these results were significantly different from non-operative controls ($p = 0.0085$ and $p = 0.0062$ respectively). Furthermore, the HA_{max} frequency distribution was significantly left-shifted, resulting in a smaller proportion of MCAs (4/16 = 25%) with an E_{max} >100%: however, the latter was not quite statistically significant ($p = 0.56$). As Fig 30 shows, the effect produced was akin to that of low-dose HA2 blockade (c.f. Fig 26). No significant right:left side difference was apparent in either E_{max} or EC_{50} value, suggesting that unilateral CCA manipulation and thread insertion were without ipsilateral effect.

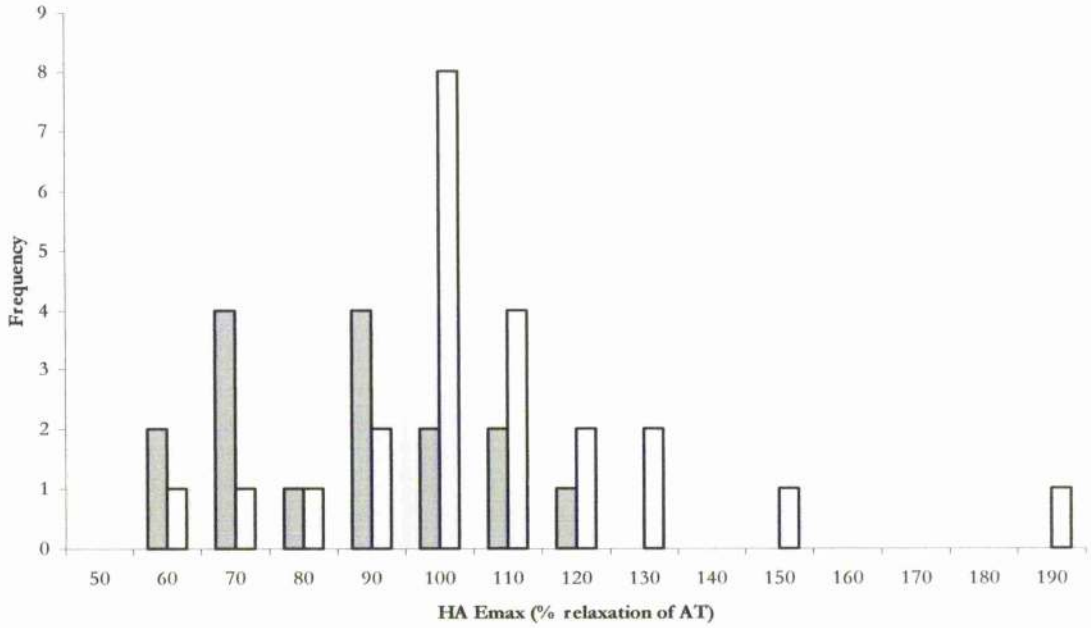


Fig 29. Combined non-operative controls and shams HA_{max} frequency histograms. Note the marked left-shift following sham operation. Note also that there are fewer numbers in the right hand tail (i.e. E_{max} >100%) *Key:* **white** non-operative controls, **grey** shams.

2.8 PPV responses

Responses were recorded in n = 16 vessels. The E_{max} was 107±16 % and the EC_{50} 2.7±0.5 μM. Neither value was significantly different from that in non-operative controls. As with non-operative controls, C-R curve abnormalities again occurred at the 50 μM level, amounting to a similar transient loss of relaxation (*see* Fig 41). No significant right:left side difference was apparent in either E_{max} or EC_{50} value, suggesting that unilateral CCA manipulation and thread insertion were without ipsilateral effect (Table 23, Appendix). The combined C-R curve is shown in Fig 39.

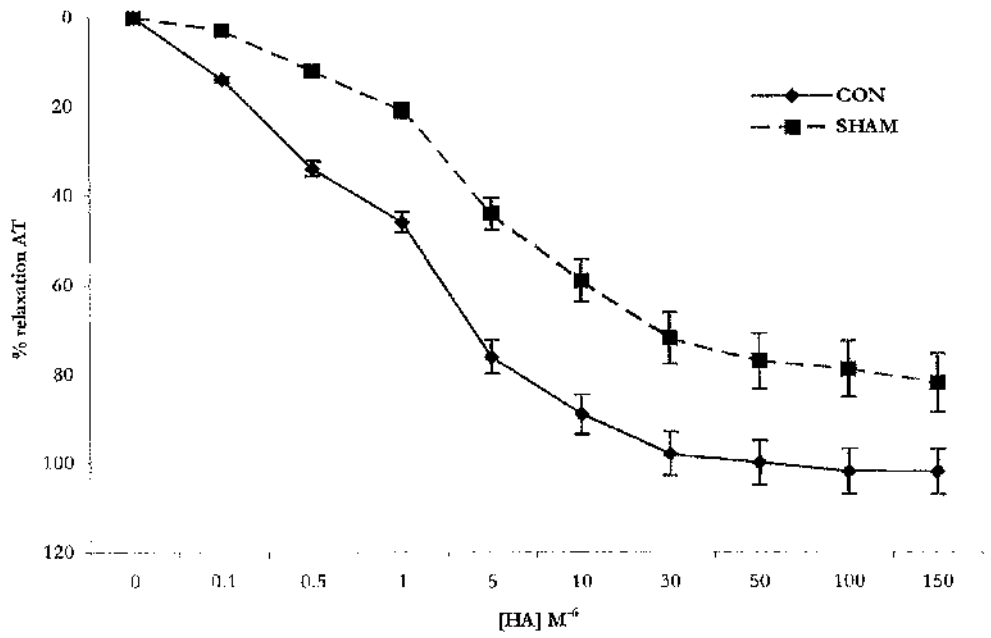


Fig 30. Combined non-operative controls and shams HA C-R curves. The significant right-shift resembles low-dose HA2 blockade (*see* Fig 26): both EC_{50} and E_{max} values are significantly different from non-operative controls ($p<0.01$).

2.9 L-ARG and L-NAME responses

Responses were recorded in n = 16 (L-ARG) and 6 (L-NAME) vessels respectively. The L-ARG E_{max} was 72±5 % and EC_{50} 4.7±1.4 μM: neither value differed significantly from non-operative controls. The combined frequency distribution shown in Fig 43 demonstrates that in very few MCAs L-ARG E_{max} > 100%. No significant right:left side difference was apparent in either E_{max} or EC_{50} value, suggesting that unilateral CCA manipulation and thread insertion were without ipsilateral effect (Table 23, Appendix).

The L-NAME mean E_{max} was 2.0±0.5 mN, which did not significantly differ from non-operative controls. No significant right:left side difference was apparent in either E_{max} or EC_{50} value, suggesting that unilateral CCA manipulation and thread insertion were without ipsilateral effect (Table 23, Appendix).

2.10 SNP responses

Responses were recorded in n = 16 vessels. The E_{max} 76±3 % was and the EC_{50} 8.8±3.2 μM: neither value differed significantly from non-operative controls. No significant right:left side difference was apparent in either E_{max} or EC_{50} value, suggesting that unilateral CCA manipulation and thread insertion were without ipsilateral effect (Table 23, Appendix). The combined C-R curve is shown Fig 46.

SECTION II
In vitro vessel responses
Chapter 3
Subarachnoid haemorrhage groups

3.1 Functional diameters

Values were recorded in n = 60 vessels. The overall SAH group mean FD_{90} was 161±39(SD) μm: the mean right MCA being 161±43(SD) μm, the mean left MCA 160±36(SD) μm. None of these values differed significantly from shams or non-operative controls. In fact, there was a remarkable degree of uniformity across these groups (p = 0.86: *see* Table 28, Appendix). This does not merely reflect an overall uniformity in MCA '*anatomical* size': MCA dimensions at 90mmHg internal pressure (FD_{90}) are determined by myogenic contraction (MT) as well as by anatomical dimensions. Thus, a uniformity in *functional* activity—at least with regard to baseline MT—is also suggested. This possibility is further—and more profoundly—suggested by the invariance of FD in n = 27 SAH MCAs throughout a range of intraluminal pressures from 70-150 mmHg (*see* Table 5 and Fig 31).

Table 5. Pressure-FD relationship values for Fig 29. (r = 0.072, p = 0.79, n = 27)

Pressure (mmHg)	70	90	110	130	150
FD±SE (μm)	157±9	143±9	152±9	160±10	150±11

Again, as with shams and non-operative controls, a significant degree of asymmetry was apparent in MCAs obtained from *individual* animals (not shown). Thus, in 15/30 (50%) of animals the right MCA FD_{90} differed from the left by at least one overall group SD (i.e. approximately 40 μm). Because of the E_{max} v FD_{90} relationship, this could have implications for experimental studies that use the contralateral vessel as the control (Part 6, Section II, 1.1.4). Nevertheless, as the overall values show, randomization for side, or—in particular—the use of larger experimental numbers, usually overcomes this error. The proportion of animals with asymmetrical MCAs (50%) did not differ significantly from either shams (50%) or non-operative controls (53%).

3.1.1. SAH sub-group analysis (*see* Tables 24-30, Appendix)

The striking degree of uniformity apparent in SAH groups overall against shams and non-operative controls was further endorsed upon SAH sub-group analysis. Thus, the FD_{90} was

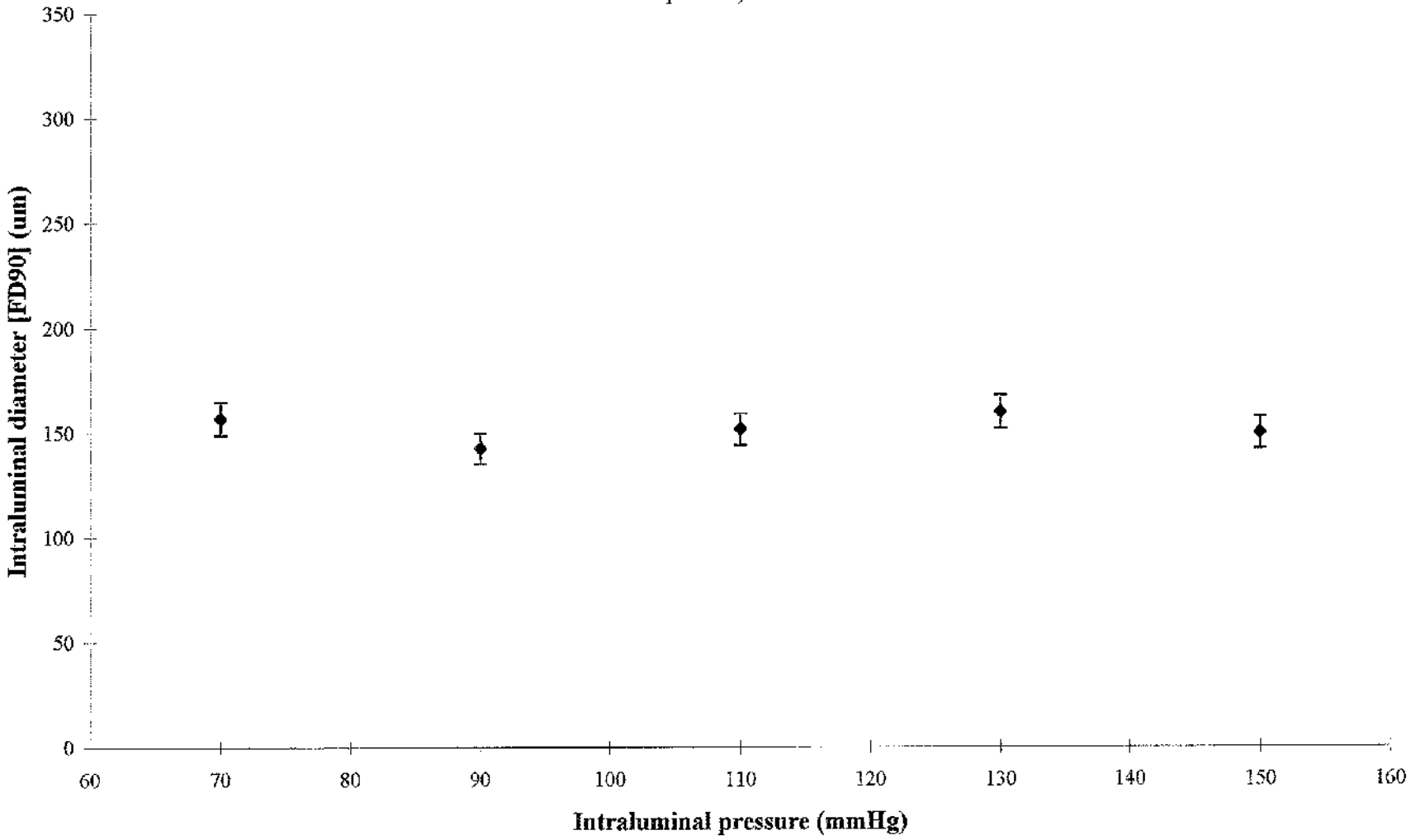
remarkably similar despite varying degrees of CCA re-clamping (i.e. SAH Groups 1-3: $p = 0.85$). Furthermore, no significant side differences were apparent in these groups individually ($p = 0.82$, 0.64 and 0.99 respectively).

In marked contrast, the FD_{90} was considerably lower in the 1 hour survival group after SAH (136±31 μm) compared to the 2 or 3 hour group. Nevertheless, this value was not significantly different ($p = 0.08$: Table 29).

The high degree of uniformity in FD_{90} was once again restored across acute pressor response groups after SAH (Table 30).

NOTE: None of the results in this section were significantly affected by excluding the two animals that were anaesthetized with hypnorm and hypnovel.

Fig 31. Invariance of intraluminal diameter with increasing intraluminal 'pressure'. (n = 27 MCAs, r = 0.072, p<0.79).



3.2 KCl responses

Responses were recorded in n = 57 vessels. The overall SAH (i.e. Groups 1-3) KCl E_{max} was 5.2±0.3 mN: this did not significantly differ from either control (Table 28). As with shams and non-operative controls, a linear relationship between E_{max} and FD_{90} was very much in evidence, though less close than with shams or non-operative controls ($r = 0.52$, $p<0.001$: Table 31, Appendix). As FD_{90} values remained remarkably unchanged after SAH (3.1 above), this implies that KCl contractions also remained *proportionately* unchanged. Thus, MCA *contractile potential* (**contractility**) remains preserved after acute SAH. Furthermore, as with shams and non-operative controls, the K_{max} remained the 'gold' standard against which to compare all other contractile responses.

3.2.1. SAH sub-group analysis (*see* Tables 24-30, Appendix)

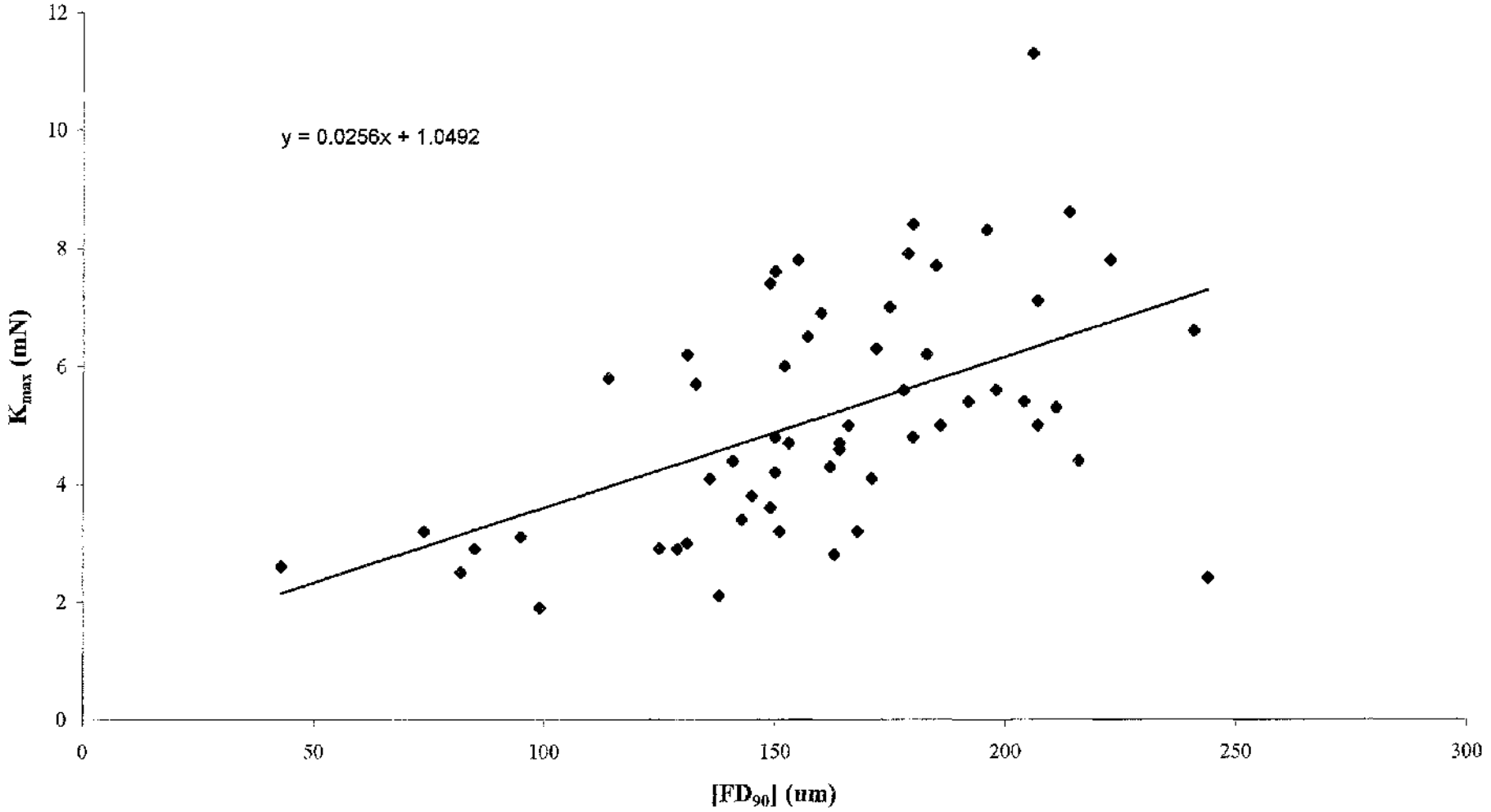
The strong degree of uniformity in KCl E_{max} values across non-operative controls, shams and overall SAH groups was also apparent across SAH sub-groups. Thus, the K_{max} did not significantly differ across SAH Groups 1-3, indicating that varying degrees of CCA re-clamping were tolerated without overt effect after SAH (Table 27). Moreover, there was a striking degree of uniformity in K_{max} values between right and left MCAs in SAH Groups 1, 2 and 3 (Tables 24-26). This was evidenced by p values of 0.90, 0.66 and 0.86 respectively which, along with FD_{90} invariance, supports the view that all other responses were essentially analyzed against a background comparison of 'like with like' in this study. It also suggests, of course, that CCA re-clamping effects did not singularly affect ipsilateral MCA reactivity: however, poor statistical *n* numbers for SAH Group 1 (n = 4 for each MCA) renders this analysis inconclusive.

The mean K_{max} was also strikingly invariant across 1, 2 and 3 hour survival groups after SAH (Table 29).

In marked contrast, the mean K_{max} appeared strongly affected by the acute pressor response (Table 30). Thus, the mean K_{max} with the type II 'invariant' pressor response (3.7±0.5 mN) almost significantly differed from either type I or type III pressor response ($p = 0.0527$) of which, each in turn, both more closely resembled shams and non-operative controls. In fact, the type II K_{max} significantly differed from type I when considered in isolation ($p = 0.025$). Furthermore, the type I K_{max} also significantly differed from the combined type II & III value ($p = 0.044$).

NOTE: None of the results in this section were significantly affected by excluding the two animals that were anaesthetized with hypnorm and hypnovel.

Fig 32. **SAH (Groups 1-3) K_{max} v FD_{90}.** More scatter is present than with controls (r = 0.52, p<0.001)



3.3 PGF2α responses

Responses were recorded in n = 55 MCAs. The overall SAH (i.e. SAH Groups 1-3) PGF2α E_{max} was 6.5±0.3 mN—this very nearly differed significantly from shams and non-operative controls (p = 0.051: Table 28). The overall SAH PGF2α EC_{50} value (5.8±0.6 μM), however, was strikingly similar to shams and non-operative controls (p = 0.88): a fact that can be appreciated in the combined C-R curve of Fig 33. Also apparent in Fig 33 is a partial loss of efficacy at supramaximal (i.e. >50 μM) concentrations. As with shams and non-operative controls, a close linear relationship of E_{max} to FD_{90} was again apparent (r = 0.632, p<0.001: Table 31, Appendix; Fig 34).

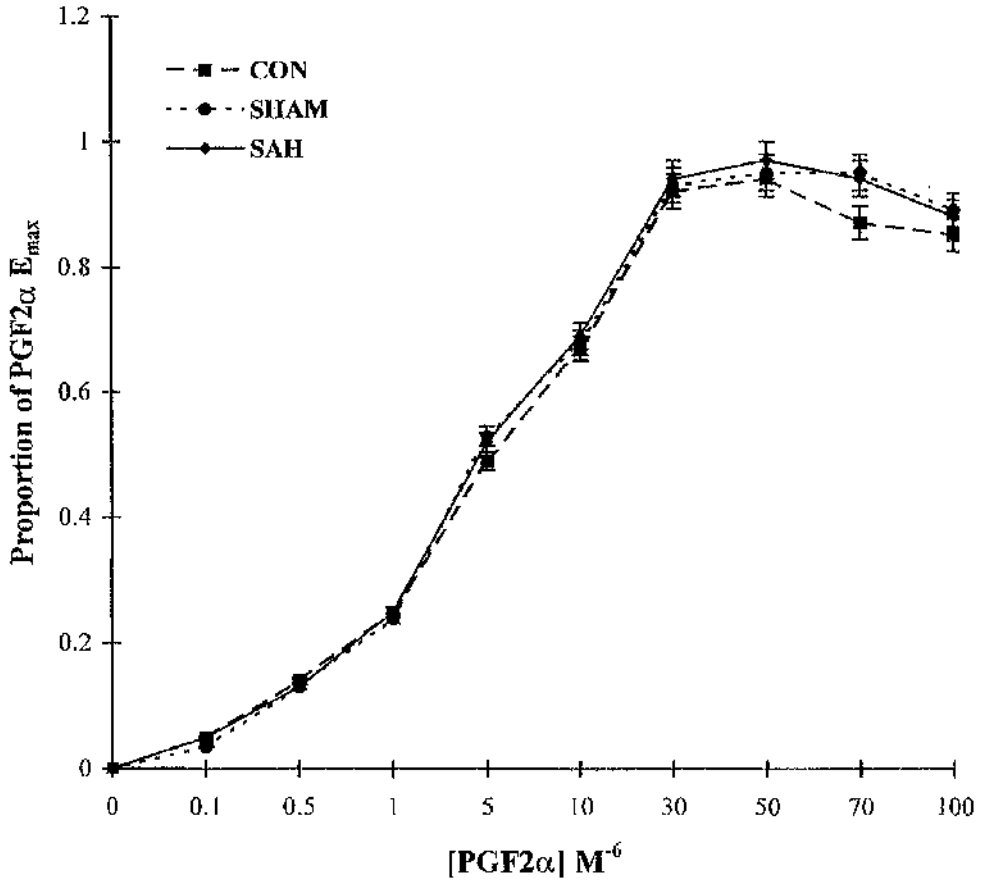
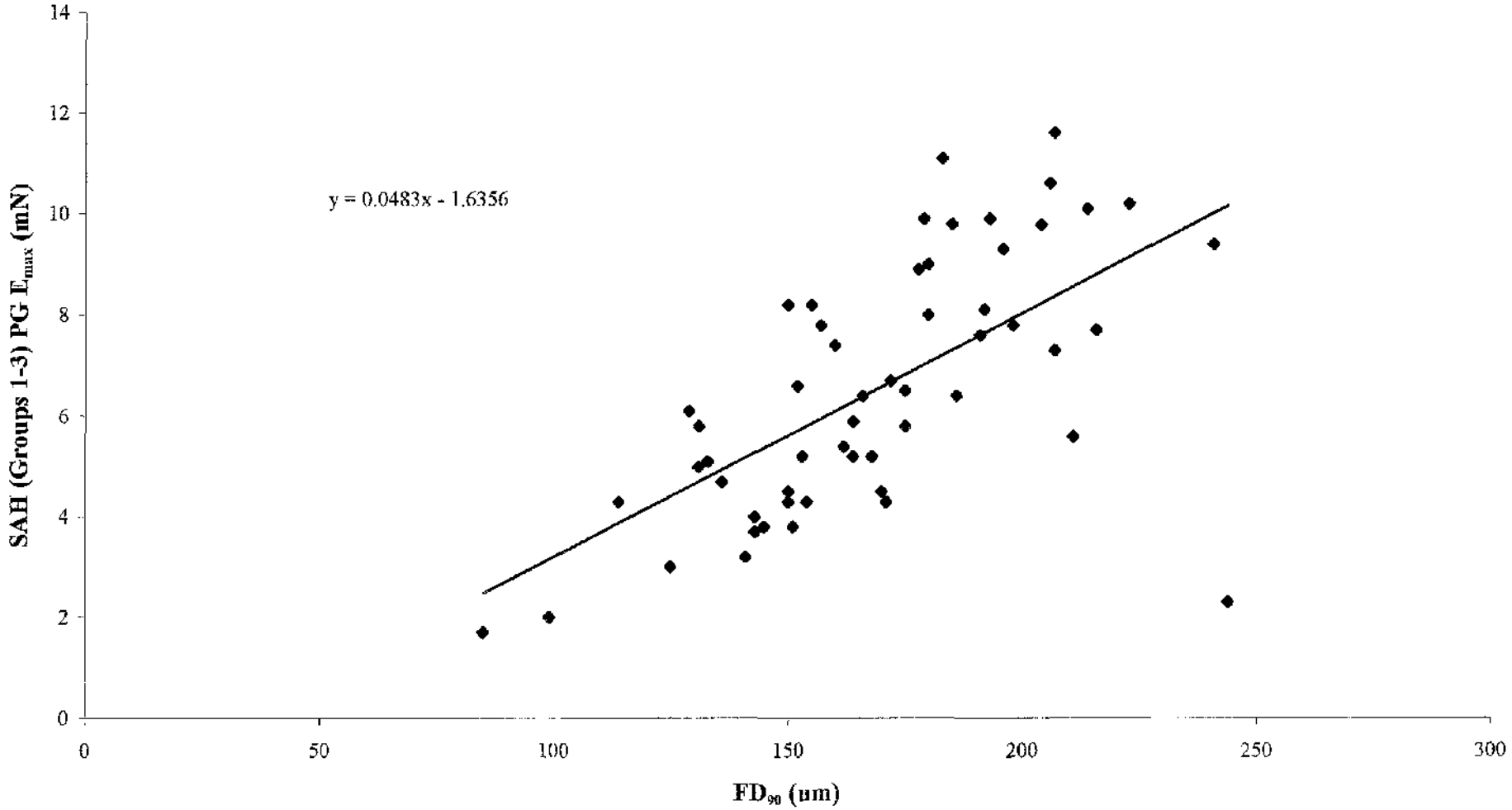


Fig 33. Combined non-operative controls, shams and SAH (i.e. SAH Groups 1-3) PGF2α C-R curves. A remarkable degree of uniformity apparent in each C-R curve. Some loss of efficacy is apparent at [PGF2α] >50 μM.

Fig 34. SAH (Groups 1-3) PGF2α E_{max} v FD_{90} plot. (r = 0.632, $p < 0.001$)



3.3.1. SAH sub-group analysis (*see* Tables 24-30, Appendix)

The PGF2α E_{max} did not differ significantly across SAH Groups 1-3, suggesting that varying degrees of CCA re-clamping were tolerated without overt effect within the confines of this study (Table 27). This fact is further suggested by insignificant side differences between right and left MCAs within each Group—particularly within SAH Group 1 (Tables 24-26). However, poor statistical *n* numbers for SAH Group 1 (n = 4 for each MCA) renders the latter analysis strictly inconclusive. Statistical significance was apparent, however, in EC_{50} values between SAH Groups 1-3. Thus, the mean EC_{50} in SAH Group 2 (3.7±0.6 μM) significantly differed from Groups 1 and 2: moreover, it *nearly* differed significantly from shams and non-operative controls (p = 0.058).

There appeared to be a progressive increase in mean PGF2α E_{max} with increased survival after SAH (Table 29). Thus, the E_{max} increased from 4.6±0.8 mN in the 1 hour group, through 6.6±0.7 mN in the 2 hour group, to 7.2±0.4 mN in the 3 hour group (p = 0.03). Furthermore, the 3 hour value significantly differed from both shams and non-operative controls (p = 0.006). However, it has already been noted that the FD_{90} was considerably smaller in the 1 hour group. Although not in itself of statistical significance (Part 5, Section II, 3.1: Table 29), a smaller FD_{90} could account for a smaller E_{max} by virtue of the E_{max}: FD_{90} relationship (Fig 32). No significance was apparent in mean EC_{50} values across this series: indeed, the mean EC_{50} appeared strikingly uniform with survival.

Mean PGF2α E_{max} and EC_{50} values did not differ significantly across differing acute pressor response sub-groups after SAH (Table 30).

NOTE: None of the results in this section were significantly affected by excluding the two animals that were anaesthetized with hypnorm and hypnovel.

3.4 5HT responses

Responses were recorded in n = 54 vessels. The overall SAH (i.e. SAH Groups 1-3) E_{max} was 5.1±0.4 mN, the EC_{50} 0.21±0.06 μM: neither value differed significantly from shams or non-operative controls (Table 28). A linear relationship between 5HT E_{max} v FD_{90} (not shown) of similar correlation to that of shams or non-operative controls was again apparent after SAH: i.e. similar to KCl or PGF2α (r = 0.56, p<0.01: Table 31, Appendix). The combined C-R curve suggests approximate conformity across the three main experimental groups (Fig 35).

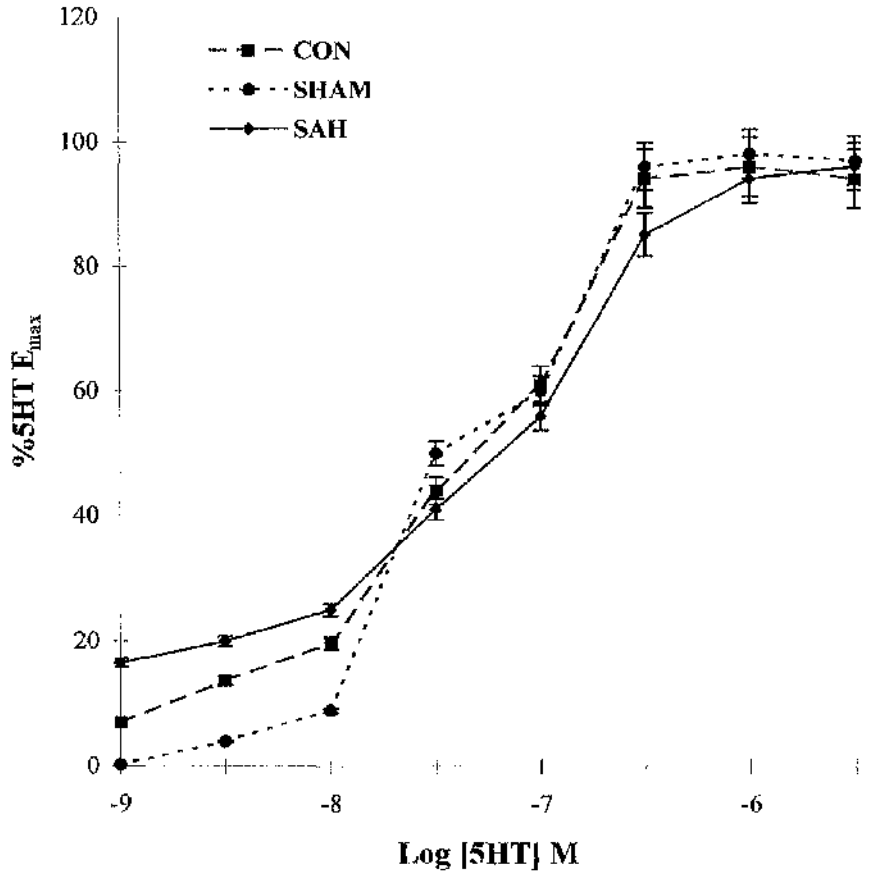


Fig 35. Combined non-operative controls, shams and SAH (i.e. SAH Groups 1-3) 5HT C-R curves.

3.4.1. SAH sub-group analysis (*see* Tables 24-30, Appendix)

No statistical significance was demonstrated in 5HT E_{max} values between SAH Groups 1-3 (Table 27): nor were there any significant side differences apparent (Tables 24-26). Varying degrees of CCA re-clamping were therefore tolerated without overt effect, although small n

numbers for Group 1 render this inconclusive. The SAH Group 1 mean EC_{50} value (0.72±0.31 μM), however, did significantly differ from SAH Groups 2 & 3 ($p = 0.0004$); as well as from shams and non-operative controls ($p = 0.0002$). No significant side difference, however, was demonstrated in SAH Group 1.

No significance was demonstrated in 5HT E_{max} values between survival groups after SAH (Table 29). However, the 2 hour EC_{50} value (0.38±0.17 μM) appeared markedly different from the 1 and 3 hour value. Although this did not reach statistical significance ($p = 0.06$), it significantly differed from either shams or non-operative controls ($p = 0.012$).

No significance was demonstrated in 5HT E_{max} values between acute pressor response groups after SAH (Table 30). The type III (CHR) response EC_{50} value (0.92±0.64 μM), however, did significantly differ from either type I & II response groups ($p = 0.002$); as well as from shams and non-operative controls ($p = 0.0003$).

NOTE: None of the results in this section were significantly affected by excluding the two animals that were anaesthetized with hypnorm and hypnovel.

3.5 NE responses

NE responses were *attempted* in n = 57 MCAs from n = 29 animals (in one animal, NE responses were not attempted due to pressures of time: *see* Part 4, 6.5). As with shams and non-operative controls, however, NE responses were singularly heterogeneous in comparison to other contractile agonists: some MCAs effectively demonstrated no response (NE_{max} ≤1.0mN) despite adequate constrictions to other agonists. Fig 36 demonstrates this in relation to corresponding KCl constrictions per each MCA. As with shams or non-operative controls, a linear relationship was apparent in 'responders' between E_{max} and FD_{90} ($r = 0.47$, $p<0.001$: Table 31, Appendix). Again, as in shams or non-operative controls, the overall SAH NE_{max} in 'responders' (3.1±03 mN) was significantly weaker than the overall mean SAH K_{max} (5.2±0.3 mN, $p = 0.000003$). However, the overall SAH 'responder' NE_{max} did not differ significantly from either shams or non-operative controls (Table 28, Appendix): indeed, all of these values were impressively similar ($p = 0.90$). The EC_{50} in responders (5.9±1.4 μM) again did not significantly differ from either control: however, sub-group analysis suggested that SAH Group 3 did statistically differ from either SAH Groups 1-2 or shams and non-operative controls (*see below*).

3.5.1 NE-'responders'

A total of 34/57 (60%) MCAs showed a response to NE in 22/29 (76%) animals (Tables 12-14, Appendix). NE responses were obtained bilaterally in 12/28 (43%) animals [in one animal the contralateral MCA was generally unresponsive to *all* agonists, thus reducing the denominator]. NE responses were therefore asymmetrical (i.e. one 'responder' contralateral to a 'subresponder') in 9/28 (32%) animals. None of these values was significantly different from shams or non-operative controls.

3.5.2 NE-'subresponders'

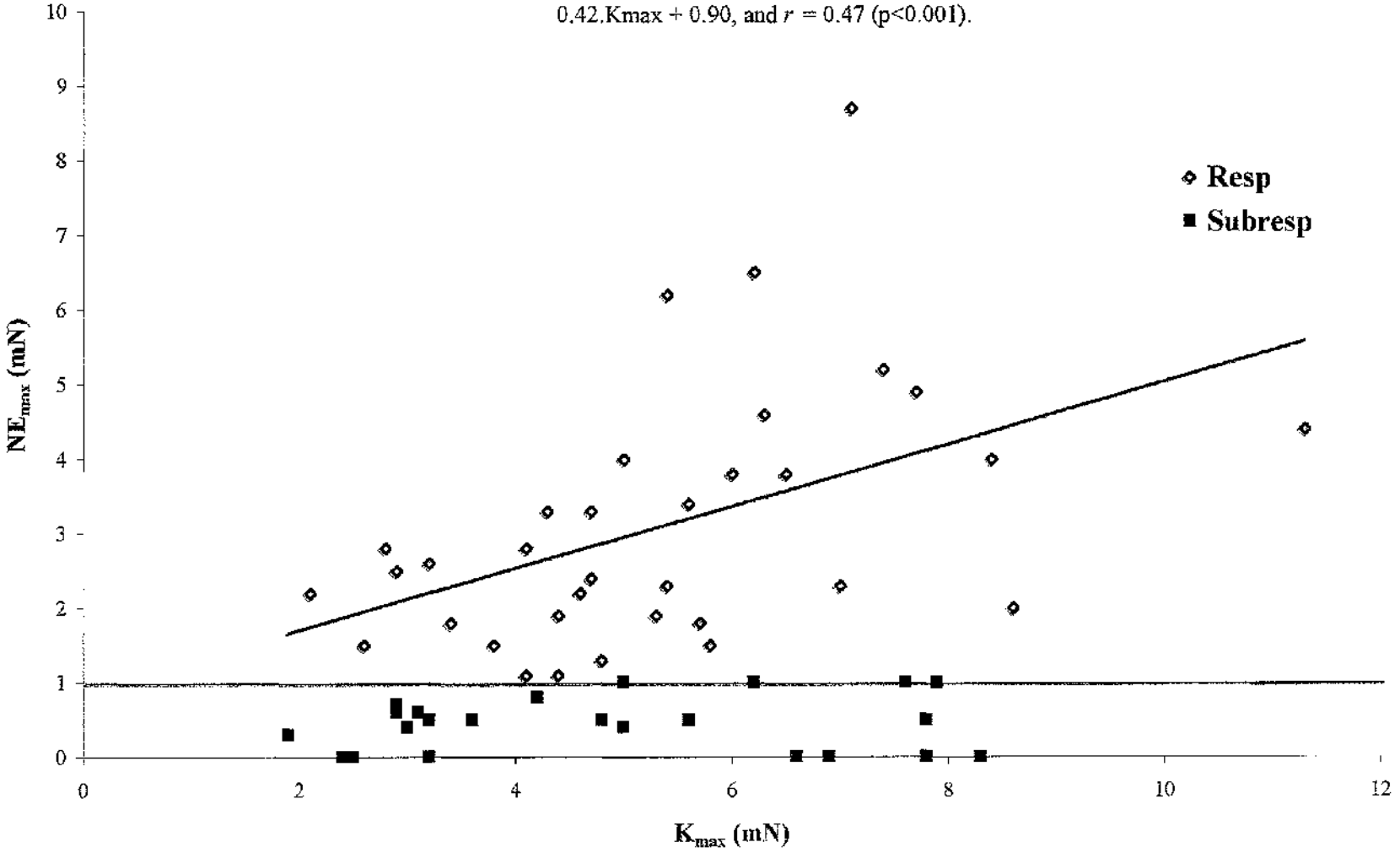
A total of 23/57 (40%) MCAs showed no effective NE response in 16/29 (55%) animals (Tables 12-14, Appendix) despite adequate responses to other contractile agonists (Fig 36). The response was bilaterally poor in 7/28 (25%) animals. NE responses were therefore asymmetrical (i.e. one 'responder' contralateral to a 'subresponder') in 9/28 (32%) animals. None of these values was significantly different from shams and non-operative controls.

3.5.3. SAH sub-group analysis (*see* Tables 24-30, Appendix)

Table 27 demonstrates the striking degree of uniformity in 'responder' NE_{max} despite varying degrees of CCA re-clamping (i.e. SAH Groups 1-3: $p = 0.97$). Note also that no significant side difference nor trend is demonstrated in any SAH sub-group based on CCA-clamping (i.e. SAH Groups 1-3: Tables 24-26): however, poor statistical *n* numbers for SAH Group 1 [$n = 4$ for each MCA] renders this analysis inconclusive. Nevertheless, the 'responder' EC_{50} in SAH Group 3 (9.2±2.3 μM) was significantly different from either SAH Groups 1 or 2, or from shams and non-operative controls ($p = 0.002$) [no EC_{50} was obtained in Group1—*see* Part 4, 6.5]. The degree of heterogeneity (i.e. proportion of NE-'subresponders':'responders') did not vary significantly across SAH Groups 1–3 (38%, 35% and 45% NE-'subresponders': $p>>0.2$).

The mean 'responder' NE_{max} appeared to increase significantly with prolonged survival after SAH (Table 29). Thus, the 3 hour NE_{max} (4.8±0.6 mN) differed significantly from the 1 and 2 hour values ($p = 0.04$); as well as from shams and non-operative controls ($p = 0.026$). However, the mean functional diameter in the 1 hour group was considerably lower than the 2 or 3 hour value and, excluding this group, no significance was apparent between 2 and 3 hour values when considered in isolation ($p = 0.09$). No statistical significance was apparent in EC_{50} values across this series. No significance was apparent in NE heterogeneity with survival (33%-Group 1, 38%-Group 2, 48%-Group 3 NE-'subresponders': $p>>0.2$).

Fig 36. Overall SAH (Groups 1-3) NE_{max} v K_{max}. All points >1.1 mN are designated NE-'responders' (**Resp**), all points below this level NE-'subresponders' (**Subresp**). The regression line for 'responders' is NEmax = 0.42.Kmax + 0.90, and r = 0.47 ($p<0.001$).



The mean 'responder' NE_{max} was strikingly invariant across acute pressor response groups after SAH (Table 30). In contrast, an equally striking degree of statistical significance was apparent in EC_{50} values across the same series: the 'invariant' type II acute pressor response group appeared distinctively abnormal here. In fact, type II acute pressor response EC_{50} values were significantly different from both type I & III acute pressor response groups ($p = 0.004$ and $p = 0.02$ respectively), as well as from shams & non-operative controls ($p = 0.0001$). However, poor statistical *n* numbers for type II and III pressor groups ($n = 4$ EC_{50} values in each case) renders this analysis inconclusive. In similar fashion, the degree of heterogeneity in type III acute pressor responses was significantly lower than that in type I or type II pressor groups (39%-type I, 60%-type II, and 0%-type III NE-'subresponders', $p<0.05$): however, the statistically small group number in the type III pressor group once again precluded this from being conclusive. Furthermore, no statistical significance was apparent in the degree of heterogeneity between the type I acute pressor response group (39%) and type II & III pressor groups (47%: $p>0.2$) when the latter were grouped together based upon their significantly higher ΔICP values [Part 5, Section I, 1.3.2].

NOTE: None of the results in this section were significantly affected by excluding the two animals that were anaesthetized with hypnorm and hypnovel.

3.6 UTP responses

Responses were recorded in $n = 55$ MCAs. The overall SAH (i.e. Groups 1-3) E_{max} was 3.7±0.3 mN, the overall SAH EC_{50} 21.3±1.3 μM: neither value significantly differed from either control (Table 28, Appendix). The uniformity in UTP contractions across each major experimental group can be appreciated from the combined groups C-R curve in Fig 37. As with shams and non-operative controls, a linear relationship between E_{max} and FD_{90} was very much in evidence (graph not shown); though less close than with shams or non-operative controls ($r = 0.58$, $p<0.001$: Table 31, Appendix).

3.6.1. SAH sub-group analysis (*see* Tables 24-30, Appendix)

No significant difference was apparent in either E_{max} or EC_{50} values across SAH Groups 1-3: indeed, there was a striking degree of uniformity apparent (Table 27). Furthermore, there were no significant side differences apparent in UTP contractions within each group (i.e. SAH Groups 1-3), suggesting that CCA re-clamping was without significant effect in this study (although small *n* numbers in Group 1 render this inconclusive: Tables 24-26).

No significant difference was recorded in either E_{max} or EC_{50} values with increasing survival after SAH (Table 29).

No significant difference was recorded in either E_{max} or EC_{50} values with acute pressor responses after SAH (Table 30).

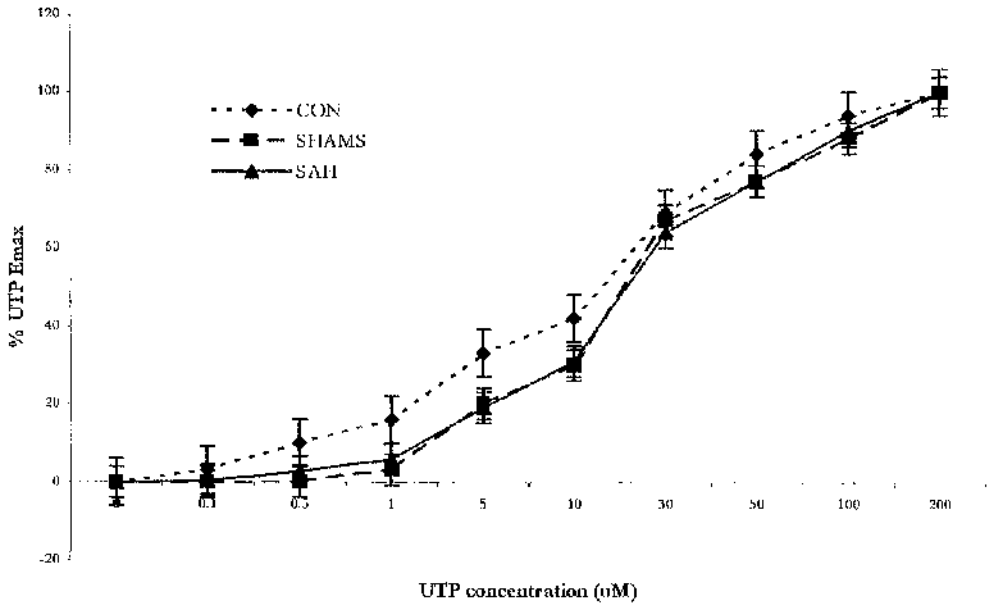


Fig 37. Combined non-operative controls, Shams and SAH (Groups 1-3) UTP C-R curves.

NOTE: None of the results in this section were significantly affected by excluding the two animals that were anaesthetized with hypnorm and hypnovel.

3.7 HA responses

Responses were recorded in n = 54 vessels. The overall SAH (i.e. SAH Groups 1-3) E_{max} was 70±4 %. Although Table 28 suggests that this may be significant, the tabulated significance is actually *across* the series: this, in fact, principally reflects the sham/non-operative control difference. Thus, no significance is apparent between [overall] SAH and shams (p = 0.093) when these are considered in isolation. The overall SAH EC_{50} was 4.9±0.5 μM. As with E_{max} values, a high degree of statistical significance was again apparent *across* the series: again, however, this principally related to the sham/non-operative control difference. Thus, no significance was apparent between [overall] SAH and shams (p = 0.14). In consequence, the increased right shift acutely after SAH—additional to that after sham operation—is not significant: i.e. most of the shift is due to sham operation (Fig 38). Nevertheless, the proportion of MCAs dilated >100% by HA decreased progressively and significantly from non-operative controls (52%), through sham operative controls (25%), to SAH Groups (11%) [p < 0.001; **Table 12** *below*].

3.7.1. SAH sub-group analysis (*see* Tables 24-30, Appendix)

Although suggestive, no statistical significance was demonstrated in E_{max} or EC_{50} values across SAH Groups 1-3 after SAH (Table 27). Furthermore, no significant side differences were apparent with either value in SAH Groups 1, 2 or 3 individually (Tables 24-26). These results suggest that varying degrees of CCA re-clamping were tolerated without significant effect after SAH: however, poor statistical *n* numbers for SAH Group 1 (n = 4 for each MCA) renders this analysis inconclusive.

Although the 3 hour E_{max} (67±4 %) seemed markedly reduced relative to the 1 hour (87±6 %) or 2 hour (77±5 %) values, this difference was—surprisingly—not statistically significant (p = 0.54: Table 29). No statistical significance was demonstrated in EC_{50} values across survival groups after SAH.

No statistical significance was demonstrated in E_{max} or EC_{50} values with acute pressor responses after SAH (Table 30).

NOTE: None of the results in this section were significantly affected by excluding the two animals that were anaesthetized with hypnorm and hypnovel.

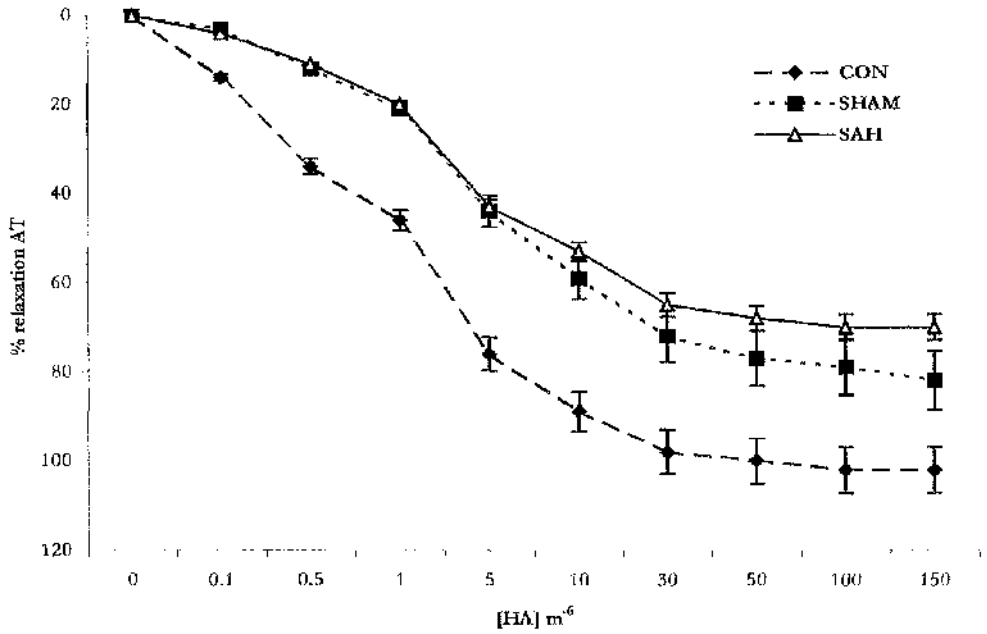


Fig 38. Combined non-operative controls, shams and SAH (Groups 1-3) HA C-R curves. Only the E_{max} is reduced—but not significantly—from shams after SAH. The greatest effect on non-operative control HA responses—a *significant* reduction in both E_{max} and EC_{50}—is achieved by sham operation.

Table 6. 2 x *k* table demonstrating proportions of MCAs with HA E_{max}>100%. The value of χ^2 across the series is 14.84 ($p < 0.001$).

	Non-operative controls	Shams	SAH (Groups 1-3)	*R*
E_{max} >100%	12	4	6	22
E_{max} ≤100%	11	12	48	71
N	23	16	54	93

3.8 PPV responses

Responses were recorded in n = 52 vessels. The overall SAH (i.e. Groups 1-3) E_{max} was 104±3 %, the overall SAH EC_{50} 2.3±0.3 μM: neither value differed significantly from shams or non-operative controls (Table 28). In fact, there was a remarkable degree of uniformity in PPV-dilatation across these groups (p = 0.95 and 0.61 respectively) which can be more fully appreciated in the combined C-R curves of Fig 39. As with shams and non-operative controls, however, C-R curve abnormalities were again prevalent in *individual* MCAs (*see* Fig 41 below).

3.8.1 Concentration-response curve abnormalities

As in shams and non-operative controls, a transient loss of efficacy was occasionally seen in SAH responses at 50 μM which soon passed off either with time, or after raising the bath [PPV] to 100 μM (Fig 41, **A**). However, in addition, another disturbance was also occasionally observed at 0.5 μM [PPV] which appeared unique to SAH responses. It amounted to a definite increase of vessel tone that negated the relaxation previously achieved (Fig 41, **B**): again, this appeared transient. Due to its small magnitude (this being within the error of baseline PGF2α-induced vasomotion [Fig 19]), the latter aberration was not quantifiable.

3.8.2. SAH sub-group analysis (*see* Tables 24-30, Appendix)

Although suggestive, the mean PPV E_{max} in SAH group 1 did not significantly differ from that of SAH Groups 2 and 3 (Table 27): therefore, prolonged CCA re-clamping after SAH did not significantly affect PPV responses. Furthermore, no significant side differences were demonstrated in SAH Groups 1-3 (Tables 24-26). However, poor statistical *n* numbers for SAH Group 1 (n = 4 for each MCA) renders this analysis inconclusive. No significance was apparent with EC_{50} values either between groups, or between right and left sides within each group.

The mean PPV E_{max} nor the mean PPV EC_{50} differed significantly with survival following SAH (Table 29).

Neither the mean PPV E_{max} nor the mean PPV EC_{50} differed significantly with acute pressor response following SAH (Table 30).

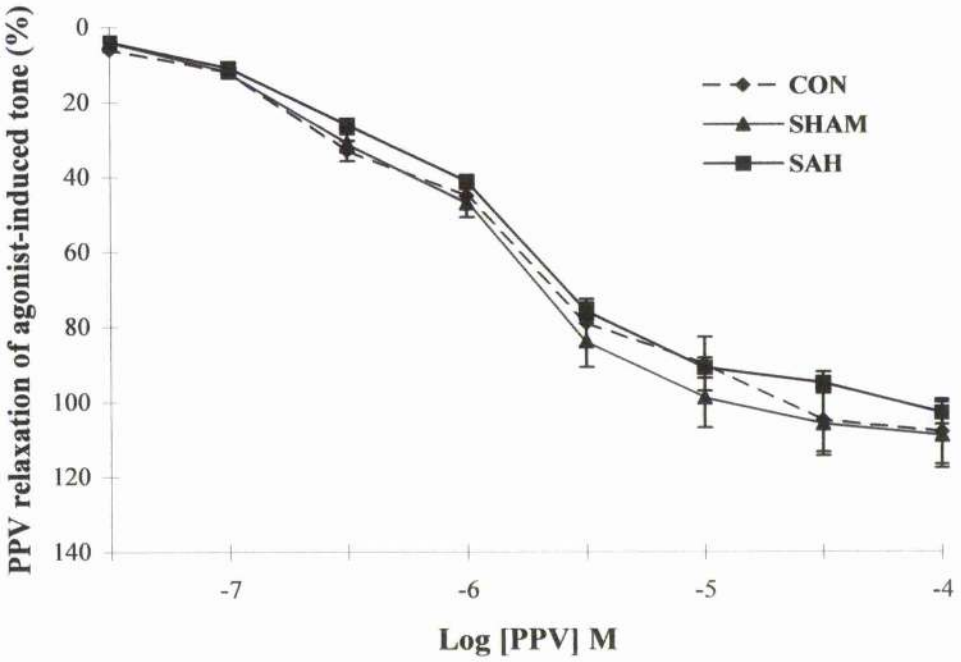


Fig 39. Combined non-operative controls, shams and SAH (Groups 1-3) PPV C-R curves. PPV relaxation remains uniform across non-operative controls, shams and overall SAH groups.

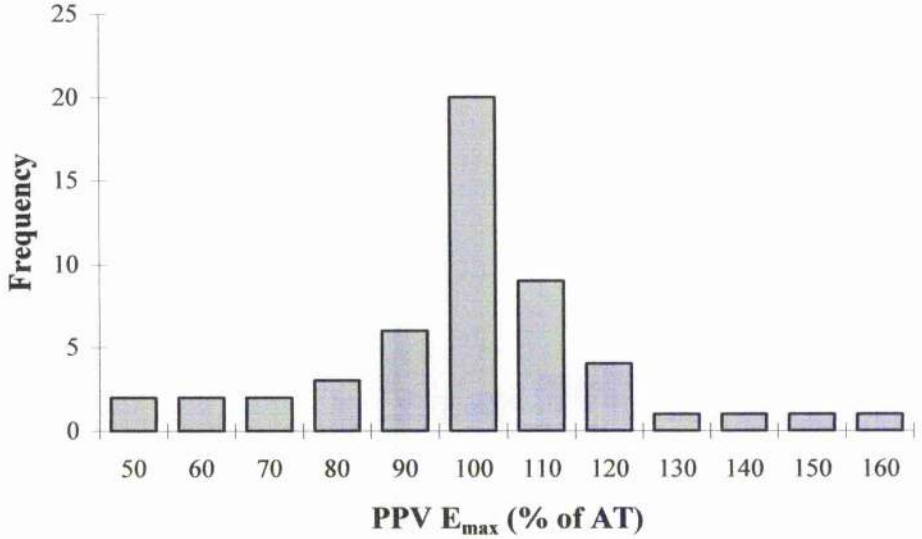


Fig 40. Overall SAH (i.e. Groups 1-3) PPV E_{max} frequency histogram. The right hand tail of the normal distribution (where E_{max} >100%) reflects the proportion of MCAs where dilation of MT must also have occurred.

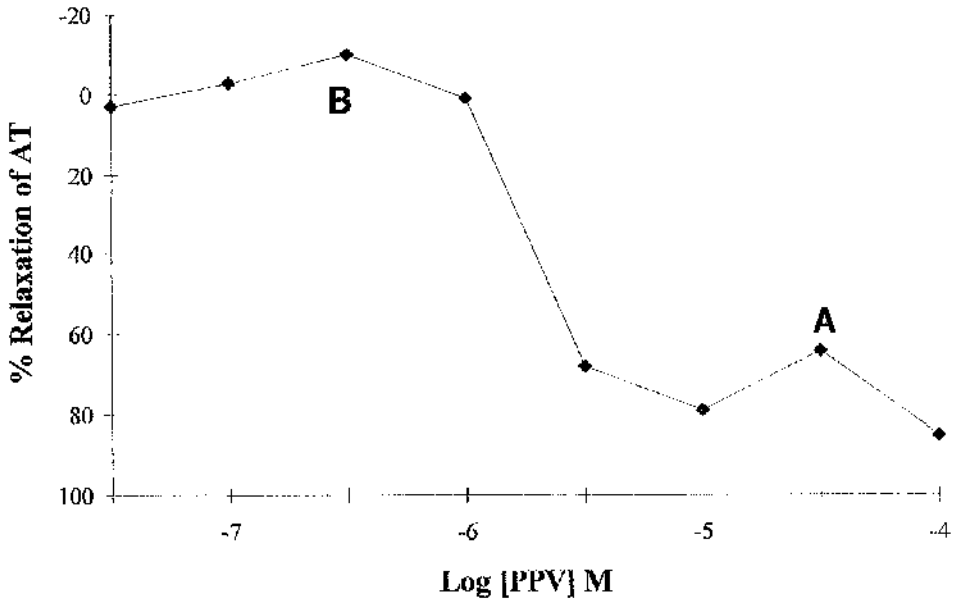


Fig 41. PPV C-R curve abnormalities. Representative SAH vessel. *Key*: **A** – *transient* loss of efficacy at 50 μM. **B** – paradoxical *transient* vasoconstriction at 0.5μM

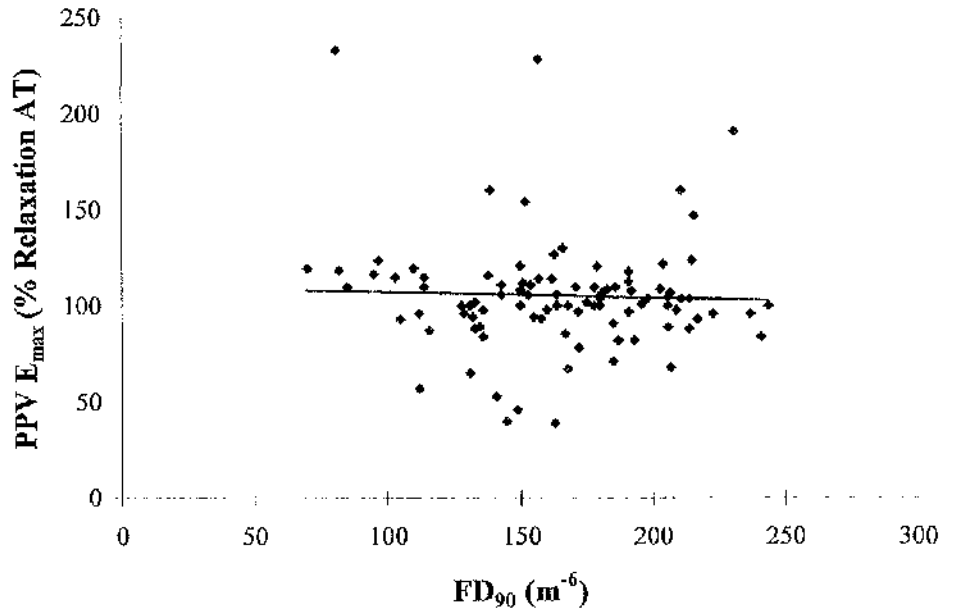


Fig 42. The relationship of PPV E_{max} to FD_{90}. Combined responses from control, sham and overall SAH groups. No correlation of E_{max} with diameter is demonstrated ($r = 0.04$).

3.8.3. Relationship of E_{max} to FD_{90}

Because of the possibility that vasodilators, with differential activity related to vessel size, may cause TIAs with VSM, it was considered prudent to assess the relationship of FD_{90} to PPV E_{max}. Fig 42 clearly shows that, in fact, no such relationship exists in non-operative controls, shams or overall SAH groups ($r = 0.04$).

NOTE: None of the results in this section were significantly affected by excluding the two animals that were anaesthetized with hypnorm and hypnovel.

3.9 L-ARG responses

Responses were recorded in n = 53 vessels. The overall SAH E_{max} was 92±5 %, the overall SAH EC_{50} 3.4±0.6 μM (Table 28, Appendix). The former value is significantly different from shams or non-operative controls (p = 0.01): this is illustrated in the frequency histogram of Fig 43. The EC_{50}—although not significant in the overall SAH analysis—was, however, significant on sub-group analysis (*see below*). The general shift in L-ARG responses after SAH can also be appreciated in the C-R curve of Fig 44.

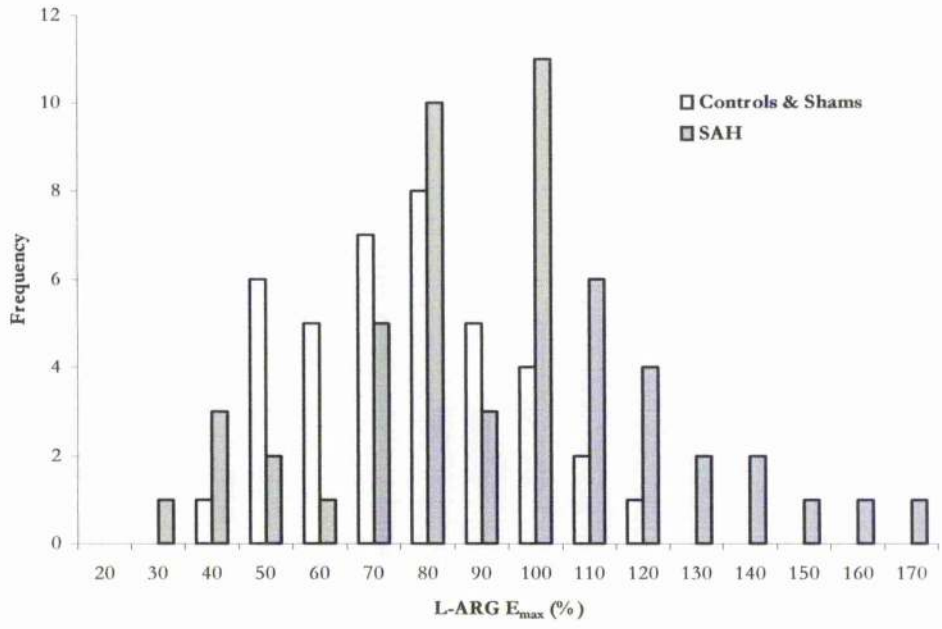
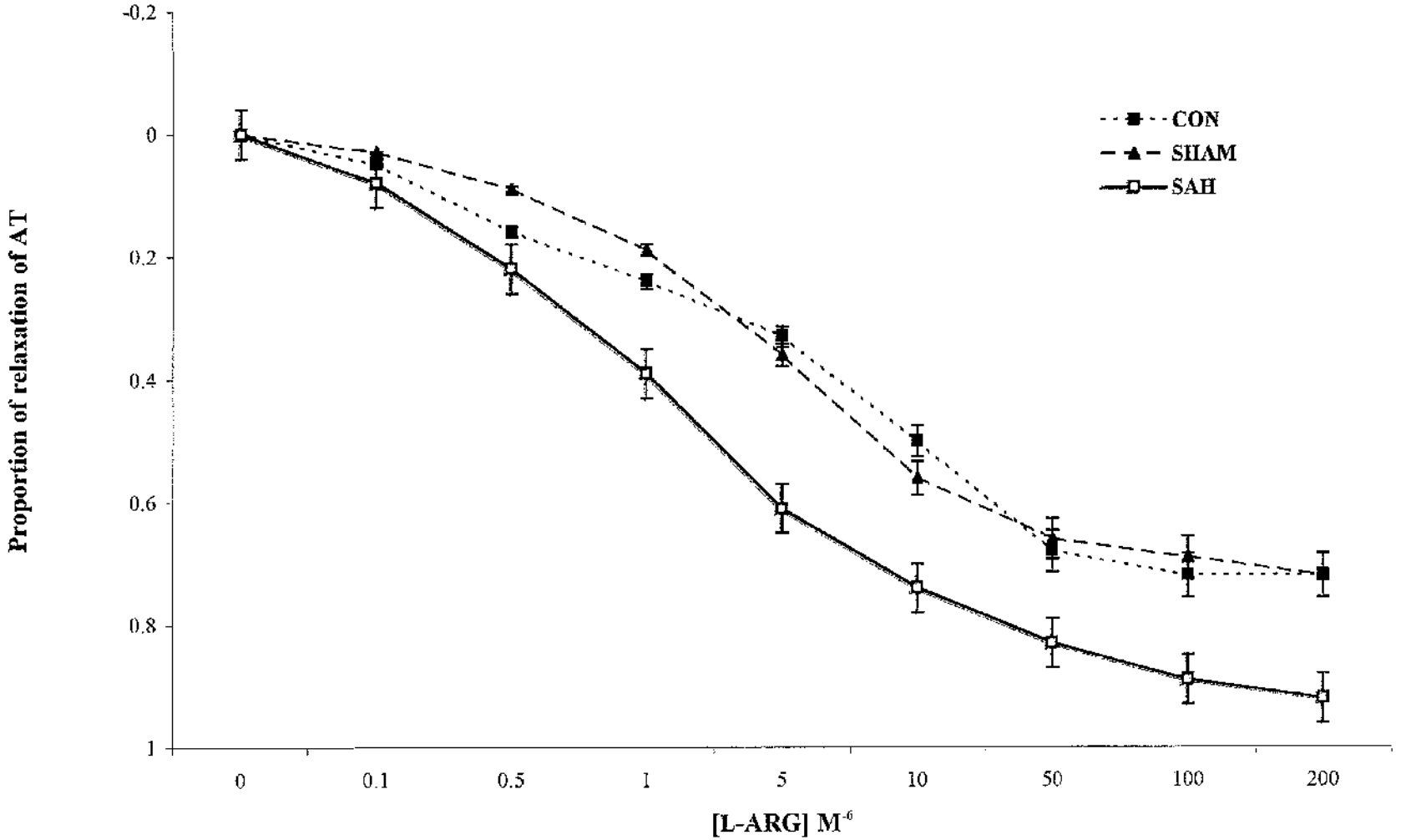


Fig 43. Combined non-operative controls, shams and SAH (Groups 1-3) L-ARG E_{max} Frequency histogram. *Key:* **white** combined shams and non-operative controls, **grey** post SAH. Note the significant right-shift in the frequency distribution after SAH. Note also that both distributions are highly representative of normal curves.

Fig 44. Combined Non-operative Controls, Shams and SAH (Groups 1-3) L-ARG C-R curves



3.9.1. SAH sub-group analysis (*see* Tables 24-30, Appendix)

No significant difference is apparent in E_{max} values across SAH Groups 1-3 (Table 27): nor is any significant side difference demonstrated in E_{max} in each group individually (Tables 24-26). These results suggest that increasing degrees of CCA-clamping did not affect ipsilateral MCA reactivity: however, poor statistical n numbers for SAH Group 1 (n = 4 for each MCA) renders this analysis inconclusive. Table 27, however, suggests that prolonged CCA-clamping significantly obtunded an underlying trend in L-ARG EC_{50} values to decrease after SAH. This is borne out by the fact that the combined EC_{50} in SAH Groups 2 & 3 significantly differed from shams and non-operative controls after exclusion of SAH Group 1 ($p = 0.036$).

Table 29 suggests a trend toward increasing E_{max} with prolonged survival after SAH. Although this was not statistically significant, only the E_{max} in the 2 and 3 hour survival groups significantly differed from shams and non-operative controls ($p = 0.007$ and $p = 0.003$ respectively). The EC_{50} values, however, do significantly differ across this series. Paradoxically, it is the 1 and 3 hour survival groups that are decreased relative to shams and non-operative controls: the 2 hour group (i.e. the statistically 'abnormal' group across the series in Table 29) appears unchanged after SAH.

No statistically significant trend was demonstrated in either E_{max} or EC_{50} values across acute pressor response groups (Table 30). However, the trend in E_{max} is nearly significant and, when type II & III acute pressor responses are considered together their combined value (101±5 %) is significantly different from type I (85±4 %: $p = 0.02$). Furthermore, the type I acute pressor response E_{max} does not significantly differ from either shams or non-operative controls, whereas the type II & III response do with a high degree of statistical significance ($p = 0.0001$). Indeed, this pattern remained significant even when allowing for 'survivors' and 'non-survivors' within this sub-group.

NOTE: None of the results in this section were significantly affected by excluding the two animals that were anaesthetized with hypnorm and hypnovel.

3.10 L-NAME responses (Table 28, Appendix)

Responses were recorded in n = 13 vessels. The E_{max} was 2.4±0.4 mN: this did not differ significantly from either control. No EC_{50} responses were possible because no C-R curves were performed.

3.10.1. SAH sub-group analysis (Table 26, Appendix)

L-NAME responses were only recorded in SAH Group 3, therefore no SAH sub-group analysis was possible. However, no significant difference was apparent in right-left MCA comparison within SAH Group 3.

No sub-group comparisons based on either survival or acute pressor response were possible due to the small n numbers involved.

NOTE: None of the results in this section were derived from animals that were anaesthetized with hypnorm and hypnovel.

3.11 SNP responses (*see* Tables 24-30, Appendix)

Responses were recorded in n = 52 vessels. The overall SAH E_{max} was 96±4 %, the overall SAH EC_{50} 3.8±0.7 μM: both values significantly differed from shams and non-operative controls (Table 28, Appendix).

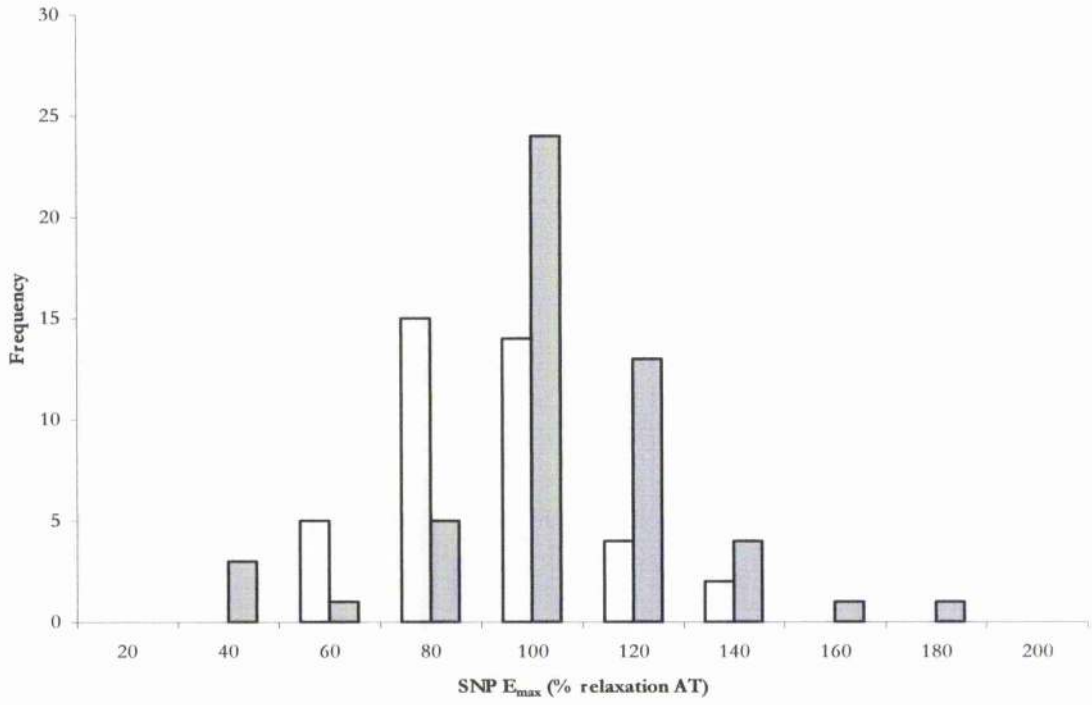
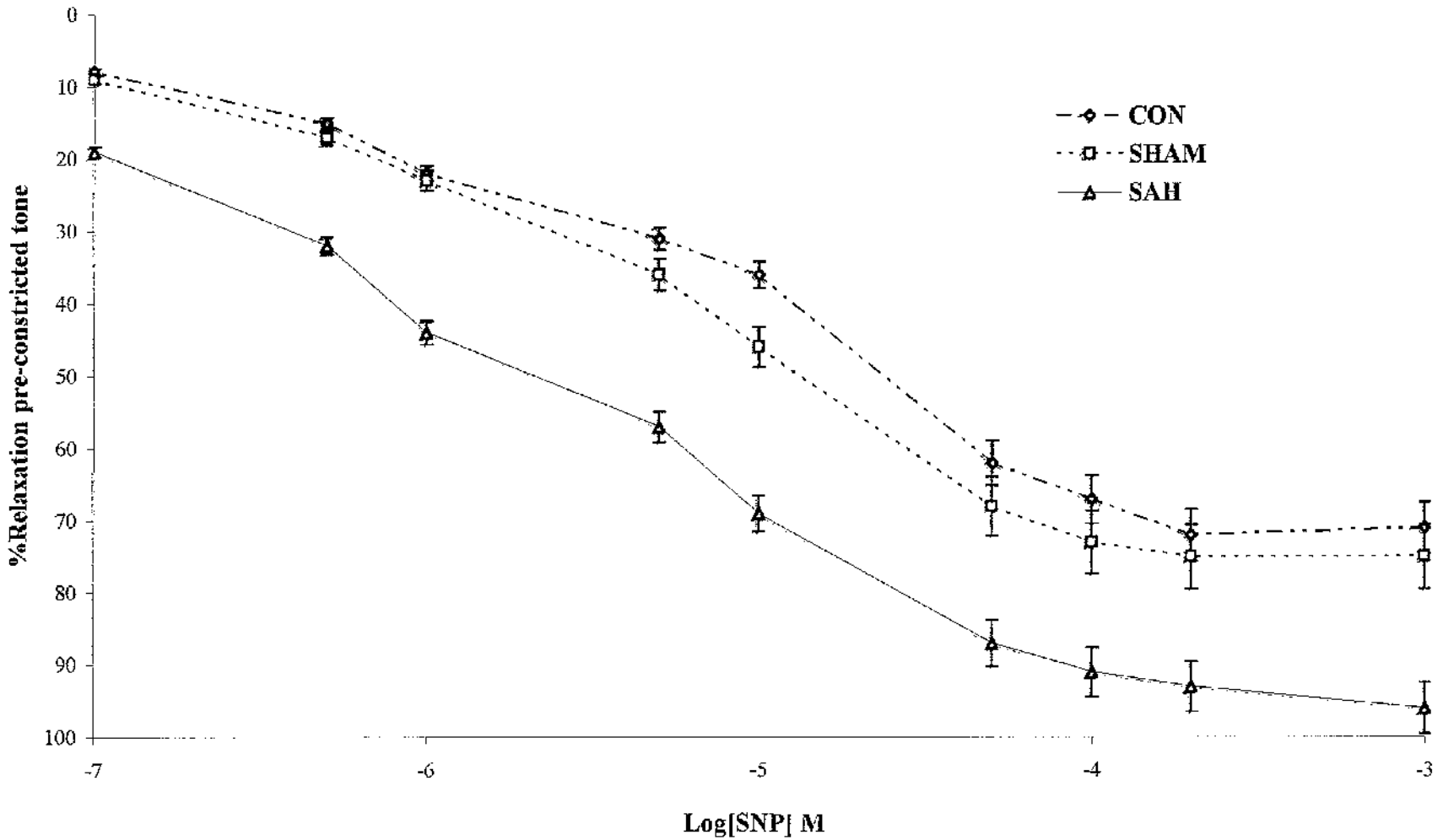


Fig 45. Combined non-operative controls, shams and SAH (Groups 1-3) SNP frequency histograms. *Key*: **white** combined shams and non-operative controls, **grey** after SAH. Note again the normal distributions and the significant right-shift following SAH. Note again how a significant proportion dilate >100% AT after SAH.

3.11.1. SAH sub-group analysis (*see* Tables 24-30, Appendix)

Table 27, Appendix, shows that—just as with the L-ARG EC_{50}—prolonged CCA-clamping (SAH Group 1) significantly obtunded the increase in SNP E_{max} that, overall, SAH (i.e. SAH Groups 1-3) otherwise produced. Thus, excluding Group 1 animals from analysis increased the overall remaining SAH group SNP E_{max} significance (i.e. in SAH Groups 2 & 3) further still [E_{max} = 99±3 %, $p << 0.0001$]. Similarly, the EC_{50} significance was also greater in SAH

Fig 46. Combined Non-operative controls, Shams and SAH (Groups 1-3) SNP C-R curves.



Groups 2 & 3, relative to shams and non-operative controls, after excluding SAH Group 1. Nevertheless, although suggestive, neither the E_{max} nor the EC_{50} were preferentially obtunded on the side ipsilateral to the clamping in Group 1 (Table 24, Appendix); nor, indeed, were any significant side differences demonstrated in SAH Groups 2 & 3 (Tables 25 & 26, Appendix). However, poor statistical n numbers for SAH Group 1 (n = 4 for each MCA) strictly render this analysis inconclusive.

Neither the E_{max} nor the EC_{50} values significantly differed with increasing survival after SAH (Table 29).

The mean E_{max} did not significantly differ across acute pressor response groups (Table 30). However, the mean SNP EC_{50} in type II & III pressor responses (1.5±0.4 μM) was significantly different from that in type I (7.1±1.3 μM; p = 0.007); which, in turn, did not significantly differ from either non-operative or sham operative controls.

NOTE: None of the results in this section were significantly affected by excluding the two animals that were anaesthetized with hypnorm and hypnovel.

PART 6

DISCUSSION

SECTION I Chapter 1
SECTION II Chapters 1-3
SECTION III Conclusions

SECTION I
Whole animal responses

Chapter 1 Intracranial and arterial pressor responses

SECTION II
In vitro vessel responses

Chapter 1 Normal MCA characteristics

SECTION I
Whole animal responses
Chapter 1
Intracranial and arterial pressor responses

1.1 Background

Blood pressure is commonly considered related to SAH only in the *elevated* state. Thus, hypertension may precipitate acute bleeds[603] and worsens outcome[132]. It is also often present in the immediate post-SAH period, where some consider it to represent the Cushing response (CHR)[163, 390]. In this context, the CHR is considered beneficial, serving to maintain CPP against elevated ICPs[163]. However, as we have already seen (p 14) the ICP usually falls to plateau values close to baseline levels within minutes following SAH[453, 454, 632]. Thus, a CHR (see below) cannot readily explain any hypertension seen at this time.

Alternatively, post-SAH hypertension may serve to maintain re-expansion of a previously collapsed cerebrovasculature necessarily incurred during acute SAH. In this context, it may thus serve to 'flush out' areas of microcirculatory collapse—some perhaps obstructed by intravascular clot—in order to subvert no-reflow phenomena (NRP). Notwithstanding, perfusion defects frequently persist, perhaps as a result of CBF restoration lagging behind ICP restoration[18, 306, 583]. Similar perfusion defects, however, are also seen in spite of increased MAP after a cardiac arrest[360]: thus, hypertension does not always appear capable of achieving its aim[180]. Indeed, especially with SAH, it may even be harmful (*see* above)[360].

1.2 Caveat: BP recording bias

BP recordings may be biased between studies by factors other than observer bias. One obvious example is the dichotomy between invasive and non-invasive BP monitoring. The majority of experimental models, for example, directly monitor intra-arterial pressure (although older experimental studies, of course, obviously did employ non-invasive cuff monitoring—Cushing's study being an example). Thus, direct inter-study comparisons of *absolute* BPs may be unreliable here. Furthermore, the site monitored may seriously affect the value obtained, since the MAP is increased in peripheral arteries relative to the aorta[663]. Moreover, almost no study has measured the value that really matters in SAH: i.e. that present within the ICA proximal to its entry to the cavernous sinus. Nevertheless, the direct inter-study comparison of *qualitative* changes—such as, for example, the frequency of acute hypo- or hyper-tension after SAH—should not be affected by such errors in quantification. In the current study, MAP was assessed from the distal aorta via the femoral artery.

Unfortunately, the current thesis has highlighted a further—hitherto little explored—error that may significantly affect both inter- and intra-study of BP magnitudes; and their display. The error discovered relates to the graphical extrapolation of absolute BPs from dynamic tracings to statically calibrated scales on a multi-channel, flat bed, pen recorder. As is demonstrated in Part 5, Section I, 1.1, such extrapolation will *always* yield erroneous systolic and diastolic BP values whenever the animal's heart rate exceeds the inertial drag of the pen on the recorder bed. Thus, the inertial drag of the pen (particularly at the speeds encountered with small animal heart rates) results in a shrinkage of the systolic BP—equal in magnitude to an inflation of the diastolic BP—when these values are extrapolated to the calibrated scale (Figs 7 a & b).

The error is incurred because the graphically calibrated scale is usually derived from static BPs via a sphygmomanometer: the pen recorder tracing, however, is produced by a dynamic BP.

As a result, the pulse pressure becomes artificially contracted with increasing HR in such displays, one possible example from this thesis deriving from the HR change observed with a CHR after SAH in Fig 11. In principle, one remedy for this error would involve some form of dynamic calibration: however, errors may continue to be incurred with further HR fluctuations throughout the study period. Other remedies are listed in Part 5, Section I, 1.1. Whatever the permutations, the quotation of systolic and diastolic BPs from such extrapolations ought to be avoided. Furthermore, the graphical displays—such as those in Figs 8, 10 & 11) of this thesis—necessarily provide contradictory information, because the systolic and diastolic peaks are uncorrelated to the scale provided. The only satisfactory way to overcome these difficulties is to employ a modern electronic digital display, with fluctuations recorded in real time and stored to hard disk.

Importantly, the MAP—*when this is obtained from the aorta*—remains correlated to the statically calibrated scale; because the MAP under these circumstances is equal to the arithmetic mean of the pulse pressure[663]. This is important, because it appears that the arithmetic mean of the pen recorder tracing—i.e. bandwidth mid-point—can continue to be extrapolated to the statically calibrated scale. This axiom simply derives from the fact that the pen must always pass through the mid-point of the two BP signals (Fig 7), even though it may not be able to reach the full extent of the excursion required by them. As a result, changes in BP throughout the study can be reliably assessed only by changes in MAP; lest considerable errors may be incurred (Part 5, Section I, 1.1). The magnitude of BP changes throughout this thesis, therefore, are based upon MAP values assessed as the bandwidth mid-point on the graphical display; and—where these were immediately written down—corroborated by the digital display from the electronic patient monitor.

1.3 Current experimental findings

In this study, SAH was most frequently heralded by the occurrence of **respiratory irregularities**, **blood-pressure disturbances** and **extensor-posturing**: a familiar triad. The latter was always a transient phenomenon. Respiratory dysfunction, however, sometimes persisted, and occasionally required ventilatory support. By far the most major disturbance was that of eventual **subacute cardiovascular collapse** (SCC: 42%). From this scenario, it would seem that a considerable SAH had been induced; yet neither a high ICP_{max} nor a CHR were *rarely* witnessed. In fact, the exact opposite pertained instead: a **moderate ICP_{max}** (38 mmHg) and an **acute hypotension** ($-\Delta MAP = 30\pm4$ mmHg; $p = 0.03$) [Part 5, Section I, 1.3]. In consequence, SCC was often preceded by the exact opposite of the sympatho-adrenal response ordinarily expected[115-117, 516]. Why was this?

The earliest experimental studies had shown that pressure on the brain could result in cardiac[109] and respiratory[636] disturbances. Following Kocher's previous ideas, Cushing presented his classical findings at the Mütter lecture in 1902[114, 115]. Here, in addition to noting respiratory irregularities and bradycardia, he proposed that an increase in intracranial tension "occasions a rise of blood pressure sufficient to overcome the high intracranial tension". Thus, the **CHR** did not occur until the **ICP** had **approached** the **MAP**: whereafter both pressures rose pari passu[114—117]. Cushing emphasized that the CHR primarily served to **maintain flow** to the medullary **respiratory centre** (it was not an attempt to restore global CBF), and that its occurrence at lower ICPs could, in fact, prove detrimental[115]. In contrast, the mean ΔICP in the current study (from 8±1 to 49±4 mmHg[236, 625]) was clearly far short of the MAP 123±5 mmHg. As a result, then, the CHR was rarely seen. But why should we have instead observed the opposite (acute hypotension)? And why was the ΔICP only moderate when SAH itself appeared severe?

In fact, in other studies where a classic CHR eventually supervened, a **$-\Delta MAP$** was additionally observed **at lower ΔICPs**[180, 533, 552]. Unfortunately, intracranial balloon expansion models were invariably used in these cases: this form of study necessarily incurring *heterogeneous* ΔICPs[115, 644]. Nevertheless, others have achieved similar results following subarachnoid saline injections (which cause homogenous ΔICPs)[258]. Indeed, acute but brief $-\Delta MAP$s clearly followed each subcritical ΔICP in Cushing's original study[115]. However, in none of the aforementioned studies was the ΔICP as rapid as it should have been in order to have mimicked acute SAH. Indeed, even in Cushing's study, the ΔICP was produced *gradually* in order to mimic the effects of space-occupying lesions or haemotomata[115, 116]. In consequence, **Cushing did not state the CHR in the context of acute SAH.**

However, Cushing did indeed refer to such a scenario. In his 'variants' to the classic response, Cushing clearly stated that **sudden ΔICPs** resulted in a complete "**checking of the heart**" for "up to thirty seconds"; from which the MAP then gradually recovered[115]. Although often infusing directly into the CM, close to the cardio-respiratory centre, Cushing also tried "many other sites" without this influencing the results[115]; and hydraulic effects would have been experienced homogeneously throughout the subarachnoid space anyway. In support of such findings, a more recent model (involving arterial rupture in cats) also recorded a –ΔMAP with ICP_{max} values of 50 mmHg[256]—a result similar to that observed in the current study. Therefore, it may be that events associated with *acute* SAH are quite distinct from those associated with more *subacute* ΔICPs, suggesting that the δ ICP/δt may be the determining factor here. Nevertheless, the CHR has been observed in some SAH studies following vessel rupture where an acute δICP/δt occurred[18, 306, 546]. In all of the latter, however, the ICP_{max} approached the MAP. Furthermore, either dogs or primates were used (often in small number)[306, 546]; whilst in some cases an *initial* –ΔMAP was clearly in evidence [18, 546]. Overall, then, the specific occurrence of hypotension following SAH may depend both upon the ICP_{max} and the δICP/δt attained. Each of these may depend, in turn, upon the species concerned; or even on the individual.

Perhaps, then, a syncopal response may reflect a less severe SAH? The problem here, however, is that post-mortem analysis regularly confirmed the general impression of extensive SAH (Plates 5, 7, 9). Clearly, then, syncope cannot relate to smaller bleeds; but, instead, to paradoxically **moderate ΔICPs** concurrent **with severe bleeds**. Hydraulic failure in the ICP monitoring can be ruled out as subarachnoid injections cause homogeneous ΔICPs[115, 258]; and blood clearly communicated with the CM posteriorly (Plates 5 and 7). Furthermore, the continuous ICP tracings obtained do not suggest obstructive interludes in this study (Figs 8, 10 & 11). Thus, the degree of SAH appears genuinely disproportionate to the moderate ICP_{max}, suggesting that the latter poorly reflects the volume of blood extravasated (V_E). In fact, the latter disparities have frequently been alluded to[221, 256, 257, 453, 561]. However, these are usually the *opposite* to that observed here: the ΔICPs in these studies is usually greatly out of proportion to the V_E [221, 453, 561]. Nevertheless, the pattern in some has been as that in this thesis[558, 625]. Therefore, these two states may represent extreme, **opposing variants** in the **ΔICP expected from** the V_E (*see below*).

1.4 Hypothetical determinants of the ΔICP after SAH

1.4.1 Small V_E : large ΔICP

Where one injected fluid produces the same ΔICP as a larger volume of another, this may reflect either inferior pressure-venting mechanisms; or the induced release of another fluid

into the confined space. The most obvious cause of the latter might seem to be more CSF: however, CSF production probably ceases acutely at 25-30 mmHg[522]. Another possibility, derives from clinical studies in which the **cerebral blood volume (CBV)** has been shown to **increase after SAH** (Part 1, 1.1.1)[222-224, 349, 453, 491]. These studies suggest an acute imbalance in inflow-outflow to the cranium. Cushing drew attention to the early closure of veins (including the large venous sinuses) and capillaries following ΔICP[115]. The latter probably occurs at ICPs around 30-35 mmHg[212]: but it may occur sooner with SAH, since α-receptor affinity is much greater within cerebral veins[237]. Such cerebrovascular 'back-damming', then, would favour a **cerebrovascular expansion**[491]. A simultaneous vasoparalysis[349, 454] occurring proximally would thus accentuate the engorgement, raising the ICP pari passu with the V_E. Such a mechanism much better explains the steep and rapid attainment of an ICP_{max}, up to the level of the MAP, from studies such as those by Voldby[632]. The rapidity with which a vasoparalysis may occur, and its occurrence with often minimal SAH, suggests a local vascular reflex[453, 651]. Since the brain parenchyma is virtually incompressible, such a mechanism would as effectively **tamponade SAH** as any other[651]. In consequence, the V_E would be expected to be much smaller with this mechanism; the ICP_{max} and the $\delta ICP/\delta t$ large.

A small V_E associated with a large ICP_{max} may therefore be explained by a non-compliant subarachnoid space; or by the presence of an acute cerebrovascular engorgement.

Larger ΔICPs were significantly correlated with type II (invariant: ΔICP = 50±6 mmHg) and type III (Cushing hypertensive: 73±4 mmHg) responses in the current study.

1.4.2 Large V_E: small ΔICP

This, of course, is the case in the current study, and must necessarily preclude any aforementioned reflex vascular expansion. It essentially suggests particularly **efficient ICP-venting**[115, 121, 453, 454]. This may involve unusually compliant meninges; or, instead, rapid CSF displacements from the subarachnoid space. In support of the latter, Seiro found it difficult to maintain the ICP_{max} in his studies as if due to some continuous CSF-leakage[541]. Indeed, Cushing had been warned (by Adamkiewicz) that such leaks, into the venous system, might threaten cardiac overload in his model[115]. Although this did not ultimately occur (only 100 mls were expressed in 30 mins in his study!) Cushing readily acknowledged this "free communication"[115]. This was nowhere more dramatically witnessed than following a burst of the intracranial balloon, whereupon mercury was subsequently discovered in the "sinuses, jugulars, right heart and lungs"![115]. Conceivably, such venting may be greater in some individuals than in others[343, 344]. This may, therefore,

account for the similar failure of other studies (with either rats[558, 625] or cats[256]) to demonstrate an ICP_{max} approximately equal to the MAP after SAH.

However, another factor may be that of reflex reductions in CBF or CBV—i.e. the opposite of the aforementioned cerebrovascular expansion. Any acute fall in the arterial driving pressure must necessarily reduce SAH from an acute jet to more that of an ooze. Such a mechanism would be expected to slow down the δICP/δt (Fig 8) as well as—by allowing more time for venting mechanisms to take hold—limiting the ICP_{max}. One mechanism here could be that of an **acute** cerebral **vasospasm.** This is certainly implied in one study, where the injection of subarachnoid blood yielded greater CBF falls reductions than did saline for the same degree of ΔICP[558]. However, another mechanism still would be that observed in the current study: i.e. **acute hypotension (–ΔMAP).**

A large V_E with a moderate δICP/δt and moderate ICP_{max} may occur where efficient subarachnoid venting mechanisms have combined with cardiovascular mechanisms to limit CBF. The latter may involve either an acute cerebrovascular spasm, or an acute systemic hypotension.

Smaller ΔICPs were significantly correlated with a type I (acute hypotensive: ΔICP = 31±2 mmHg) responses in the current study.

1.4.3 The ICP_{max}: MAP_{final} ratio

The significant –ΔMAP produced in the current study [–ΔMAP = 30±4 mmHg] significantly elevated the ICP_{max}:MAP ratio from a possible 31±3 % (had hypotension not occurred) to 41±3% after SAH [p = 0.02]. Importantly, the latter ratio was only slightly significantly different from either type II or III pressor ICP_{max}:MAP ratios (p = 0.46), whereas the former value was markedly different (p = 0.001: Part 5, Section I, 1.3). This suggests that relatively modest ICP_{max}:MAP ratios—perhaps only in the range 40-60%—are required to stanch SAH. It may be crucial that this ratio is obtained at **lower absolute values** for both ICP_{max} and MAP where a syncope (i.e. –ΔMAP) has occurred. Thus, SAH will not only be stanched with less compressive cerebral strain, but also with less shear stress to the supplying cerebrovasculature. Quite clearly, syncope could be advantageous with SAH. The **correlation** between **–ΔMAP and ΔICP** in this study (Fig 9) suggests that syncope may be especially associated with efficient ICP-venting mechanisms: thus, it may serve to aid a naturally 'weak' tamponade. Certainly, a CHR could prove counterproductive where efficient ICP-venting prevails; this, perhaps, explaining its absence where ICP_{max}<<MAP (Fig 9).

1.5 Mechanisms of arterial pressor responses after SAH

To explain the pathophysiological mechanisms involved some have postulated a progressive march of ischaemia cranio-caudally throughout the CNS with progressive Δ ICP. In the first phase (ICP_{max} <MAP), the **cerebrum** alone is **ischaemic**[180, 343, 344, 546]; this resulting in decreases in HR, MAP and in cardiac output[516, 533, 552]. The latter effects are attributable to **'vagal activation'**: certainly the bradycardia can be prevented by sectioning of the vagi[115]. As the pressure wave progresses (ICP_{max} ~MAP), the **upper brainstem** becomes **ischaemic**, this causing a mixed vago-sympathetic output manifest as the **CHR** (bradycardia, hypertension and respiratory irregularity). Eventually (ICP_{max} >MAP) the **lower medulla** becomes **ischaemic** where (somehow) the parasympathetic response is singularly lost, this allowing the SNS alone to dominate. The latter is manifested in a general **hyperdynamic state** of increasing heart rate and MAP: a common agonal feature of brain death[516, 533, 552]. The current results—i.e. acute hypotension (64%)-invariant (30%)-acute hypertension (6%)—certainly suggest that two opposing 'forces' (i.e. one 'depressor' [–ΔMAP] and one 'pressor' [+ΔMAP]) may be in operation such that either one may predominate (depressor = hypotension: pressor = hypertension); or that both 'forces' may be in balance (invariant).

The first stage, then, resembles the syncope observed in the current model; where modest Δ ICPs may only have affected hemispheric flow. The term 'vaso-vagal syncope' (VVS) was coined by Lewis, in 1932, to explain the slow pulse and hypotension accompanying a faint[229]. However, VVS is certainly an autonomic hybrid: the patient is also pale and clammy—frequently with dilated pupils. Indeed, the bradycardia—as in the current study—may even be absent, or replaced by tachycardia[229]. Furthermore, VVS can be triggered by emotional factors and pain—stimuli which ordinarily activate the sympatho-adrenal axis[229]. **VVS** is characterized by both a **sudden hypotension** and a **failure of cerebral autoregulation**: thus, cerebral infarcts may accompany mistaken attempts to prop the patient up[136]. VVS most likely results from excessive dilation in splanchnic and skeletal muscle vascular beds[229]; thus implying a loss of sympathetic tone. Certainly the –ΔMAP seen with sympatholysis closely resembles that seen with sympatholysis in cord shock (Part 2, 2.6). That Cushing noted the opposite—"anaemic bowels"—with the CHR, suggests a fundamental linkage between the two processes[115]. The occurrence of VVS with emotional shock is of uncertain teleological value[229]: its occurrence with SAH, however, may be entirely appropriate; serving to augment the stanch of the bleed. It is not without relevance that in some cases of SAH, where headache (and therefore ICP-loading) appeared minimal, the presentation was actually that of syncope[36]. It is important to remember that the **current experimental findings** were recorded in an animal that was normothermic, previously cardiovascularly stable, but above all **supine**: the **effects** of

SAH-syncope would clearly be **more marked** given contrary circumstances—in particular, with an animal in the **erect position**.

1.6 Subacute cardiovascular collapse (SCC) after SAH

SCC occurred in 42% of animals and resulted in the premature termination of the study within the arbritary three hour period. All animals that survived into the third hour did not suffer SCC. It has been argued above that SAH-syncope (i.e. type I acute pressor response) may be theoretically less stressful both to the cerebrovasculature, as well as less stressful to the cerebral parenchyma. Nevertheless, SAH-syncope did not appear capable of protecting against a subsequent SCC (Table 20, Appendix). In fact, SCC appeared equally as likely irrespective of the acute pressor response that preceded it. The apparent 'severity' of SAH—as determined by a subsequent SCC—therefore cannot be predicted by the type of acute pressor response that precedes it. Indeed, the very fact that SCC supervened—in the vast majority of cases—in the *absence* of the CHR appears contrary to the view that SCC (and, therefore, early mortality) necessarily results from activation of the hypothalamo-sympatho-adrenal axis[74, 158, 380, 412, 446, 460, 462, 485, 493, 516, 552, 602, 656, 665]. However, that a CHR—and, therefore, a sympathetic "storm"—has been recorded in numerous other studies, hints at the likelihood of either species (or even individual) variation. It must be remembered, however, that all clinical recordings have necessarily witnessed only secondary SAH, which may be an entirely separate 'statistical' event.

In many ways the results of the current study accord, then, with those obtained following head injury. In some of these studies, an initial –ΔMAP—with +ΔMAP rebound—had finally given way to one of SCC (modest ΔICPs occurring throughout)[69, 180, 214, 258]. Such results, therefore, favour non-catecholaminergic mechanisms, such as those recently proposed involving cytokine-release— and, possibly, cytokine-mediated inductions of NOS (Part 3, 1.8)—within the myocardium. In the latter site, NO has been shown to obtund β-mediated adrenergic contractility[26, 93, 125, 242, 410]. It is interesting that high levels of cytokines have been recorded systemically in cases of SCC[26, 535], as they have also been within the CSF after SAH[439, 443]. The normal findings on routine histopathological (or electron microscopic) sectioning of the myocardium (Part 5, Section I, 2.3) are not surprising in this context, since others have assessed that at least 6 hours are required for their development[331]: this was beyond the scope of the present study.

1.7 Conclusions

- Abrupt δICP/δts after SAH in rats may elicit acute hypertension, acute hypotension, or no BP change at all (invariant).
- The most common acute pressor response after SAH in rats is acute hypotension (64%): hypertension is rare (6%). An invariant pressor response is seen acutely in the remainder (30%). These results directly agree with Cushing: hypertension was not stated with *abrupt* δICP/δts—hypotension was stated instead.
- The height of the ICP_{max} correlates with the acute pressor response seen. Hypotension predominates with ΔICPs ≤ 65 mmHg: hypertension prevails with ΔICPs ≥ 65 mmHg.
- The ICP_{max} does not correlate with the V_E.
- Subacute cardiovascular collapse (SCC)—and early mortality—after SAH is equally likely (approximately 40%) irrespective of the type of acute pressor response observed.
- The prevalence of acute hypotension with SCC suggests that non-catecholaminergic mechanisms may be primarily responsible.

SECTION II
In vitro vessel responses
Chapter 1
Normal MCA characteristics

1.1 MCA diameter asymmetry and its effect upon agonist response

1.1.1 Background

When setting about the study of isolated vessel responses, it is usual to restrict investigation to a particular branch, such as that of the "proximal middle cerebral artery". This is a statement inferring the ultimate comparison of 'like with like' between the different groups considered. Usually the diameter-range is given in such studies but, since the same branch is used, it is invariably assumed that they will all be comparable; even where size differences may be appreciable.

It is frequently the case in small vessel studies, however, that a large variance of agonist response is obtained; with correspondingly large standard deviations (SD). This renders statistical analysis less attractive, forcing either large samples or large response differences in order to prove statistical significance. Such variance in agonist response may largely be due to natural variation in, for example, receptor-density on the vessel wall. However, it may also result from a failure to compare 'like with like'.

1.1.2 K_{max} varies linearly with FD_{90}

As is commonly the case, the variance of K_{max} values within each experimental group appeared large and did not very closely approximate to that of a normal distribution (Fig 16). However, the distribution itself was symmetrical, with similar mean (5.5 mN) and median (5.4 mN) values. It was felt throughout the study, however, that the diameter had appeared important in determining the efficacy of contractile responses obtained. A clue to this became apparent upon correlating the FD_{90} (μm) with the K_{max} (mN) in each vessel used. Here a frequency histogram much more closely resembling that of a normal distribution was obtained (Fig 17). This apparent dependence of agonist response upon vessel diameter was demonstrated more clearly still by simply plotting K_{max} against FD_{90} (Fig 18). Over the range of diameters encountered, this approximated to a linear relationship, and conformed to the regression equation:

$$\mathbf{K_{max}} = (0.031).\mathbf{FD_{90}} + (0.6)$$

The correlation obtained was highly significant ($r = 0.75$, $n = 34$, $p<0.001$).

Such a relationship gained further credence upon consideration of other groups and agonist responses. For example, for KCl responses in the sham operative group (Fig 28) the equation was again quite similar:

$$\mathbf{K_{max}} = (0.038).\mathbf{FD_{90}} - (0.2)$$

and with even less spread about the regression line ($r = 0.78$, n = 16, $p<0.001$).

Receptor-mediated constrictions also yielded linear relationships to similar degrees of statistical significance. Table 31, Appendix lists all such correlations and significance values for individual regression plots in each agonist concerned. From this, it can be seen that up to 76% of the variation in certain agonist E_{max} (i.e. PGF2α in shams) may be explained merely by variations in FD_{90}. From the above equations it can be noted that, for example, the K_{max} values predicted at 200 μm (6.7 mN and 7.4 mN respectively) are both nearly twice that predicted at 100 μm (3.6 mN and 3.6 mN respectively). That is, an **MCA** that is **twice the size** of another may be expected to **produce twice the contractile force** in response to a membrane depolarization. How might such findings tally with simple physical considerations?

1.1.3 Theoretical implications

The tension developed within the walls of elastic structures when subject to intraluminal pressures exerted radially is described by the law of Laplace as:

$$T = \frac{P.a}{th}$$

This implies a linearity in tension development in response to passive distension, and is demonstrated in the distension-diameter study obtained with pulmonary arteries in Fig 13. Such vessels function purely as passive capacitance vessels within the systemic circuit, the FD increasing proportionately with increasing luminal pressure ($r = 0.98$, n = 13, $p<<0.001$). A similar background passive tension must necessarily be apparent with the MCAs here as these have also been subject to distension. But unlike pulmonary arteries, MCAs develop **myogenic tone** (**MT**) to distension (Part 2, 2.2). This was reflected in the **spontaneous vasomotion** of all vessels used, this being a feature of pre-capillary sphincters, and concords with other studies[22]. In such vessels, MT increases proportionately with increased distending pressure (Fig 14), this serving to maintain stable diameters and, therefore, stable flow (autoregulation). In consequence, each **MCA** is likely to possess a certain amount of both **passive and active tension prior to** each KCl depolarizing **constriction.**

The circumference at 200 μm is, of course, twice that at 100 μm (since this is π.diam.). If VSMCs are arranged in a predominantly concentric fashion within the tunica media then, from first principles, we would expect twice as many to be present at 200 μm as at 100 μm. This, of course, assumes that each VSMC is approximately similar in length, and that each is arranged in series. If this is the case, then one would not be too surprised to find the overall constricting force present at 200 μm to be twice that at 100 μm. Furthermore, since this will be additive to any passive elements, the overall tension developed at 200 μm ought to be double that at 100 μm, given the appropriate stimulus. Thus, the $\mathbf{K_{max}}$ at **200 μm ought** to be **twice** that at **100 μm**. Furthermore, if each VSMC is the same size (and has the same properties as any other), then we might expect that it shortens to the same degree as its fellows given the same pharmacological stimulus. Thus, following appropriate stimulation, luminal size will continue to be, in the larger vessel, twice that of the smaller one. As a result, it can be seen that the simple **relationship** between **agonist response and functional luminal diameter** is in **accordance with** correspondingly simple principles based upon a **concentric arrangement** of the **VSMCs** present within the **arterial walls**. Indeed, although as a general principle one should not extrapolate outside of the regression range obtained, it is clear from the graphs that the regression lines invariably pass close through the origin; which, with greater experimental numbers, may possibly become even more apparent. If so, then this would again accord with simple physical considerations, as a vessel with zero diameter would not be expected to produce any tension.

1.1.4 Practical implications

In **most experiments** on small vessels, agonist responses between different experimental groups are often only compared by virtue of their summary statistics, there being **little attention** given **to either** the **diameter or** the **anatomical side** from which the vessel is derived. In large samples, and with randomization for side, such errors ought to be minimized (as was shown in this study: Part 5, Section II, 3.1) . However, with cerebral arteries even this may not be the case. Thus, in a recent cadaver study of 145 vessels[347] the mean right vertebral artery diameter (4.5 mm) of the group was some 13% larger than the left (4.0 mm), with the largest difference being 280% (2.5 mm against 7 mm)! Such differences in size (an increase of x1.3 will result in a x3 increase of flow: Part 2, 2.4) may have appreciable effects in vivo. However, they will also have important—and unwanted—effects with in vitro experimental studies. Thus, although the mean FD_{90} remained remarkably constant across both experimental groups in the current study (Table 5), this belies the fact that there was **significant asymmetry** present in up to 50% of the animals used. As Table 9, Appendix, shows, a difference in size between right and left

MCAs was apparent in these cases, similar to that seen in the overall results of the aforementioned study[347].

The importance of the response-diameter association herein established may therefore apply to *any* small vessel study where small numbers are involved. But it especially applies, however, to **unilateral studies** of **cerebral vessels** in which the **contralateral side** is used to supply the **paired control**. Such models are particularly common with unilateral stroke study, and are currently gaining credence with type IIb SAH studies using craniotomy (Part 4, 1.2.2). For example, from the non-operative controls in Table 9, by taking only the combinations where the right MCA exceeded the left by ≥1xSD (bold type) we obtain a mean right MCA of 171±21 μm compared to a left of 111±26 μm. From the regression equation (Fig 18) it can be seen that the K_{max} will be significantly larger in the right MCA (5.9 mN) than the left at 4.0 mN ($p<0.01$). Thus, by making the systematic error of choosing these animals for a unilateral 'paired control' study, we might quite wrongly attribute a significantly greater *right* MCA response to the effects of the procedure performed. But in reality, this simply resulted from the fact that the right MCA *diameter* was significantly greater ($p<0.001$), the increased K_{max} being simply a consequence of this. As a result, studies of both in vitro and in vivo vasoreactivity must therefore take into account the highly significant degree of diameter-asymmetry that is present within the cerebral vasculature.

1.1.5 Conclusions

Although the large variance obtained in agonist response with small vessel studies may be attributed to such factors as receptor density, this study clearly shows that another factor is that of diameter-variance; at least within cerebral arteries. In fact more than **half** of the **variance** in **agonist response** obtained with agonists such as KCl, 5HT and PGF2α may be **explained** simply by **variance** in **vessel size**. In these cases variance in factors such as receptor or ion-channel density appear less significant. However in other cases, such as responses obtained with 5HT or, especially, NE (*see below*), receptor-mediated factors appear much more significant. In consequence, this study emphasizes the **regular quotation** of **diameters** in **all studies** comparing agonist responses (especially those involving KCl, 5HT and PGF2α), but **in particular** with **unilateral studies** using the **contralateral vessel** as the **paired control**. However, by obtaining **response-diameter plots** in non-operative controls, due allowance can be made for diameter-effects by noting **deviations** in '**observed from expected**' **responses**. Indeed, in some cases, such plots may indicate diameter effects to be almost disregardable (NE responses, *see below*). The failure to quote diameters in many current SAH small vessel studies might be a critical

factor in explaining some of their failure to explain—or to procure efficacious treatment strategies for—either delayed VSM or DCI.

1.2 Heterogeneity of NE response: pathophysiological implications

1.2.1 Introduction

The role of the SNS in governing CBF is largely unknown. Its role in the systemic circulation is largely one of redistribution[663], although it also serves to maintain MAP. The latter is well evidenced upon its sudden removal, as in spinal cord transection, where acute decreases in MAP to 40-60 mmHg can occur[663]. Nevertheless, most have found adrenoceptors to mediate much poorer responses within the cerebrovasculature (Part 2, 2.6). Because of the brain's vital function, a limited role for sympathetic regulation—and, therefore, a limited means to divert flow elsewhere—may thus be considered a convenient adaptation. Nevertheless, sympathetic regulation may be more prominent with extremes of MAP; or with intense tissue metabolism (Part 2, 2.6).

1.2.2 Current experimental findings

The current study confirmed relatively poor NE_{max} values within rat MCAs (Figs 23 & 24; Table 31, Appendix). Thus, the mean 'responder' NE_{max} at 3.2±0.3 mN was significantly weaker than either the K_{max} (5.5±0.3 mN, $p = 0.0001$) or the $PGF2\alpha_{max}$ (5.1±0.4 mN, $p = 0.0006$). A novel finding from this thesis, however, was that NE responses were singularly **heterogeneous**. Thus, whereas a poor E_{max} to one agonist was invariably associated with an equally poor response to other agonists, responses to NE were frequently poor despite adequate E_{max} values to other agonists (NE-'subresponders'). The plot of NE_{max} v FD_{50} initially yielded a wide scatter with minimal linear correlation: indeed, as little as 10% of NE_{max} variance could be attributable to FD_{50}.

Clearly, the major factor distorting the E_{max} v FD_{50} correlation was the presence of numerous NE-'subresponders' (Figs 23 & 24). Upon their exclusion, a linear relationship—with correlation equal significance to that with other agonists—could be restored ($r = 0.62$, $p<0.01$). It was therefore decided to exclude such 'subresponders' from analysis of continuous data, but to analyze them instead as **categorical data**: a manoeuvre also justified by 'subresponder' NE_{max} values being within the range of baseline vasomotion (Fig 12). NE 'subresponders' are clearly discernible in the lower portion of Fig 23 where with $NE_{max} \leq 1.0$ mN. Because 'subresponders' could occur contralateral to a 'responder', NE responses could be **markedly asymmetrical** in some cases; whilst **absent** altogether **in others**.

That poor NE responses were genuine, and not the result of a faulty solution, was confirmed in three ways. Firstly, in nearly a half of these the contralateral vessel displayed an adequate response, in the same bath (Tables 10-14, Appendix). Secondly, whenever bilaterally poor, a freshly made up solution also failed to elicit a response. And thirdly, a 'poor-response solution' when kept and subsequently tried again (on another pair of vessels) usually produced normal—or even supranormal—responses. Thus, it appeared that a genuine subgroup of subresponders was present in this sample.

1.2.3 Theoretical implications

A clue to explaining such heterogeneity was proffered by Tsukahara et al who attributed weaker animal NE_{max} values to a greater preponderance of $\alpha 2$ receptors[607]. The more efficacious response in human vessels, therefore, related to a greater $\alpha 1$:$\alpha 2$ ratio—the $\alpha 1$ receptor allegedly mediating larger responses[607]. Unfortunately, the actual explanation may be somewhat more complicated, since β adrenoceptors are also present within the cerebrovasculature[151, 379, 511]. In fact, all three receptor types may be present upon the same VSMC (Part 3, 1.3): this alone giving rise to eight possible combinations (with five differing types of net effect :Table 7). Indeed, the situation may be further complicated still, as NE also elicits the **simultaneous release** of **endothelial-derived constricting** *and* **relaxing factors**[237, 299, 320, 321] (Part 3, 1.3). It is quite possible, in fact, that $\alpha 2$-EDRF release explains diminished responses, as this may be the basis behind similarly reduced responses in the coronary resistance circulation[299].

The ultimate effect in any one target cell thus depends upon the precise combination of receptors and effects present, this explaining almost all of the NE_{max} variance[131]. For example, the presence of β receptors may possibly explain asymmetrical NE-responses since in one study left-sided β receptors were extraordinarily fickle to minor insults[379]. However, β receptors are rare in the **proximal MCA**, most evidence indicating instead a **predominance** of **$\alpha 1$ receptors** (in both rats and humans)[237]. This suggests, then, that more specific factors are important, such as **relative α receptor density** and the prevalence of **receptor uncoupling**[237, 620]. Although there is theoretically greater potential for 5HT receptor heterogeneity (Part 3, 1.4), it is interesting that in this thesis 5HT responses were more closely linear in their E_{max} v FD_{50} correlation than NE (Figs 21 & 23).

1.2.4 Conclusions

In addition to the anatomical irregularities and asymmetry that abound within the cerebrovasculature[87, 253, 347, 396], the current study also provides evidence for a similar degree occurring at receptor levels. Thus, even after allowing for diameter-effects—and even after restricting study to a small segment of a major cerebral artery (thus reducing

gross receptor-variance[237, 620])—considerable variance was obtained in NE-mediated responses. Bevan et al have recently demonstrated that such poor responses may relate to both a sparse innervation, as well as to a poor receptor-mediated response[50].

The current results suggest that in 12.5% of individual rats (i.e. the proportion of bilateral 'subresponders' in non-operative controls: Table 10, Appendix), the regulatory role of the SNS on CBF through MCAs may be significantly compromised by a relative failure in agonist-receptor response. Furthermore, in a further 12.5%, SNS regulation may only be present on one side: a global sympathetic activation producing perversely asymmetrical effects. Since the larger part of CBF is conducted through the MCA (Part 2, 1.1), this finding could have significant physiological consequences. Eccles & Eccles have commented upon autonomic asymmetry in the clinical setting with head and neck structures[145]. At present, the SNS is thought likely to play its part only at extremes of CBF; with myogenic and metabolic regulation controlling the CBF between these limits (Part 2, 2.4 & 2.5). Should the current results extrapolate to humans and to other cerebral arteries, it is conceivable that an **absence** of **protective adrenergic constriction** (i.e. presence of NE-'subresponders') may be responsible for the bounding CBF of an acute **migraine**, since SNS regulation may be more critical in a setting of gross metabolic dilatation (Part 2, 2.6). It is interesting that migraine afflicts 15-30% of the population in this regard[642], since this is the proportion of animals possessing either one or both MCAs subresponsive to NE (migraine can, of course, be either a unilateral or bilateral affectation; depending on the individual).

Table 7. Theoretical adrenergic receptor combinations on VSMCs

Receptor types on VSMC	Effect
1. —	0
2. α1	2+
3. α2	1+
4. β	–1
5. α1.α2	3+
6. α1.β	1+
7. α2.β	0
8. α1.α2.β	2+

Assuming that α1 receptors mediate more powerful constrictions than α2 receptors[607], and that β receptors mediate dilatory effects comparable in magnitude to α2 constrictions, the following ranks of net response can be obtained: α1 = +2, α2 = +1, and β = –1. (—) denotes an absence of receptors. (**NB endothelial-derived relaxing and constricting effects have *not* been considered**). Note that, overall, only five differing types of net effect are produced.

The failure to note the presence of discontinuous agonist responses—herein epitomized by NE response heterogeneity—in many current SAH small vessel studies might be a critical factor in explaining some of their failure to explain—or to procure efficacious treatment strategies for—either delayed VSM or DCI. Such discontinuity precludes normal statistical analysis, since this requires that data are normally and continuously distributed. As a result, discontinuous data should be analysed separately from continuous data.

1.3 NO responses and autoregulation

NO was assessed in this study both by its endothelial production from L-ARG—and the latter's subsequent inhibition by L-NAME—as well as by its effectiveness as a substrate for VSMC GC by analysis of SNP. The fact that in this study the L-ARG E_{max} ~ SNP E_{max} suggests two major points (Table 28, Appendix). Firstly, it suggests that the endothelium was not unduly affected by the mounting procedure, in spite of the risks involved[431]. Thus, NO released directly into VSMCs by SNP donation achieves the same effect as that produced by endothelial release. Secondly, it indirectly supports the concept that agents such as SNP do indeed achieve their effects by the direct liberation of NO within the target cell (Part 3, 1.10). The fact that the endothelium remained intact may not necessarily relate to exquisite handling as the cerebrovascular endothelium (due to the relative importance of the BBB: Part 2, 1.3) may conceivably be more 'robust' than it is systemically.

There are numerous reports in the literature concerning the role of NO in governing CBF autoregulation. The results of the current study suggest that NO rarely affects baseline MT (i.e. E_{max}>100%, *white* right hand tails: Fig 43). Since numerous studies have shown that NO does influence CBF, this suggests that factors other than MT largely control CBF in vivo (i.e. AT!). NO is, however, co-released in vivo in submaximal amounts concurrent with both AT and shear stress (Part 3, 1.8 & 1.9). The results of the present thesis suggest that L-NAME would elevate the mean PGF2α E_{max} in normal MCAs from 5.1 to 6.4 mN, producing a value for '**basal NO release**' at **26%** of the **total NO** MCA **release possible** (Part 5, Section II, 1.10). The latter value is well within the range (20-40%) of that seen systemically. However, the relative importance of 'basal NO release' in vivo will obviously depend on the extent to which overall MCA tone is dictated by the AT:MT ratio. Since SNS tone may ordinarily be as small as 5% (Part 2, 2.6), 'basal NO release' would not be likely to influence matters much here. Nevertheless, the role of NO would certainly increase as the role of the SNS does under more extreme circumstances (Part 2, 2.6).

The precise source of AT in CBF regulation in vivo appears to be largely unknown. One possible factor, however, not entertained in most NO-CBF studies, is that of the anaesthetic agent in creating an artefactually *abnormal degree* of background AT. This would have

serious implications for the perceived role of NO in autoregulation. This criticism, of course, underlines one of the major problems of in vivo studies in general (Part 4, 4.1). But if VSM after SAH does relate to the inhibition of NO-mediated VSMC relaxation, then it would seem from the above that—at least with SAH—considerable AT must also be present within the cerebrovasculature. Such AT could, of course, derive from agonists within the clot surrounding the vessels after SAH. What does seem apparent from the current results, however, is that MT *alone* is not likely to be the major source of tone, simply because NO—even in maximal amounts (e.g. [L-ARG] = 200μM)—rarely appears to affect it (i.e. L-ARG E_{max}<100%): at least not in normal control vessels (Part 5, Section II, 1.10).

1.4. Hypoxia, HA, PPV and myogenic tone (MT)

1.4.1 Hypoxia

Of all the factors suggesting that MCAs develop MT to luminal distension, the relaxation achieved with at least 15 mins hypoxia provides the most substantive. With this, MCAs were progressively dilated from baseline conditions without any preconstriction from PGF2α or any other agonist. Such baseline dilation must ipso facto represent MT dilation. Since hypoxia achieves cerebral vessel relaxation via non-NO EDRF release—the agent presumably being PGI_2[193]—this, then, provides further confirmation for continued endothelial function in spite of the perceived risks of the mounting procedure incurred (*see above*). More specifically, it implies that hypoxia may be a powerful effector of dysautoregulation within vessels where MT represents a major source of overall tone.

The value obtained by hypoxic dilation also allows us to assess the relative contribution of spontaneous MT to that of the overall constricting power extant in MCAs. Thus, taking a maximal constrictory power in normal MCAs as that obtained from the KCl E_{max} (5.5 mN), and taking the total MT as being that obtained from hypoxic dilation (3.6 mN), **MT** can be seen to **represent 40%** of the **total tone possible**. This value for MT is in fact close to that obtained (20-40%) in other studies (Part 5, Section II, 1.13). The ratio of MT:AT within isolated MCAs is, therefore, approximately 2:3 from this study.

1.4.2 HA and PPV

As each respective frequency distribution shows in Figs 25 and 40, both PPV and HA in maximal concentrations are likely to risk dysautoregulation in vivo in approximately 35-50% of normal MCAs because, in such cases, some amount of background MT is dilated in addition to the complete abolition of AT. Such a distribution may be reflected, then, in the wide variety of responses obtained with such agents across different studies. For example, injection of HA has been noted by one group to cause cerebrovascular dilatation and

headache[73]: this possibly reflecting dysautoregulation. In similar fashion, one other group demonstrated an increased CBF and ICP following a PPV infusion[400]. In marked contrast, no observable effect was apparent in one other study in which HA was infused directly in to the CCA[452].

Since HA mainly achieves vasodilation via HA2 receptors, in both rats and humans (Part 3, 1.11 & Fig 26), this would imply that HA2 blocker therapy, such as with ranitidine or cimetidine, might be potentially protective against sudden massive central releases, such as may occur with a cerebral anaphylaxis. Thus, HA2 blockers may be conceivably prophylactic, for example, with cerebral trauma. Indeed, such therapy may prove particularly efficacious in tumour neurosurgery, as tumour vessels appeared unusually HA2-responsive in one study[452]. The implications of PPV usage will be considered, in turn, in more detail later.

1.5 Interaction between PPV and SNP: evidence for synergism?

PPV and SNP may often be present in significantly high concentrations at around the time of aneurysmal clipping. This is because PPV is often administered routinely by some groups at craniotomy closure following a successful clipping; and because SNP is often administered during aneurysm manipulation (for example, both manoeuvres represent standard Neurosurgical practice in the Royal Hallamshire Hospital, Sheffield). SNP and PPV both effect relaxation by the two cyclic nucleotide mechanisms possible (Part 2, 3.5). Thus, PPV vasodilates via cAMP-PKA mechanisms; whilst SNP dilates via cGMP-PKG mechanisms. As both PKA and PKG share homologous biochemical structures and catalytic properties, it is not surprising that evidence of a possible interaction between the two has increasingly accumulated (Part 2, 3.5).

In the current thesis, it was clearly found that responses obtained with such a PPV-SNP combination were more pronounced at each stage, confirming such interaction. Thus, the combined C-R curve (Fig 27) was clearly left-shifted relative to either individual control curve, with both significantly enhanced efficacy (E_{max}) and potency (EC_{50}) [$p = 0.01$ and 0.014 respectively]. Apart from providing some evidence for an interaction at the subcellular level, these results also present important clinical ramifications. Most importantly, they suggest that combined maximal concentrations **seriously threaten cerebral dysautoregulation** in a manner comparable to that seen with hypoxia. Thus, a profound **cerebrovascular paralysis** may result, the CBV passively expanding to the dictates of the MAP. This may threaten the microcirculation, causing cerebral oedema formation (Part 2, 2.5).

Alternatively, however, a PPV-SNP combination could be used, in more moderate concentrations, to distinct advantage. Thus, PPV may be used to diminish SNP concentrations, so obviating the risks of SNP toxicity. Furthermore, the two may be introduced simultaneously into the angiographic catheter to locally reverse VSM. As a result, such a combination may potentially have both beneficial as well as deleterious effects, clearly dependent upon the concentrations achieved.

SECTION II
In vitro vessel responses

Chapter 2
Effect of sham operation upon MCA responses

2.1 The effects of peripheral trauma upon central vasoreactivity: current knowledge

Cerebrovascular dysfunction is well recognised following a variety of cerebral insults (Part 1, 2.6). In particular, alterations in both *metabolic regulation* (e.g. CO_2 regulation) and in *autoregulation* have been documented following acute SAH[224-226, 257, 289, 307, 453, 590, 615, 632], along with widespread alteration in *receptor-mediated function*[2, 48, 123, 309, 367, 368, 574, 595, 598, 604, 635]. Little is known, however, regarding the effects of **peripheral injury** upon cerebrovascular function. In one recent study, clear evidence of cerebral **dysautoregulation** following limb trauma (ischaemia-reperfusion injury) was demonstrated, with CO_2 regulation remaining intact[227]. This concords with the view that metabolic regulation is more 'robust' than myogenic autoregulation (Part 1, 2.6). Haemorrhagic hypotension, however, which often accompanies trauma, does not affect either metabolic regulation or autoregulation[378]; suggesting this to be a lesser insult. Yet haemorrhagic hypotension is known to cause receptor-mediated dysfunction[335]. This tends to suggest that peripheral trauma, as in the study of Hadfield et al[227] above, may also have affected receptor-mediated vascular function. Direct evidence for such conjecture, however, is currently lacking.

2.2 Current experimental findings

In the first part of this study, HA responses were analyzed in non-operative control MCAs. Because prolonged anaesthesia without adequate monitoring (i.e. without arterial blood gas and fluid balance analysis) raises serious ethical issues, a true anaesthetic control group here was not possible. Following prolonged anaesthesia (4-6 hours) and surgical trauma, HA relaxation of AT was significantly reduced. Thus, HA_{max} was reduced from 102±5 to 82±5% ($p = 0.0085$), whilst the EC_{50} increased from 2.3±0.6 to 6.8±1.6 μM ($p = 0.0062$: Figs 29 & 30). The marked decrease in both affinity and efficacy appeared to resemble that of low-moderate concentration HA2 blockade (Fig 26). The resultant left-shift in the frequency distribution (Fig 29) signified that a smaller proportion of MCAs would suffer additional dilation of MT following complete abolition of AT (i.e. fewer HA E_{max}>100%:). Thus, the theoretical risk of dysautoregulation was also significantly reduced in MCAs after sham operation.

2.3 Theoretical and therapeutic implications

Although urethane is singularly ineffective in affecting cardiovascular physiology ordinarily[128], and is not known to release HA from MCs, it remains entirely possible that the continued effects of this anaesthetic agent were responsible for the adverse effects observed on HA relaxation. Clearly, a future study would be indicated in order to analyze further this possibility. However, it would be difficult to maintain anaesthesia for up to 6 hours without direct access to the animal's circulation; and considerable trauma is applied during this manoeuvre.

Surgical trauma was induced in four main areas in this study (Part 4, 3.3):

1. The left groin and associated neuro-vasculature
2. The posterior cervical musculature and cisterna magna (CM)
3. The central anterior neck region (i.e. tracheostomy)
4. The right carotid sheath and extra- / intra- cranial ICAs (i.e. thread insertion)

All of these areas could, theoretically, have been individually *or* collectively responsible for the effect observed. It seems unlikely, however, that manipulation of the right carotid sheath—with thread insertion intracranially within the ICA (No. 4 above)—played any significant role. Thus, no significant difference was observed in either HA E_{max} or EC_{50} between right (*study side*) and left (*contralateral paired control*) MCAs. This strongly suggests that the manoeuvre:

[Right carotid sheath manipulation] + [ECA obliteration] + [Intracranial ICA thread insertion]

exerted no significant lateralizing effect upon MCA reactivity within the study period. **Indeed, no significant differences between right and left MCA responses were found to *any* of the agonists used in this study**; again reinforcing the view that unilateral manipulation was without lateralizing effect (*see* later). Further support for this notion is obtained from the "Sheffield Model" of SAH, where rCBF remained unaffected by right carotid sheath manipulation[625].

The relative significance of factors 1-3 above, however, cannot be further elaborated upon here. Although penetration of the CM permits a direct mechanism by which MCA responses to HA may be affected, the degree of **peripheral trauma** suffered in Nos 1-3 have more evidence to support *their* possible contribution in this regard. In particular, the fact that various degrees of limb ischaemia were incurred following arterial cannulation must rank highly. For example, Stoner et al showed in 1984 that **limb ischaemia** could **influence central NE pathways via** nociceptor afferents ascending in the **femoral nerve**[562]. This, in fact, explained the levels of hypertension frequently seen during limb

ischaemia, a fact known since 1937[4]. Indeed, **hypertension** was frequently observed in the current study (MAP being 154/96 mmHg), this being abolished by severance of the femoral nerve (Part 5, Section I, 1.2 & 1.3). A similar neurovascular mechanism may thus pertain with HA down-regulation. Thus, combined [central and peripheral] surgical trauma plus ischaemia may have been the factors involved here; coupled to the possible effects of prolonged anaesthesia.

2.4 Conclusions

The results of this study appear to provide evidence that **peripheral trauma** may play a role in the **acute down-regulation** of cerebral **HA-mediated vasodilation**. If so, then this would be one of the first studies to demonstrate that peripheral injury may affect centrally receptor-mediated function. That such an effect might occur ought not necessarily to appear surprising, since Kóvach et al (1985) demonstrated that haemorrhagic hypotension could achieve a similar effect[335], whilst peripheral ischaemia certainly appears to affect more robust autoregulation[227]. The possible mechanisms involved (in addition to nociceptive afferent activation) will be discussed later (*see later*).

The fact that no significant differences were found between right and left MCA responses in sham operative controls to *any* of the agonists used in this study suggests that EF-SAH models do not introduce any significant 'side bias' upon ipsilateral MCA reactivity by virtue of the potentially confounding combination: *1)* unilateral carotid manipulation, *2)* unilateral EF insertion or *3)* potentially ipsilateral intracranial rupture. Such models may therefore provide the substrate for post-SAH cerebral vessel study without fear of this potentially confounding bias.

SECTION II
In vitro vessel responses
Chapter 3
Effect of acute SAH upon acute MCA responses

3.1 FD_{90} values and KCl responses

Tables 24-30, Appendix, clearly show that in spite of continued individual diameter asymmetry, and in spite of the stresses of surgical trauma and acute SAH incurred, overall mean FD_{90} values remained remarkably unaltered *across* all experimental sub-groups considered; as well as between right and left MCAs *within* each sub-group. This, to a first approximation, suggests that MT has also remained unimpaired, since MCA diameter at 90 mmHg internal pressure (FD_{90}) is dictated by myogenic contraction as well as by 'passive' anatomical factors. This is an important finding, because it not only confirms the comparison of 'like with like' MCAs; but it also suggests that MT—the fundamental aspect of 'autoregulation' that Bayliss initially conceived—is a somewhat 'robust' entity. Its continued presence was also appreciated visually by the continued presence of baseline vasomotion (Fig 12) within MCAs after SAH.

That MT was fundamentally unaffected by SAH is, however, more specifically supported still by the invariance of FD in n = 27 MCAs subjected to a range of intraluminal pressures from 70-150 mmHg after SAH (Figs 14 & 31). Thus, at least within three hours of SAH, the 'stimulus-response' ability of MCAs to increase MT in order to counteract increased distension appears to have remained largely unaltered. In consequence, it seems likely that such vessels ought to be able to 'autoregulate' normally in vivo, since this is an intrinsic property of such vessels unrelated to other physiological inputs (Part 2, 2.2-2.4). As a result, the current study suggests that the degree of **SAH** incurred is **not** likely to have **affected** the **basic mechanism** of **autoregulation** in MCAs. This is clearly contradictory to the popular view (Part 1, 2.6), and appears surprising in view of the apparent ease with which HA receptor-mediated function was clearly affected following mere sham operation (*see* previous section).

A further important finding from the current results is that the ability of MCAs to contract to a non-specific depolarizing stimulus (i.e. 120 mM KCl) also remained remarkably preserved after acute SAH (Table 28, Appendix: *see* also Fig 32). Indeed, the same degree of linear correlation between KCl E_{max} and FD_{90} was as apparent after SAH as before in shams or non-operative controls. Furthermore, K_{max} remained strikingly invariant across SAH Groups 1-3 and, thus, despite varying degrees of CCA re-clamping. These findings confirm that the basic contractile machinery of the MCA wall has remained fundamentally

unaffected by the combined stresses of sham operation and acute SAH. It also confirms that whatever other effects may be apparent in, for example receptor-mediated function after SAH, these have essentially occurred against a background comparison of 'like MCAs with like'.

The current results are completely contradictory to those that have demonstrated a loss of contractile power following acute SAH[94, 289, 472, 599]. They also, specifically, contradict Tsuji and Cook's finding of reduced KCl responses within 4 hours of SAH[604]. It is possible that the current findings may also—along with FD_{90} invariance—contradict evidence that the VSMC membrane becomes acutely depolarized in the early hours following SAH[641]. If the latter was the case, then one might expect in an increase in the MT:AT ratio to result; this resulting in reduced contractility (c.f. Tsuji and Cook and others[306, 309, 599, 635]). This result derives from Harder's finding that MT was achieved largely by way of VSMC membrane depolarization[240]. Waters and Harder proposed in 1985 that VSMC depolarization was one of the first significant physiological changes to occur following the application of blood to the BA[641]: others have since concurred[681]. Notwithstanding, the current findings do not support the view that PSCs within the VSMC membrane have been specifically damaged by acute SAH[604].

One exception to the pattern of otherwise unchanged K_{max} after SAH was apparent in one sub-group analysis of the current study. Thus, the mean K_{max} following a type II pressor response appeared markedly reduced in comparison to that following a type I or III response (Table 30). Although this did not prove to be statistically significant, it nearly did ($p = 0.0527$); whilst an isolated comparison of the type I K_{max} against the type II or against a type II/III combination (*see* Part 5, Section I, 1.3: types II & III acute pressor responses appeared similar and distinct in their AICP than type I) did reveal statistical significance ($p = 0.025$ and 0.044 respectively). These findings can be interpreted as demonstrating that, in comparison to the type II acute pressor response, the type I response appeared to 'protect' the K_{max} from the effects of SAH; maintaining its equivalence to either shams or non-operative controls. One possible explanation for this finding could relate to the fact that the type I response stanches SAH with less cerebrovascular shear stress, and also less compressive cerebral strain (Part 6, 1.4.3). Although the K_{max} in the type III group alone does not support this contention, it must be remembered that this group contained a statistically small number ($n = 2$ animals); and so might not be representative. Furthermore, other evidence from this study (Part 5, Section I, 1.3) suggests that the type II and III response groups can be considered together against type I. Reduced type II acute pressor K_{max} values after SAH suggest that higher ΔICPs and higher ICP_{max} values may damage VSMC contractility. If this is so, then it would provide some support for similar

findings with delayed VSM clinically, as observed in the study of Onoue et al discussed later [Part 6, Section III][472].

Conclusions

Two basic properties of autoregulatory arteries—the ability to develop MT in response to intraluminal distension, and the ability to constrict to a non-specific depolarizing stimulus—remain remarkably preserved acutely after SAH. These findings suggest that the basic mechanism of autoregulation remains intact, and contradicts studies that have demonstrated an acute vasoparalysis after SAH. The remarkable degree of uniformity in K_{max} and FD_{90} suggest that 'like with like' comparisons may continue after SAH; and further suggest that the K_{max} may remain as the 'gold standard' against which to compare other responses: as in shams or non-operative controls. In comparison to the type II acute pressor response, or to type II and III responses together in combination, the type I pressor response appeared protective to the K_{max}, maintaining its equivalence to shams and non-operative controls. It is possible that this protective effect in some way relates to the decreased cerebrovascular shear and decreased cerebral compressive strain that must accompany an acute hypotension with SAH. If proven, this would be a significant finding because it supports the possibility that higher ΔICPs and higher ICP_{max} values might damage VSMC contractility. Future studies utilizing greater group numbers may be required to assess this matter further.

The fact that no significant differences were found between right and left MCAs in either their diameters or in response to a non-specific depolarizing stimulus suggests that EF-SAH models do not introduce any significant 'side bias' upon ipsilateral MCA reactivity by virtue of the potentially confounding combination: *1)* unilateral carotid manipulation, *2)* unilateral EF insertion or *3)* potentially ipsilateral intracranial rupture.

3.2 PGF2α responses

The majority of previous studies have demonstrated enhanced PG-induced constrictions following SAH[635]. Furthermore, many studies have demonstrated increased PG concentrations in SAH CSF, particularly following secondary haemorrhage. Therefore, both enhanced concentrations and enhanced E_{max} values would make PGs strong candidates for agonist-induced VSM. This may be particularly so with PGF2α, since this causes a prolonged constriction (Part 3, 1.5) and is present in elevated concentrations at around the time of clinical DCI[479]. As a result, many studies have isolated PGs as the dominant vasoconstrictory force within SAH-CSF (Part 2, Ch 4), this correlating with the findings of enhanced PKC mechanisms associated with vessels in spasm[633].

Neither the PGF2α E_{max} or EC_{50} was significantly altered by SAH when SAH Groups 1-3 were considered either together or in isolation. Indeed, a remarkable degree of uniformity was apparent in the C-R curves obtained from non-operative controls, shams and overall SAH groups. This degree of uniformity also extended to the apparent loss of efficacy at supramaximal PG concentrations (*see* Fig 33): a phenomenon that might relate to cross-reactivity of PGF2α with inhibitory receptors. A closer analysis of Table 29, Appendix, however, suggests that a progressive increase in E_{max} had occurred with increased survival following the acute ictus. Thus, the E_{max} in the 3 hour group was significantly greater than that in the 1 and 2 hour survival group; as well as being greater than in shams or non-operative controls. Some of this effect could have derived from the fact that the FD_{90} in the 1 hour group was considerably smaller than in the 2 or 3 hour groups which, by the E_{max} v FD_{90} relationship, could have distorted this group's value. Nevertheless, the corresponding group K_{max} was not reduced. The possibility exists, therefore, that the progression observed is genuine, and that it might continue outwith the arbitrary three hour period—a possibility certainly compatible with the findings of others[633]. Further studies would be required to establish or refute this possibility. Such increased efficacy with survival could relate to the continued prolonged effects of some biochemical 'trigger' associated with the acute ictus: however, it could also reflect a property peculiar to 'survivors'. To exclude the latter, a future study would be required in which one group of animals developing an SCC are artificially kept alive by inotropic support: any significant differences in PGF2α efficacy between this group and those naturally surviving would therefore support the contention that the 'efficacy' effect was peculiar to 'survivors'. Notwithstanding, PGF2α remains at least as efficacious as it was in shams or non-operative controls at three hours following acute SAH.

A further finding on sub-group analysis was that the EC_{50} in SAH Group 2 was significantly smaller than either SAH Groups 1 or 3 (Fig 28). However, it did not differ significantly from shams or non-operative controls (although it nearly did: $p = 0.058$). As a significant trend across these groups, this result is difficult to explain. In principle, SAH Group 2 represents an intermediate category between the multiple CCA clamp-release→ICP re-rise→CCA re-clamping cycles of SAH Group 1, and the 'ideal' CCA clamp-release→immediate reperfusion category of SAH Group 3. We are therefore lead to the conclusion that any difference between Group 2, and Groups 1 & 3 together simultaneously, is not likely to relate to the ipsilateral effects of CCA clamping; nor to graded events associated with the ICP re-rise (and what this actually represented!). However, SAH Group 1 clearly appeared unique for numerous other reasons in this study—not least of all because its small statistical number (*see* Part 6, Section II, 3.8)—so that, upon Group 1 exclusion, we may perhaps concentrate upon differences between

Groups 2 & 3. The principal difference between the latter two groups was that SAH Group 2 contained an animal that had sustained a type III acute pressor response [*see* Table 22]. However, since this animal's mean PGF2α EC_{50} was 5.9 μM, the observed CHR cannot be held responsible for this group's deviant PGF2α EC_{50} value.

Conclusions

PGF2α constrictions are at least as potent and efficacious as they are in either shams or non-operative controls after SAH. Moreover, the trend suggests that there is a progressive increase in efficacy with prolonged survival after SAH. The prolonged and powerful constrictions that PGF2α produce, coupled with evidence for its relative abundance within the SAH-CSF and the possibility that its efficacy may increase with prolonged survival, makes PGF2α a strong candidate for the agonist responsible for reversible VSM. PGF2α constrictions appear to be significantly more potent following a reperfusion-induced ICP re-rise and subsequent CCA re-clamping for <5 mins, than after immediate reperfusion without an ICP re-rise, after acute SAH.

The fact that no significant differences were found between right and left MCAs in response to PGF2α suggests that EF-SAH models do not introduce any significant 'side bias' upon ipsilateral MCA reactivity by virtue of the potentially confounding combination: *1)* unilateral carotid manipulation, *2)* unilateral EF insertion or *3)* potentially ipsilateral intracranial rupture.

3.3 5HT responses

5HT may be important in both structural and functional VSM: thus, 5HT may promote myoproliferative change within the vessel wall (Part 3, 1.4). Most 5HT in blood is contained within platelets which are, of course, in plentiful supply within the local clot[276, 323, 610]. However, 5HT is also present within serotonergic neurones and mast cells (Part 3, 1.4). The latter source may be of crucial importance, as these are curiously prevalent within blood vessel walls in the local rupture vicinity (Part 2, 1.4.2). A recent study has demonstrated that perivascular sympathetic boutons take up and store local accumulations of 5HT, metering them out in turn over the ensuing days[578]. Furthermore, 5HT may be co-released from sympathetic terminals with NE[237, 620]; and the two may even interact[237]. In this context, serotonergic neurones may feature heavily in the DSS phenomenon (Part 3, 3.3). Interestingly, the presence of blood can directly augment the 5HT response[316, 338], as can also endothelial denudation[156, 286].

Most studies have demonstrated an increased responsiveness of cerebral vessels to 5HT after SAH. Interestingly, Debdi et al (1993) demonstrated—in a type II SAH model—that

increased 5HT responses could occur as early as 10 mins after SAH[123]. Similarly, Tsuji and Cook demonstrated increased responses within 1 hour which were significantly resistant to nimodipine dilation[604]. Others have demonstrated similar findings—unfortunately all within Bas[574, 635]. The results of the current study, however, do not support these findings. In general, 5HT responses remained uniform between non-operative controls, shams, and overall SAH groups; with no statistically significant differences in either E_{max} or EC_{50} values. This fact can be appreciated in the combined groups C-R curve of Fig 35. Although sub-group analysis of survival after SAH appears to support a trend toward an increase in E_{max} with survival, this trend was not statistically significant (Table 29, Appendix). In fact, the E_{max} did not significantly differ either between SAH Groups 1-3 (Table 27), or between acute pressor response groups (Table 30); nor was there any significance between right and left MCAs within each sub-group (Tables 24-26). As in shams or non-operative controls, the 5HT E_{max} was comparable in magnitude to both the KCl and PGF2α values after SAH, but was much larger than either the UTP_{max} or NE_{max} in 'responders'. However, the EC_{50} value was an order of magnitude smaller than that of all other agonists excepting UTP (from which it differed by two orders of magnitude). In consequence, 5HT responses are especially potent in MCAs: in non-operative controls, shams and after acute SAH.

Because E_{max} values were so invariant, it was somewhat surprising to find that the EC_{50} values frequently differed significantly amongst SAH sub-groups (Table 27). For example, the EC_{50} in SAH Group 1 (0.72±03.1 μM) was significantly larger than that in either Groups 2 and 3; or that in shams and non-operative controls (Table 28). No significant side difference within SAH Group 1, however, was apparent (Table 24). This suggests that SAH Group 1 responses significantly differed by factors not directly related to prolonged unilateral CCA clamping per se: however, the small n numbers for side comparisons make this analysis strictly inconclusive. Nevertheless, other factors that could have been important include the fact that prolonged re-clamping was employed in response to repeated ICP re-rises with each attempted reperfusion. Thus, Group 1 could have differed primarily because of multiple re-bleeds. This factor also ties in with another distinguishing feature of this group, namely that of early mortality. Thus, SCC occurred within the arbritary three hours after SAH in 4/4 (100%) of this group. However, one other important factor relating to this group was its statistically small group number: thus, only n = 8 MCAs were analyzed amongst n = 4 animals [*see* Part 5, Section II, 1.5; and Part 6, Section II, 3.8].

The 5HT EC_{50} also appeared markedly increased in the 2 hour survival group relative to the 1 and 3 hour groups (Table 29). This difference, however, did not prove to be

statistically significant. The 5HT EC_{50} in the type III acute pressor response group, however, was highly significantly different from either the type I and II groups ($p = 0.002$); or from shams and non-operative controls ($p = 0.0003$: Table 30). As with all statistical analyses attempted on the type III acute pressor response sub-group, no firm conclusions can be drawn simply by virtue of their small group number. Nevertheless, it is certainly conceivable that a CHR could have been detrimental to the 5HT receptor-mediated response, particularly in view of the greater cerebrovascular shear stress—and greater cerebral strain—that this group must necessarily have incurred. Further studies with, hopefully, greater CHR sub-group numbers, would be required to resolve further this important issue.

Conclusions

5HT responses—both E_{max} and EC_{50}—are, overall, insignificantly altered after SAH. The 5HT E_{max} after SAH, as in shams and non-operative controls, is comparable in magnitude to that with KCl and PGF2α and, therefore, considerably stronger than that with UTP or NE-'responders'. The EC_{50} is an order of magnitude more potent than with most other agonists. Sub-group analysis suggests that 5HT affinity is significantly decreased in the prolonged CCA re-clamped group, this perhaps relating to the repeated ICP re-rises—and subsequent early mortality—suffered by this group: however, it may also represent an artefact of this group's small statistical number. Sub-group analysis also suggested that the CHR was detrimental to the affinity of the 5HT response, diminishing this significantly from either shams and non-operative controls, or from acute pressor response types I & II. However, as with SAH Group 1, this effect could also have been an artefact of a small group number.

The fact that no significant differences were found between right and left MCAs in response to 5HT suggests that EF-SAH models do not introduce any significant 'side bias' upon ipsilateral MCA reactivity by virtue of the potentially confounding combination: *1)* unilateral carotid manipulation, *2)* unilateral EF insertion or *3)* potentially ipsilateral intracranial rupture.

3.4. UTP responses

UTP, like 5HT and NE, is present in copious amounts within the platelets of the SAH clot. It may also be present as neurotransmitter within the CNS, and is richly present within cerebral tissue. Little is known about its role although evidence suggests that this may be specific to the CNS (Part 3, 1.6). Few SAH studies have used UTP as a response agonist. In those that have, conflicting reports once again abound. For example, Debdi et al demonstrated increased UTP responses within 10 mins in a type IIb SAH model[123], whilst

Kim et al demonstrated diminished responses in a similar model but with the use of ring myography[326]. However, both of these studies employed the BA for analysis.

As Table 31, Appendix shows, UTP responses were singularly different from all other contractile agonists in that the E_{max} v FD_{90} correlation *in non-operative controls* was not significantly linear. Nevertheless, linearity—with a high degree of correlation and significance—was once more restored in shams and overall SAH groups (i.e. all other groups). No adequate explanation is offered for the aberrant finding in non-operative controls, apart from the somewhat banal one of 'sporadic receptor heterogeneity' or, possibly, 'sampling error'.

UTP responses remained singularly unaffected by SAH in this study. Thus, neither the E_{max} nor the EC_{50} were significantly altered by either the degree of CCA re-clamping (SAH Groups 1-3: Tables 27 & 28); the degree of survival (Table 29); nor the type of acute pressor response sustained after SAH (Table 30). These results strongly suggest that the receptors involved—and receptor coupling to intracellular second messengers (Part 3, 1.6)—have remained unaffected by the acute ictus: at least within the short period after SAH analyzed in this study. The UTP_{max}, like non-operative controls (but unlike shams), was significantly weaker than the corresponding K_{max} after SAH ($p = 0.0001$). In terms of scale, the UTP_{max} ranked more akin to NE than to KCl, PGF2α or 5HT. Therefore, UTP constrictions are not as powerful as PGF2α or 5HT constrictions. Furthermore, with an EC_{50} value an order of magnitude higher than with these agonists, UTP constrictions are also significantly less potent.

Conclusions

UTP responses remained as potent and efficacious after SAH as in shams or non-operative controls: however, in both of these parameters, UTP responses are significantly weaker than either KCl or PGF2α. SAH sub-group analysis does not reveal any exceptions from this rule. If, during delayed VSM, a local increase of [UTP] occurs, then the current findings at up to three hours —should they persist for several days after SAH—suggest that UTP may mediate equally as potent and powerful constrictions as in shams or non-operative controls. However, such constrictions are significantly weaker and less potent than with other likely agonists.

The fact that no significant differences were found between right and left MCAs in response to UTP suggests that EF-SAH models do not introduce any significant 'side bias' upon ipsilateral MCA reactivity by virtue of the potentially confounding combination: *1)*

unilateral carotid manipulation, *2)* unilateral EF insertion or *3)* potentially ipsilateral intracranial rupture.

3.5 NE responses

3.5.1 Background

One factor involved with VSM may be excessive adrenergic activation[190]. Thus, myonecrosis is frequent in the outer medial layers[669] which represents the most functionally active layer adjacent to the adrenergic supply[639]. Furthermore, high local concentrations of catecholamines have been shown experimentally to produce a myonecrosis[7], as well as to produce endothelial denudation[300, 301] and meningeal reaction[669] also commonly seen. Animals pre-treated with reserpine (RSP), however, do not develop these changes[255, 555]; nor do animals treated with blood from a donor similarly pre-treated with RSP (where RSP also depletes platelets of NE)[584].

An acute sympathetic activation often accompanies SAH. Hence the CHR (resulting from activation of resistance vessels[659] and the myocardium[213]), and the development of cardiac dysfunction[218, 331]. Indeed, the cardiac lesions incurred with the CHR often resemble those of an acute catecholamine overload[218, 331], and surges in catecholamine levels often correlate with the height of ICP elevations[113, 665]. Furthermore the widespread occurrence of adrenergic nerves spent of neurotransmitter[123, 162, 367, 368, 434, 472] appears testimony to the aftermath of such excessive activity. There are varying reports concerning the effect of SAH upon NE-responsiveness in cerebral vessels. By far the majority have demonstrated that NE efficacy is increased after SAH[123, 162, 367, 368, 434, 472]. Some, though, have shown no change at all[254]; whilst others have even demonstrated decreased responses[48, 529, 599]. Unfortunately, in most of these studies the model used was either a type IIb, or the vessel study was unrepresentative (either in design, or because of the use of the BA).

3.5.2 Current experimental findings

The distinguishing feature of NE constrictions in non-operative controls—i.e. the marked **heterogeneity** of response between 'responders' and 'subresponders'—persisted through shams and to all SAH groups. In fact, although the degree of heterogeneity (% NE-'subresponders') remained statistically invariant across this series, the trend actually suggested that it increased (non-operative controls 24%, sham operative controls 12.5%, SAH groups 55%: $p>0.05$). NE heterogeneity also did not significantly vary across:

- SAH Groups 1-3 (38%, 65% and 55% NE-'subresponders': $p>>0.2$)
- Survival groups (33%-Group 1, 38%-Group 2, 48%-Group 3 NE-'subresponders': $p>>0.2$)

- Acute pressor response type I (39% NE-'subresponders') and type [II & III] (47% NE-'subresponders': p>>0.2)

NE heterogeneity therefore appears to have persisted despite SAH or any of its possible sub-divisions. The vessels designated 'subresponders' were specifically so toward NE—responses to other agonists were normal. The NE_{max} in 'responders' remained remarkably uniform between non-operative controls, shams and overall SAH groups; with a significance value approaching unity (Table 28, Appendix). This uniformity was even greater still across SAH Groups 1-3 (Table 27). Therefore, both the degree of heterogeneity and 'responder' efficacy remained generally invariant after SAH. Furthermore, the other feature of NE responses—i.e. their significantly weaker efficacy relative to KCl or PGF2α—also persisted after SAH (Part 5, Section II, 3.5).

SAH sub-group analysis, however, suggested that NE efficacy progressively increased with prolonged survival after SAH. Thus, the 3 hour value (4.8±0.6 mN)—impressively similar to the corresponding K_{max} (4.9±0.4 mN)—significantly differed from either the 1 or 2 hour value ($p = 0.04$); or from shams and non-operative controls ($p = 0.026$). As with PGF2α survival efficacy, some of this effect could have derived from the fact that the FD_{90} in the 1 hour group was considerably smaller than that in the 2 or 3 hour groups which, by the E_{max} v FD_{90} relationship, could have distorted group 1's NE_{max}. Nevertheless, no such distortion was apparent in the corresponding group K_{max} value. If, then, the progressive increase in NE_{max} with survival is real and does persist—as with the progression in $PGF2\alpha_{max}$ (3.2 above)—then both of these agonists could appear as key players in the development of 'reversible' VSM: whether acute or delayed. Several studies have demonstrated an increased NE_{max} after SAH consistent with the delayed time course of VSM.

Although no significance was actually demonstrated, a progressive increase in the EC_{50} value was also suggested from non-operative controls, through shams, to overall SAH groups (Table 28: $p = 0.08$). Analysis of SAH sub-groups, however, revealed that the immediate reperfusion group (i.e. SAH Group 3) was largely responsible for this deviation (Table 27). Thus, the Group 3 EC_{50} (9.2±2.3 μM) was significantly greater than that in SAH Group 2 (2.4±0.7 μM, $p = 0.01$); or than that in shams and non-operative controls ($p = 0.002$). Unfortunately, no EC_{50} value was obtained from SAH Group 1 for a more complete comparison: nevertheless, the difference was quite striking. SAH Groups 3 differed from Group 2 in that normal reperfusion was restored immediately upon thread withdrawal after SAH induction: in SAH Group 2, however, the clip was repositioned for up to 5 mins in response to an ICP re-rise (Part 4, 3.3). The fact that no significant right:left side differences were apparent in either group suggests that the difference

between the two groups' EC_{50} value does not relate to CCA re-clamping effects per se (i.e. to ipsilateral CCA ischaemia). It would seem more likely, therefore, that the 'protective' effect in SAH Group 2 related in some way to the vexed issue of the 'ICP re-rise', and what this actually represented (Part 6, Section I, 1.4). However, in view of the similarly increased NE EC_{50} after a type II pressor response (*see below*), one other possible explanation relates to the fact that SAH Group 3 contained a higher proportion of type II acute pressor responses (43%) than did Group 2 (27%): although this difference was not statistically significant (Table 22).

As aforementioned, the NE EC_{50} was also strikingly increased after a type II pressor response (15.3±5.0 μM) relative to either type I alone, or to types I & III in combination (Table 30); or to shams and non-operative controls (Table 28). If this is so, then it suggests that the type II acute pressor response is detrimental to NE responses. One likely explanatory factor here could be that of the higher ΔICP and ICP_{max} incurred (Part 5, Section I, 1.3). Unfortunately, the experimental numbers from the type II and III groups were too small for this hypothesis to be conclusive. Nevertheless, put together, these sub-group findings suggest that NE affinity in 'responders' may, in some cases, be significantly reduced after SAH. It is interesting, in this context, that a cadaver study has demonstrated specific α2 receptor damage in patients at various stages after SAH[607]. Similar findings have also been reported for cerebral α1 receptors following cerebral trauma[498]. It is certainly possible that these effects are the consequence of acute surges in catecholamines in response to SAH, since this may result in high-dose receptor uncoupling[54] (Part 3, 3.2). Whatever the fate of the EC_{50}, however, it is clear that the NE_{max} continues to remain unaffected: indeed, the current evidence suggests that it increases with survival. Thus, maximal local NE concentrations will still achieve maximal responses in those vessels still capable: this is an important point (*see* below).

3.5.3 Implications of NE heterogeneity for VSM

Intracranial adrenergic activation may arise in *localized* fashion via two separate mechanisms: platelet-borne NE deriving from sequential clot lysis; and ipsilateral sympathetic denervation causing a delayed DSS (Part 3, 3.3). Both appear likely mechanisms after SAH, since both develop with some delay—the latter often being maximal after at least 5 days (Part 3, 3.3). Although functionally 'denervated' by being bereft of neurotransmitter (NE), copious amounts of NE, of course, continue to prevail locally within clot platelets. Can delayed super-sensitivity (DSS) occur with such a scenario? As described in Part 3, 3.3, the neurotransmitter is not the only substance required to 'subdue' a target cell. Thus, in many cases, the co-release of a "trophic factor" along with the neurotransmitter occurs to raise target cell threshold. Any depletion of the

trophic factor, then, will cause DSS *to the neurotransmitter* in spite of the latter's continued presence. Increased circulating catecholamines occurring 5-7 days after SAH would, thus, worsen these prospects[485]; and, furthermore, haemoglobin itself augments the NE response by a variety of mechanisms (Part 1, 1.4 & Part 3, 1.3).

Any animal containing at least one NE-'responder' can potentially produce VSM in that vessel where VSM is mediated by contractile agonists acting via α adrenoceptors[190, 313, 576]. The proportion of animals capable of producing VSM, therefore, includes both symmetrical and asymmetrical NE-'responders' (Tables 12-14). From the results of this thesis, a maximum of 22/29 (76%) animals could develop VSM in MCAs after SAH under this scheme. However, only in those animals where both MCAs unequivocally respond to NE may VSM develop irrespective of clot localization (symmetrical responders, Tables 12-14)—if subarachnoid clot is assumed to represent the major source of the contractile agonist required[177]. Thus, 12/28 (43%) animals could develop VSM irrespective of clot localization under this scheme.

VSM does not occur in every patient. Attempts to prognosticate development and severity of VSM are, most commonly, based upon clot site and size[177]. Nevertheless, widespread anecdotal clinical evidence supports published findings that VSM often defies such prognostication[530, 583]. The correlation is therefore not perfect[530]: clot may be present without local VSM, and vice versa. As Fisher's landmark study clearly showed, VSM may be witnessed at some distance from the clot (Fisher's cases 14, 17, 33, and 47) as well as being minimal or absent within it (Fisher's cases 12,13, 16, 18, 25, 28, 29, 31, 34, 36-38, 40, 42-46) [177]. In fact, VSM only occurs in 43-67% of cases of proven SAH[134]. Such disparities have been attributed to "individual differences" in cerebral artery susceptibility[63]: however, no underlying mechanism has yet been proffered.

Clearly, it would be premature to extrapolate the above findings to the clinical scenario of delayed VSM. However, if it is assumed that NE heterogeneity—with possibly increased NE_{max} responses in survivors remaining—does persist into the period of delayed VSM after SAH; and if one assumes that NE acting at α receptors is instrumental in VSM aetiology[190, 313, 576]; then exceptions to the 'clot = VSM' hypothesis can easily be explained. Thus, based on the results of this thesis, a maximum only of 75% animals would be capable of developing VSM under this scheme, *irrespective of clot size*. Since, however, only bilateral 'responders' exist in 43% animals after SAH, only this proportion could sustain VSM *irrespective of clot lateralization*. It is interesting that this range—i.e. 43-76%—is comparable to that obtained from large epidemiological studies of clinical VSM[134]. Although such extrapolations are of dubious scientific merit, the underlying principle—i.e.

that of a dichotomy of agonist response in MCAs—could easily explain exceptions to Fisher's axiom. Furthermore, the decrease in NE affinity amongst some sub-groups after SAH would serve to localize VSM still further: since significant responses would only be expected in areas of higher agonist concentrations—i.e. within the clot itself.

3.5.4 Conclusions

The degree of NE_{max} heterogeneity, and NE-'responder' efficacy, remained generally invariant after SAH, although the NE_{max} continued to be significantly weaker than either the K_{max} , $5HT_{max}$ or the $PGF2\alpha_{max}$. The significantly increased NE_{max} at 3 hours, as with the similarly increased $PGF2\alpha_{max}$ at this time, suggests that NE responses may be more efficacious with prolonged survival after SAH. The significantly increased EC_{50} in SAH Group 3, and the significantly increased EC_{50} after a type II pressor response, is difficult to account for; however, one possibility is that it derives from the greater ΔICP and ICP_{max} experienced in these groups. Significantly diminished NE affinity in these cases suggests that SAH may be detrimental to α adrenoceptors in some cases. Persistent heterogeneity provides a conceptual mechanism to explain exceptions to Fisher's axiom where VSM may be mediated by NE acting at α adrenoceptors. Diminished affinity in some 'responders' could explain why VSM is only apparent with high NE concentrations within the clot.

The singular existence of discontinuous response heterogeneity with NE precluded their use in demonstrating an absence of 'side bias' in EF-SAH models.

3.6 HA responses

When acute SAH was superimposed against the background conditions of sham operation (Part 2, Section II, 3.7), HA responses appeared diminished further still. This can be appreciated visually in the combined C-R curve of Fig 38. Nevertheless, neither the HA E_{max} nor the EC_{50} significantly differed from sham values. Thus, at up to three hours after SAH, the major effect upon HA responses continues to relate to the underlying effects of 'sham operation' upon non-operative control responses. It is entirely possible, however, that, with time, a further (and perhaps significant) reduction in E_{max}—or increase in EC_{50}—could ultimately be seen: future studies would be indicated to confirm or refute this hypothesis. Some support for the latter suggestion may be gleaned from the present thesis, where the HA E_{max} appeared to progressively decrease with survival: however, this did not prove to be statistically significant. A similarly insignificantly increased EC_{50} value at 3 hours also suggests this possibility (Table 30). Furthermore, the proportion of MCAs dilated >100% by HA progressively and significantly decreased from non-operative controls (52%), through shams (25%), to SAH Groups (11%) [$p < 0.001$; Table 6]: nevertheless, once again, most of this effect related to the effect of sham operation. Direct

support for a continued significant reduction in HA responses after SAH is, however, already extant from some studies[667].

Conclusion

Despite the trend suggested by the combined C-R curves (Fig 38), and despite the highly significant ANOVA values across the control-sham-SAH series, HA responses are not significantly affected by SAH at up to three hours. Furthermore, no significant differences were apparent either within SAH sub-groups, or between right and left sides within each sub-group. As with sham operative controls, the significant difference in HA E_{max} and EC_{50} with non-operative controls presumably, in some way, relates to either the trauma sustained (with SAH itself being included here) or to the prolonged effects of the anaesthetic agent used. Again, as with the 'sham operative findings', should it prove—in subsequent studies—that trauma represents the unifying mechanism, then this would justify analysing the role of HA2 blockers in such a setting in future studies.

The fact that no significant differences were found between right and left MCAs in response to HA suggests that EF-SAH models do not introduce any significant 'side bias' upon ipsilateral MCA reactivity by virtue of the potentially confounding combination: *1)* unilateral carotid manipulation, *2)* unilateral EF insertion or *3)* potentially ipsilateral intracranial rupture.

*** Excluding the two animal's anaesthetized by hypnorm and hypnovel did not alter these findings**

3.7 PPV responses

PPV is being increasingly used locally to reverse VSM, particularly via the catheter at angiography[99], with dramatic improvements often being reported[378, 400, 538, 622, 608]. Nevertheless, PPV does not always work[99, 89, 365], suitable responses perhaps being obtainable in around 50% of cases[373]. Moreover, PPV may even cause adverse effects in this setting[102, 391], many with direct relevance to the condition being treated. For example, the development of a severe immunologically-mediated thrombocytopenia[418] theoretically increases the risk of re-rupture in those cases still awaiting definitive surgery. However, it is with the development of TIAs that most concern is currently being voiced[102, 391].

Detrimental effects with PPV have so far occurred in a total of 9 out of 68 (13%)[102] VSM cases reported: these have included episodes of blindness[99], brainstem depression[391], hemiparesis[102, 317] and seizure activity[99]. In some cases there have been associated increases in ICP reported[400], this perhaps implying a failure of autoregulation. Although VSM may vary from an active vasoconstriction to one of a fibrotic non-

compliance[668]—thus explaining PPV's effectiveness in some cases and not in others[638]—this hardly seems able to account for iatrogenic TIAs. Instead, it appears that intrinsic properties related to PPV itself are implied since other mechanisms, such as those related to the mode of administration, appear unlikely[102, 293].

The major feature of PPV responses in the current study was that they remained as potent and efficacious after SAH as they were in either shams or non-operative controls. Furthermore, efficacy and potency were not significantly affected by either the degree of CCA re-clamping, the degree of survival, or by the type of acute pressor response sustained after acute SAH (Tables 24-30). These findings are not likely to surprise the majority of neurosurgeons. Thus, in the majority of cases, PPV completely abolished AT without disrupting background MT: induced VSMC levels of cAMP are, thus, likely to have remained unchanged across each group—irrespective of the procedure performed.

These results, although agreeing with the popular view (and with the recent findings of others[608]), are significantly at variance from studies that have demonstrated a **paradoxical vasoconstriction** at certain PPV concentrations[293, 521]. For example, Jin et al[293] demonstrated such constriction at **50 μM** in cortical pial vessels of rats following pre-constriction with a phorbol ester. At 100 μM, however, PPV caused a vasodilation much as expected. Jin et al hypothesized that such a biphasic response could be a possible explanation for poor angiographic-clinical correlates with VSM and, potentially, for the inadvertent development of aforementioned TIAs[293].

One obvious difference between the Jin et al's study and the current one is that *apparently* smaller vessels were used in that study (30-70 μm). However, since the vessels in the former study were not exposed to intraluminal distension they would, of necessity, appear smaller anyway. Furthermore, such vessels would also possess little or no MT (Part 2, 2.2). It is also important to note that no 'true' SAH-model was utilized in the former study: a phorbol ester-induced constriction alone was considered to closely resemble VSM. In addition to creating a more physiological SAH, MCAs were pre-constricted in the current study with PGF2α. The latter activates PKC in a similar fashion to that of a phorbol ester (Part 2, 3.5). Finally, significant differences in the bath temperature were apparent between the two studies. The temperature of 33°C used by Jin et al[293] was unphysiological: any results therefore obtained would therefore not be directly comparable to those produced at 37°C.

In spite of these shortcomings, it was intriguing to have observed similar disturbances in the C-R curve at *precisely* the same (**50 μM**) concentration in the current study, both in

control groups as well as after SAH (Fig 41 **A**). Thus, a point of inflexion occurred in the otherwise sigmoid curve negating some of the relaxation achieved at preceding, lower concentrations. However, in marked contrast to the previous study, the 50 μM anomaly did not amount to an actual vasoconstriction, but rather to a **transient loss of efficacy**. It amounted to a loss of about 10-15% of previously-dilated tone. Therefore, since dilation of AT was still extant, this disturbance **cannot** be considered as a possible **cause** for **TIAs**—although it occurs at intriguingly the same position on the C-R curve.

In support of the previous study, however, a small proportion (10-15%) of MCAs appeared to develop a definite—yet similarly transient—potentiation of pre-constricted tone at a [PPV] of **0.5 μM** (Fig 41 **B**). The latter anomaly certainly could be classified as a **'paradoxical vasoconstriction'**. However, the plateau of tone reached following PGF2α-induced constriction in many vessels was, like the prior baseline level, fluctuant (Figs 12 & 19). Therefore, a precise quantification of this phenomenon was not possible. In the few cases where the 0.5 μM anomaly may assume some significance—i.e. in terms of magnitude and duration—it is certainly possible that it may produce a TIA. However, it must be emphasized that this phenomenon was not widespread and, invariably, was extremely *transient* (i.e. seconds). Furthermore, it did not occur at the concentrations observed by Jin et al. In conclusion, then, the current findings do not *generally* support those of the aforementioned study.

Other possible causes for TIAs were, in turn, considered. One putative mechanism concerns a differential dilation achieved dependent on vessel size. For example, where a vasodilator specifically acts at the microvascular level—leaving larger arteries unaffected—a vascular 'steal' may result where there exists an undistendable, diseased segment. Thus, certain nitrosovasodilators such as dipyrimidole (which specifically dilates the smaller *coronary* arteries) may, in fact, promote an episode of angina rather than alleviate it[247]. In spite of the fact that only MCAs were studied, a rather large range of vessel diameters was obtained; thus providing some scope for such investigation. However, no definite relationship was, in fact, demonstrated between vessel diameter and PPV response obtained (Fig 42). This was also the case with PPV in systemic vessels of another study[2, 13]. In consequence, a differential PPV-effect would not appear to be a likely mechanism for TIAs following PPV infusion. Interestingly, such differential vasodilation has also been observed with nimodipine in cerebral vessels[64, 634]; and a recent case report also appears suggest that some degree of this might have occurred with PPV[102].

A finding from the current study that much more likely explains the occasional episode of TIA with PPV concerns the proportion of MCAs where PPV dilated MT in addition to all

AT (i.e. in those MCAs where E_{max}>100%). As can be seen from the normal distribution of SAH vessels (Fig 40), the right-hand tail reflects those vessels (n = 17, **33%**) where—at least in principle—a **dysautoregulation** may occur in vivo: however, this would be particularly significant in approximately 15%. The significance obviously depends on the relative influence of MT which was, in turn, assessed at 40% in non-operative controls of the current study (Part 5, Section II, 1.13). Thus, in MCAs where PPV dilates ≥130% of pre-constricted tone, a 50% reduction in MT will result, this causing considerable increases in flow in vivo (Part 2, 2.4).

Potentially, the effect of PPV on systemic **pre-capillary vessels** can reveal exactly what can be expected within cerebral vessels when such tone is also abolished. The latter vessels serve to maintain intracapillary pressures close to 15 mmHg (Part 2, 2.5). However, PPV is regularly used experimentally to **paralyze** such vessels[407]—after which **intracapillary pressures** of up to **36 mmHg** may be seen. Under such circumstances, large amounts of plasma can be transudated as oedematous fluid[407]; whilst high intravascular pressures threaten architectural disruption (Part 2, 2.5). It is conceivable, therefore, that such a powerful **mechanism** could easily underlie the precipitation of a **TIA** with PPV were this to also occur within the cerebrovasculature. That PPV infusion has caused both excessive vasodilation[211] as well as ICP-elevation[400] in some studies, is supportive of this assertion. Moreover, given the possible interaction with SNP, a PPV-SNP combination would seriously threaten this complication when both agonists may be present in significant concentration: as, for example, during aneurysm surgery.

Conclusion

This study thus re-affirms PPV as a powerful and potent vasodilator of major cerebral arteries such as the MCA. However, when MT is additionally dilated (approximately 15% vessels) cerebrovascular dysautoregulation may ensue, and threaten capillary perfusion. Should the pre-capillary sphincters themselves also prove susceptible (as they are within the systemic circulation), then TIAs could follow systemic surges in MAP. However, since in the majority only non-MT is affected (i.e. only superimposed AT), this would explain why approximately 50% of patients show clinical improvement with PPV[305, 400]; and why such improvement occurs without complication (because autoregulation itself remains intact). Two other possible mechanisms, however—that of a low-dose (0.5 μM) paradoxical vasoconstriction, and that of a vascular 'steal'—cannot be ruled out from the present study; although both appear unlikely. A prolonged and powerful constriction at 50 μM[293], as promoted by Jin et al, can be ruled out; since this was not observed in any experimental group. Nevertheless, the transient loss of efficacy observed at 50 μM requires further study and explanation.

The fact that no significant differences were found between right and left MCAs in response to PPV suggests that EF-SAH models do not introduce any significant 'side bias' upon ipsilateral MCA reactivity by virtue of the potentially confounding combination: *1)* unilateral carotid manipulation, *2)* unilateral EF insertion or *3)* potentially ipsilateral intracranial rupture.

3.8 L-ARG and L-NAME responses

NO is produced by NOS using L-ARG as substrate. The major form of NOS within the vasculature is the constitutive variety, cNOS, occurring within endothelial cells. However, other sources include VSMCs and adventitial nitroxidergic neurones. The NO produced acts as the substrate for GC within VSMCs (Part 2, 3.5 & Part 3, 1.8): as a result, VSMC relaxation occurs following an increase of intracellular cyclic guanosine 3',5' monophosphate (cGMP) and subsequent PKG activation.

NO is inferred to be co-released with tonic activity in systemic arteries, both in vivo and in vitro[75, 502]. Thus, co-inhibition of NO release during AT results in increased AT. Such co-inhibition has been used to explain pathologically increased tone, such as that observed in hypertension[372], prinzmetal's angina and in atherosclerosis[156]. The common initiator in all of these cases is assumed to be that of endothelial dysfunction. It has also been suggested that inhibition of 'basal' NO release is responsible for the occurrence of VSM following SAH. Whilst endothelial dysfunction may similarly be responsible here, another possibility would also include NO-scavenging by the surrounding clot[244, 272, 325]. Several studies have documented evidence for both mechanisms, although most favour the latter[318]. Alternatively, some have demonstrated that the surrounding clot directly affects GC activity within the VSMCs themselves. Whatever the case, inhibition of basal NO release by L-NAME in the current study elevated effective AT by approximately 26% (Part 5, Section II, 1.10).

In the current study, L-ARG efficacy was significantly increased after SAH compared to shams and non-operative controls. Observation of the C-R curve Fig 44 suggested that the EC_{50} might also be significantly affected: however, no significance was demonstrated on ANOVA. Observation of the combined frequency histogram in Fig 43 shows that the proportion for which E_{max}>100% is significantly greater after SAH (32%) than in shams (6%) or non-operative controls (9%: $p<0.05$). Thus, maximal [L-ARG] may threaten dysautoregulation in a proportion of MCAs after SAH, in a similar fashion to that deduced for PPV and HA (*see* above). This is important, because increased NO production has frequently been observed following ischaemia and ischaemia-reperfusion insults[486]. One of the perceived *raison d'êtres* for this effect relates to beneficial vasodilatory effects within

the ischaemic penumbra. The results of the current study suggest that the L-ARG→NOS→NO→GC→cGMP sequence may be more efficacious in this role after SAH.

Significantly enhanced L-ARG efficacy in overall SAH groups, and significantly enhanced affinity in n = 26 animals from SAH Groups 2 & 3 (*see* below), suggests that the L-ARG→NOS→NO→GC→cGMP pathway is in some way stimulated acutely after SAH. This is a remarkable finding, because it completely contradicts the plethora of reports that demonstrate that this pathway is inhibited. This dichotomy of evidence may derive from the fact that most models used in SAH studies are type II models (Part 4, 1.2): thus, they do not create a truly 'physiological' ictus. However, another key difference is that such studies have not examined responses in the acute period; whilst the current study, in turn, has not examined the delayed period following a physiological ictus. Thus, it is entirely possible that an acute early activation might become translated into a depressed one several days after the acute ictus: further studies would be indicated to confirm or refute this.

One possible explanation for L-ARG→NOS→NO→GC→cGMP pathway activation after SAH is that of *c*NOS activation. The most likely tissue sources for increased *c*NOS production or activation would include:

- MCA endothelium
- MCA VSMCs
- MCA adventitia—fibroblasts and/or myofibroblasts
- Perivascular glial tissue
- Perivascular neurones (particularly nitroxidergic neurones)

Numerous studies have confirmed that an early activation of the L-ARG→NOS→NO→GC→cGMP pathway with ischaemic or ischaemia-reperfusion injuries most frequently relates to *c*NOS activation. If this is so, then current evidence suggests that this 'early' response is predominantly 'neuroprotective'[486]. Thus, with ischaemic or ischaemia-reperfusion injuries, excess consitutively-released NO ameliorates penumbral zone ischaemia via vasodilatory and anti-platelet aggregatory functions[486]. Another neuroprotective mechanism would be that of nitrosoyl inhibition of glyceraldehyde-3-phosphate dehydrogenase function, which would lead to a diminished lactate production[486]. A further 'protective' mechanism still may concern NO's role in stabilizing mast cells[201, 389]. We have already noted that such cells are curiously prevalent around sites of aneurysmal rupture (Part 2, 1.4.2), as they are also within coronary atherosclerotic plaques where they are thought to mediate plaque rupture[201]. NO-mediated mast cell stabilization may therefore prevent MC discharge of lytic agents; as well as ameliorating the effects of MC-induced ischaemia-reperfusion injury[312, 313]. Either way,

*c*NOS activation is thought to result from ischaemia-induced calcium mobilization, although nitroxidergic neuronal activation would also achieve this[486]. Whatever the mechanism, the current results clearly suggest that greater MCA dilation will result with L-ARG acting as substrate. It is interesting, in this regard, that increased CSF L-ARG levels are apparent after SAH[304]. However, for *c*NOS activation to be tenable, we would have to accept that:

- Acute SAH in some way triggers widespread calcium mobilization (c.f. head injury)
- The 'calcium trigger' deriving from SAH continues within the MCA wall into and throughout the period of myography

Clearly, the current study cannot provide sufficient evidence to speculate further upon either of these requisites: future studies would be indicated instead.

Another possible explanation for L-ARG→NOS→NO→GC→cGMP pathway activation is that of de novo *i*NOS production within the vessel wall (Part 3, 1.8). Most studies have suggested that *i*NOS activation is characteristically delayed, since changes in genetic transcription are implied. However, it must be remembered that MCAs were immediately transferred to a bath at normal body temperatures containing PSS: thus, a continued incubation of MCAs already genetically 'switched-on' is conceivably possible here. Support for the latter possibly derives, in the current study, from the trend toward increased E_{max} at 2 and 3 hours' survival—only these values differed significantly from sham operative or non-operative controls (Table 29, Appendix). Further support may also derive from the fact that L-NAME efficacy remained invariant after SAH; whilst L-ARG efficacy was increased (Table 28, Appendix). This result suggests that an alternative source of NOS has been created by SAH that is not tonically released. However, in reality this extra source may equally as likely represent *c*NOS as *i*NOS: in fact, no results from the current study can further aid in differentiating this 'extra' NO source.

*i*NOS can only be activated by endotoxin or cytokine stimulation (Part 3, 1.8). It is clearly possible that operation could have introduced Gram negative bacteria into the rat's internal *milieu*, and so elaborated endotoxin. One such source could be that of the plastic cannulae used for arterial or intracranial pressure monitoring. Another source is that of the 3/0 Prolene® thread inserted into the ICA: especially on those occasions where the same thread was used in different animals. However, all of these materials were—as with the operative instruments—regularly cleaned with detergent and, just prior to surgery, cleaned with sterile alcohol wipes and allowed to dry. **Furthermore, sham operative animals underwent exactly the same manoeuvres excepting intracranial arterial rupture**. The current results therefore tend to implicate, a priori, cytokines rather than endotoxin as the trigger toward putative *i*NOS production. It is interesting in this regard that several studies

have demonstrated the presence of cytokines within the CSF of SAH patients[439, 443]. Furthermore, Beasely et al demonstrated that interleukin-1 lead to a prolonged L-arg-dependent increase in cGMP production in rat VSMCs[37], whilst Smith et al have directly demonstrated interleukin-1 within human cerebral vessels after SAH[555]. Circumstantial evidence for central cytokine liberation is also extant in the form of the prolonged central pyrexia[163, 518] and drowsiness[163, 396] that is frequently observed in SAH patients (Part 1, 1.1.2). Such *i*NOS activation is usually harmful and delayed (Part 3, 1.8), and will be discussed later (Part 6, Section III). However, to reiterate: **the current study cannot attribute enhanced L-ARG efficacy—in the presence of unchanged L-NAME efficacy—to either *c*NOS or *i*NOS activation.**

SAH sub-group analysis revealed that the failure of SAH L-ARG EC_{50} to attain overall significance was largely due to the confounding effects of SAH Group 1 (Table 27, Appendix). Thus, when the remaining overall SAH EC_{50} from Groups 2 and 3 was re-assessed—with Group 1 excluded—statistical significance was obtained against shams and non-operative controls. It therefore seems that SAH Group 1 in some way obtunded the general effect which, overall, SAH had activated in MCAs (*see* **3.9** *below*). The possible explanation for this confounding effect must relate to one or more of the following factors:

- Prolonged unilateral CCA re-clamping (1-2 hours) and potential unilateral ischaemia
- Repeated ICP re-rises (the *raison d'être* for re-clamping)
- Early mortality (100% in Group 1)
- Protective effect of type I acute pressor response (75% in Group 1)
- Small experimental group numbers distorting statistical significance

It is interesting that a procedure normally associated with NOS induction—i.e. carotid ischaemia—might be, when combined with acute SAH, conceivably 'anti-NOS inductive'. However, the evidence that CCA clamping—for even 1-2 hours—in rats necessarily incurs ischaemia is not convincing. Thus, studies that use rats as a substrate for carotid ischaemia invariably co-administer profound hypoxia in order to achieve this result[210], since the CoW in rats is amongst the most completely developed of the animal kingdom. Moreover, the Sheffield Model demonstrated that CCA clamping did not significantly affect ipsilateral CBF and, more specifically, concluded that "...perfusion in the distribution of the ipsilateral MCA territory is not affected by clipping the CCA on the ipsilateral side" [625]. Finally, Rosenblum has demonstrated, in an in vivo pial model, that vasoreactivity to agonists similar to those used in the current study was not affected by CCA ligation for periods of up to 6 hours[513]. Such findings endorse the findings of insignificant side differences in the current study (Tables 24-26, Appendix) and suggest that the degree of CCA clamping utilized in SAH Group 1 did not alter ipsilateral vasoreactivity. It seems

unlikely, therefore, that carotid ischaemia could have been responsible for the 'normalizing' effect upon SAH-induced increased L-ARG efficacy.

In contrast, the possibility that early mortality was responsible seems—at least superficially—more attractive. For example, *i*NOS activation (*see* above) probably requires changes in genetic transcription, which would take some time to evolve: death within 1-2 hours could, conceivably, have arrested this process. As a result, no increased L-ARG efficacy would have been expected to have been observed, despite the presence of the 'adequate stimulus' in the form of acute SAH. However, as aforementioned, MCAs were immediately expropriated to an oxygenated PSS bath that could easily have permitted a continued incubation for a period of several hours (Part 4, 6.1).

The possibility that the 'normalizing' effect of SAH Group 1 on L-ARG→NOS→NO→GC→cGMP pathway activation after SAH related to repeated ICP re-rises after attempted reperfusion is also difficult to entertain conceptually. Transient disturbances in CBF and CPP notwithstanding, such episodes would hardly be long enough to constitute 'ischaemia-reperfusion' injuries. However, they may be short enough to have constitute **ischaemic pre-conditioning**: interestingly, NO—from *c*NOS as source—has recently been implicated in this role (Part 2, 2.6.2). Repeated re-bleeds, on the other hand, would more feasibly be detrimental, since these would imply a greatly magnified stimulus than that of a solitary SAH-with-reperfusion. However, as is discussed in Part 6, Section I, 1.4, an ICP re-rise may not necessarily imply a re-bleed: it may also reflect an acute cerebrovascular engorgement. The occurrence of the latter phenomenon, in the acute setting of SAH at craniotomy, has been succinctly described by West[651]. An acute cerebrovascular engorgement could be 'protective' in SAH by, tamponading SAH with a *smaller* V_E; and by holding vessels 'open' thus preventing no-reflow phenomena.

It seems most likely, then, that a small statistical group number—combined with a 100% group mortality—represented the most likely source for the confounding effects of SAH Group 1 on a significant increase in L-ARG affinity after SAH. However, one other possibility deserves serious consideration: 75% of Group 1 animals experienced an acute pressor response type I. Interestingly, this pressor group yielded an E_{max} that was statistically insignificantly different from shams and non-operative controls: the major overall 'SAH effect' instead deriving from acute pressor response types II and III ($p = 0.0001$: Table 30). Thus, it is entirely possible that the type I acute pressor response represented the 'protective' effect of SAH Group 1. If so, then the staunching of SAH at lower cerebrovascular shears, and lower cerebral compressive strains, seem somewhat convenient explanations.

L-ARG affinity was significantly increased in the 1 and 3 hour survival groups, but unchanged from either shams or non-operative controls in the 2 hour group (Table 29, Appendix). The major feature of the 2 hour survival group was that 4/8 (50 %) animals derived from SAH Group 1. We have already seen that this group presents many features of a 'statistically separate' group from other SAH sub-groups. It would therefore appear that the unchanged affinity of the 2 hour sub-group reflects the undue influence of SAH Group 1 upon the general trend toward an increased L-ARG affinity after SAH.

No statistically significant trend was demonstrated in either E_{max} or EC_{50} values across acute pressor response groups (Table 30). Nevertheless, the trend in E_{max} is nearly significant and, when type II & III acute pressor responses are considered together their combined value (101±5 %) is significantly different from type I (85±4 %: $p = 0.02$). Furthermore, the type I E_{max} does not significantly differ from either shams or controls, whereas the type II & III response does so with a high degree of significance ($p = 0.00005$). Because the type II & III arterial pressor response groups suffered a significantly higher AICP than those of type I, there is some justification in treating these groups separately. With such a high degree of significance, it would seem likely that the increased cerebrovascular shear experienced with the former groups was a factor in their significantly increased E_{max} values. If this is so, then it constitutes a major finding in the current thesis, since it strongly suggests that the acute physiological disruption does indeed exert a direct and significant effect upon cerebrovascular reactivity that is clearly contradictory to current results emanating from 'clot-VSM' (i.e. type II) SAH models.

Conclusion

L-ARG efficacy is significantly increased after SAH as compared to shams and non-operative controls. Furthermore, a significantly greater proportion of MCAs are dilated beyond baseline MT after SAH than in shams or non-operative controls. If SAH Group 1 is excluded, L-ARG affinity is also significantly greater after SAH than in shams or non-operative controls. A similar effect is seen with survival, where the 2 hour group—disproportionately represented by SAH Group 1 (50 %)—does not differ from shams or non-operative controls; whilst the 1 and 3 hour group (in which no SAH Group 1 animals occur) did so. Overall, such results suggest an activation of the L-ARG→NOS→NO→GC→cGMP pathway. Such activation could derive from either *c*NOS or *i*NOS activation. Currently, *c*NOS activation is thought to be 'early' and 'neuroprotective' with cerebral insults; *i*NOS 'delayed' and harmful. The results from the present study suggest that L-ARG will lead to greater vasodilation of MCAs after SAH: this may prove beneficial in the reperfusion of ischaemic penumbras, and in offsetting post-SAH no-reflow phenomena. Increased L-ARG efficacy, in the presence of unchanged L-

NAME efficacy, suggests that the alternative source of NOS produced after SAH is not tonically released.

Significantly increased L-arg E_{max} after SAH appears specifically restricted to animals exhibiting a typeII/III arterial pressor response. Significantly increased L-arg E_{max} after SAH also appeared more likely in 'survivors'. If this is so, then it constitutes a major finding in the current thesis, since it strongly supports the principle hypothesis that the acute physiological disruption exerts a direct and significant effect upon cerebrovascular reactivity that is independent of later chronic clot lytic effects. Both of these effects were reiterated with SNP (*see* below).

The fact that no significant differences were found between right and left MCAs in response to L-arg also suggests that EF-SAH models do not introduce any significant 'side bias' upon ipsilateral MCA reactivity by virtue of the potentially confounding combination: *1)* unilateral carotid manipulation, *2)* unilateral EF insertion or *3)* potentially ipsilateral intracranial rupture.

3.9 SNP responses

Further evidence from this study that the L-ARG→NOS→NO→GC→cGMP pathway had become activated after SAH derives from the results with SNP. Thus, both the efficacy and affinity of SNP were significantly increased overall after SAH in relation to either shams or non-operative controls (Table 28). This fact can be appreciated in both the combined frequency histogram of Fig 45, and the combined C-R curves of Fig 46. Furthermore, similar to L-ARG, the prolonged CCA re-clamp group (i.e. SAH Group 1) appeared to obtund the increase in both efficacy and affinity that, overall, SAH otherwise produced (Table 27, Appendix). Thus, by excluding SAH Group 1, the significance of these values over shams and non-operative controls was increased further still.

Once again, no significant side differences were found within SAH Group 1 (nor, indeed, were any side differences demonstrated in either Group 2 or 3 for that matter: Tables 24-26, Appendix). This, superficially a least, suggests that the obtunding effect did not relate to the unilateral clamping per se. However, the n numbers here were even smaller still (n = 3 MCAs on each side), so this deduction cannot possibly be conclusive. Nevertheless, it does prompt one to strongly consider other explanations, as discussed previously with L-ARG (*see* 3.8 above).

As with L-ARG responses, a trend toward increased SNP efficacy with prolonged survival after SAH was apparent, although not of statistical significance (Table 29, Appendix).

Further studies would be required to confirm whether or not this trend continued to significantly increase outwith the arbritary three hour period. SNP affinity, however, was significantly increased after a type II or III acute pressor response relative to either a type I response, or shams and non-operative controls (Table 30, Appendix). This suggests that most of the 'SAH effect' upon SNP affinity relates primarily to the invariant and Cushing hypertensive responses: the type I response appears 'protective'. This is an interesting observation because, as was seen above, the type I pressor response also appeared 'protective' toward the L-ARG E_{max}—maintaining its equivalence to both shams and non-operative controls. Again, as with the latter finding, we may speculate that the staunching of SAH at lower cerebrovascular shears, and lower cerebral compressive strains, were important contributors in this regard.

3.9.1 Therapeutic implications for SNP after acute SAH

Hypertension is clinically apparent in the early post-acute period. This is most likely due to either pre-existing hypertension, or to nociceptive afferent stimulation deriving from the meninges, in a fashion after Stoner[562]. As has already been noted, increased levels of MAP at this juncture may be detrimental, in spite of the fact that they may represent a mechanism to re-open a closed microvasculature[132]. Several other aspects of SNP's effects may in fact prove beneficial in this context. Firstly, SNP is more efficacious toward cerebral vessels in the early hours after SAH. As a result, SNP may **augment autoregulatory dilation** of the cerebrovasculature at the **lower limit** of the **MAP range**. Furthermore, by virtue of its anti-platelet and fibrinolytic effects (Part 3, 1.8 & 1.10), SNP may also serve to **protect against NRP**; whilst its mast cell stabilizing properties may also limit ischaemia-reperfusion injuries, as well as—potentially—re-rupture.

Table 8. Therapeutic possibilities for SNP after acute SAH

Pharmacological property	Therapeutic protection offered
Antihypertensive	Vulnerable microcirculation
	Re-rupture
Antiplatelet	No-reflow phenomena
Fibrinolytic	No-reflow phenomena
	Reduce clot size - offset vasospasm
Mast cell stabilizing	Ischaemia-reperfusion injury
	? Re-rupture

The fact that no significant differences were found between right and left MCAs in response to SNP suggests that EF-SAH models do not introduce any significant 'side bias' upon ipsilateral MCA reactivity by virtue of the potentially confounding combination: *1)* unilateral carotid manipulation, *2)* unilateral EF insertion or *3)* potentially ipsilateral intracranial rupture.

SECTION III
Conclusion

The endovascular filament (EF) model used in this study created a SAH that closely resembled the clinical scenario. This is not the case with the vast majority of models currently employed. Although others have also begun to use similar models[40, 41, 236, 302], **no EF-SAH study has simultaneously analyzed isolated cerebral vasoreactivity: the current study appears to be the first to do so. Moreover, isolated cerebral vasoreactivity was analyzed in this study in all cases immediately upon animal sacrifice: no vessels were fridge-kept for use on subsequent days**. The SAH produced in this study was frequently severe, both in terms of V_E as well as in terms of the significant mortality (42%) incurred. It is curious that many might see such a mortality as a potential weakness of the model used, given that SAH is a significant cause of sudden death clinically[130, 374, 472]. Thus, between 40-50% patients with SAH may die before reaching hospital[477]. More specifically, Bederson et al recently recorded a similar *early* mortality (57%) in an experimental SAH study using endovascular filament rupture in 21 rats[41]. A similar figure (50%) was also recorded in an earlier study with 22 rats: in that study, 25% of early mortality occurred *within the first two hours*[40]. In one other study, a 16% mortality was observed *within the first hour* following basilar artery rupture in 94 rats[302]. Finally, in the previous study from the author's laboratory —in which the same endovascular filament model was used as in the current study—a 53% mortality was recorded *at three hours* in 43 rats[625]. The plethora of current SAH models (in reality, 'clot-VSM' models: Part 4, 1.2), therefore, that deliver survival rates in excess of 90% into the first week—whilst certainly being *efficient* in terms of experimental loss—utterly betray the reality of the acute ictus.

The potential devastation of the acute ictus, then, is obviously a factor completely overlooked by current 'clot-VSM' (i.e. type II) SAH models. Potentially, then, factors associated with the acute ictus might go someway toward explaining why type II models fail to deliver clinically useful information. By showing that, in the current study, MCAs from 'survivors' differed significantly from shams and controls—at least in some of their physio-pharmacological properties—then at least one possible explanation for the current failings of type II models might, therefore, be advanced. Thus, the vessels subjected to chronic clot lysis in type II models are indistinguishable from controls, when—as the the current results clearly suggest—vessels from 'survivors' of an acute ictus are significantly 'altered' from controls. Because it is eminently conceivable that other differences will also subsequently be found with other agonists, it seems likely that type II models might be fundamentally flawed because they do not commence study from the correct 'baseline'.

The current results, therefore, suggest that chronic 'clot lytic' effects ought to be superimposed upon cerebral vessels *that have been significantly altered from controls by virtue of 'acute ictal' effects: they should not be merely superimposed on vessels indistinguishable from controls*. The principle hypothesis of the current thesis is therefore endorsed: i.e. that events associated with acute SAH elicit changes in MCA physio-pharmacology that are independent of chronic clot lytic effects; but which may, nevertheless, subsequently interact with the latter effects to potentially explain both delayed VSM and DCI.

Notwithstanding, by noting that 'survivors' significantly differed from shams and controls, at least one potential conundrum immediately arises. Thus, it could be reasoned that responses obtained from 'non-survivors' ought to be even more altered than 'survivors'; because—it could be naturally presumed—the acute ictus in 'non-survivors' might have been more 'extreme' than with 'survivors'. Notwithstanding, several rational explanations can immediately be advanced to counteract this conjecture. Firstly, in animals dying acutely, it is conceivable that insufficient time could have elapsed for the processes required in generating the physio-pharmacological alterations to have become established. Secondly—and probably far more likely—these very processes may subsequently have become negated by the secondary effects that the acute ictus has subsequently wreaked: in particular, the secondary confounding effects of a hypotensive state (i.e. SCC) upon MCA reactivity[335]. Finally, it proved extremely difficult—if not impossible to identify any parameter that correlated with the concept of 'severity' of SAH in this study. In particular, neither the V_E (Part 5, section I, 2.1)—nor the ΔMAP or ΔICP (Table 22, Appendix; Part 5, section I, 1.4)—appeared to correlate with 'survival' or acute mortality in this thesis.

In addition to the potential importance of ictal 'survivors' in explaining the current scientific failure of type II models, another factor also relates to the dichotomy of acute arterial and intracranial pressor response observed after SAH—with it's subsequent effect upon acute cerebrovascular reactvity. Indeed, a major distinction in the current results from those described by others relates to the fact that a massive ΔICP—with a correspondingly high ICP_{max} and a CHR—proved extremely rare (6%). In fact, the opposite response—an acute hypotension—was the most frequent observation (63%): with an invariant response making up the remainder. Interestingly, the acute pressor response observed correlated with the extent of the ICP_{max} and, therefore, with the ΔICP. More specifically, acute hypotension correlated with lower ΔICPs, whilst a CHR was only observed at ΔICPs over 65mmHg. The latter finding, in fact, is compatible with previous findings[114, 115, 180] and suggests the presence of central pressure (or mechano-) receptors which are only triggered at, or above, such an ΔICP.

Of particular importance to the central hypothesis of the current study, however, was the correlation of the type of acute ΔMAP/ΔICP observed with significantly altered vasoreactivity to certain agonists. In particular, significantly increased L-arg E_{max} after SAH appeared restricted to a typeII/III arterial pressor response, whilst the type I response did not significantly differ from shams or controls. Importantly, this fact remained so even after allowing for 'survivors' and 'non-survivors' within this category (indeed, there was no correlation between the acute pressor response and mortality in this thesis: Table 20, Appendix). Thus, the type I response appeared to exert a 'normalizing' effect upon an otherwise overall 'drive' of acute SAH toward eliciting a significantly increased L-arg E_{max}. One potential explanation for this effect relates to the known effect that endothelial shear stress has upon the cerebrovasculature under normal circumstances: i.e. the 'tonic' release of NO basally[75, 502]. Thus, given that the endothelial shear is likely to be much greater after a type II/III response (where both MAP and ICP are significantly greater [Part 5, Section I, 1.3.1; also *see* Fig 9]) than after a type I response, it is conceivable that this mechanism may have been 'up-graded'—and rendered somewhat more persistent—as a result of the acute ictus. Notwithstanding the precise mechanism, however, the dichotomy of acute ΔMAP/ΔICP after SAH demonstrates another factor—i.e. distinct from that determining acute mortality—associated with the acute ictus that is able to alter MCA reacitivity from that of shams and controls.

Moderate acute ΔICPs and negative ΔMAPs—as evidenced with a type I arterial pressor response—also appeared to be associated with potentially 'beneficial' effects towards other agonists. For example, similar 'normalizing effects' were also seen with the EC_{50} values of SNP, 5HT and NE (*see* below) which also significantly differed from controls after SAH only with a type II/III pressor response. Nevertheless, as aforementioned, the type I arterial pressor response did not appear prophylactic toward an eventual SCC—and, therefore, toward an acute mortality. In fact, SCC was equally as likely irrespective of the precise acute pressor change that preceded it (Table 20, Appendix). It would seem, therefore, that SAH per se—irrespective of arterial and intracranial tensions created—is peculiarly prone to producing cardiovascular dysfunction. Furthermore, the frequent occurrence of SCC in the absence of a CHR (94 % in the current study) must necessarily implicate non-adrenergic mechanisms in this response—a conclusion that is clearly contrary to current neurosurgical dogma (Part 1, 1.3.2.1). It would seem, therefore, that cardiovascular dysfunction after SAH may not necessarily correlate with cerebrovascular dysfunction.

The finding that L-arg E_{max} is significantly increased after SAH suggests the L-ARG→NOS→NO→GC→cGMP pathway is activated acutely after the acute ictus: this is

completely contradictory to a majority of extant publications[244, 272, 318, 325]. However, it must be remembered that the current results only pertain to the first three hours after SAH: thus, it is entirely possible that the acute findings could ultimately become reversed chronically. Notwithstanding, an acute early activation of the L-ARG→NOS→NO→GC→cGMP pathway—which most frequently relates to *c*NOS activation—is currently believed to be 'neuroprotective'[486]. For example, with ischaemic or ischaemia-reperfusion injuries, excess consitutively-released NO ameliorates penumbral zone ischaemia via vasodilatory and anti-platelet aggregatory effects[486]; diminishes lactate production via nitrosoyl inhibition of glyceraldehyde-3-phosphate dehydrogenase[486]; and stabilizes mast cells (thereby off-setting reperfusion injury)[201, 389]. *c*NOS activation is thought to result from ischaemia-induced calcium mobilization, or to nitroxidergic neuronal activation[486]. By whatever mechanism, the current results suggest that this pathway is more efficacious after SAH. The increased proportion of MCAs where L-ARG E_{max} >100% may, in addition, explain the degree of **vasoparalysis** that is sometimes observed after SAH (particularly in the poorer clinical grades)[257, 453]—and the increased CBV that may be recorded in consequence[222-224].

Nevertheless, it is not easy to see how such acute findings (i.e. with L-arg E_{max}) might ultimately become reversed chronically; to thus become compatible with results from 'clot-VSM' models[244, 272, 318, 325]. One potential explanation, however, derives from consideration of an alternative explanation for the mechanism of enhanced L-arg E_{max} in the current study. Thus, one other explanation for L-ARG→NOS→NO→GC→cGMP pathway activation is that of de novo *i*NOS production within the vessel wall (Part 3, 1.8). The combination of *i*NOS-NO with other toxic agents, such as free radicals (e.g. with superoxide leading to **peroxynitrite formation**[39]—a potent liberator of further free radical formation: Part 3, 1.8), may result in the progressive inhibition of VSMC metabolism. Interestingly, haemoglobin—i.e. such as released during chronic clot lysis—can augment cytokine-mediated *i*NOS production within VSMCs[573]. Since VSMC levels of ATP and GTP are known to be depleted following the mere application of surface blood to cerebral vessels[633], the effects of *i*NOS (Part 3, 1.8) may therefore be exacerbated. If so, then a cellular metabolic exhaustion could result, and lead to a 'rigor-type' increase in VSMC tone[566] (Part 2, 3.3). Such a mechanism is clearly compatible with the **myonecrosis** that accompanies VSM[204-206] (Part 1, 1.3.1.2). Because, in large numbers of VSMCs in this state, acto-myosin bonds would be permanently engaged (Part 2, 3.3), such vessels would appear **rigid, contracted** and **hypo-responsive**[472,623, 655]. However, because myonecrosis is typically patchy, a partial responsiveness to both contractile agonists and vasodilators would also be expected[472]. The production of a 'latch-state'[566] as a consequence of a clot-product/NO interaction could, therefore, account for cases of **'irreversible' VSM** observed

clinically. *Such reasoning therefore provides a mechanism that potentially links acute changes in post-SAH MCA reactivity (as found in this thesis) with the subsequent effects of chronic clot lysis (not analyzed in the current thesis).*

The current results suggest that relatively modest ICP_{max}:MAP ratios—perhaps only in the range 40-60%—are required to staunch SAH (Part 6, Section I, 1.4.3). If this is correct, then it suggests that ratios in excess of this—as are necessarily indicated by a type II & III response—may be unnecessary; and, possibly, detrimental. Excessive ICP_{max}:MAP ratios after SAH would certainly encourage a complete cerebrovascular closure: however, they would also risk the development of no-reflow phenomena (NRP) upon—and despite—reperfusion. The triggering of the CHR at AICPs of approximately 65 mmHg—as found in the current study—may serve to offset this effect. The CHR may, therefore, denote an evolutionary mechanism that serves primarily to ameliorate NRP where intracranial pressure venting has, in turn, permitted a rapid and excessive ΔICP. The CHR would not be 'necessary', however, where a high ICP_{max} related to a cerebrovascular expansion[222-224, 349, 453, 454, 491, 651] (Part 6, Section I, 1.4). Clearly, cerebrovascular expansion—by holding vessels open—would per se limit NRP just as effectively as it would tamponade SAH. This state of affairs would be analogous to the use of positive-end-expiratory-pressure (PEEP) to prevent atelectasis during pulmonary ventilation. Such a mechanism would also serve the expedient of limiting V_E and, therefore, the delivery of contractile and myoproliferative agonists into the subarachnoid space. On the debit side, however, is the greater cerebrovascular shear—as well as the greater cerebral parenchymal strain—that a 'cerebrovascular expansion-with-CHR' necessarily implies.

Subtle differences in MCA reactivity notwithstanding, it is also worth noting that the results from the current study demonstrated that MCA *contractility* remained largely preserved despite the nature and severity of the acute ictus incurred: i.e. there was no gross disruption of MCAs consequent upon the acute ictus. For example, the spontaneous development of myogenic tone (MT) to intraluminal distension—the basis behind Bayliss' original concept of 'autoregulation'[35]—appeared uniformly intact after SAH; as well as remaining remarkably invariant across each SAH sub-group considered. MT was manifest in the continued, rhythmic, tonic variation of mounted MCAs; as well as in the invariance of functional diameters between sub-groups functional diameters (FD_{50}). However, it was particularly manifest in the invariance of MCA FD with increasing intraluminal distension (Figs 14 & 32). In addition, maximum MCA contractile power (*contractility*) to non-specific depolarizing stimulation (i.e. KCl) also remained remarkably uniform after SAH; as well as between numerous SAH sub-groups. MCAs pre-constricted with AT from PGF2α also dilated to drugs, such as PPV, in a manner indistinguishable to those of

controls. A major conclusion from the current study, therefore, is that whilst vasoreactivity to some agonists certainly was altered after SAH, no *gross* derangement of contractile or dilatory *potential* was demonstrated.

Ironically, this fact is also at variance with the findings of certain others where profoundly diminished cerebrovascular function has been found following a type I (i.e. arterial rupture) SAH[94, 289, 306, 435, 472, 599]. For example, most groups studying in vivo responses have demonstrated acute alterations in CO_2 reactivity[289, 306, 408], metabolic regulation[306, 408], and/or autoregulation[289, 306] after SAH from arterial rupture. In particular, some have shown an acute vasoparalysis[289]. However, as noted in Part 4, 4.1, all in vivo studies suffer from the potential setback of the continuing effects of the anaesthetic agent: furthermore, many of these studies were thwarted not only small group numbers[289, 408] but, in addition, by even smaller numbers of shams[289, 408]. More specifically, however, other groups have demonstrated global vessel hypofunction following a type I SAH on isolated vessels in vitro[599]. Unfortunately, such studies have invariably created arterial rupture via a previous craniotomy (with all it's potential setbacks [Part 4, 1.1]), as well as having utilized decidedly unphysiological small vessel study models (Part 4, 4.1)[599]. In contrast, our results were obtained via in vitro myography following non-craniotomy arterial rupture.

It is interesting that Bokowski et al recently recorded a similar preservation of acute cerebrovascular contractility after midline, fluid percussion, total brain injury in rats[56]. The limitations of the latter study, however, rendered the authors unable to state whether or not MT may also have been preserved. Although acute SAH, in principle, resembles a fluid percussion injury; it is clearly less severe. The current findings—that both MT and maximum constrictory/dilatory *potential* remained intact after SAH—would not appear, on this comparison, to be surprising. What the current findings do imply, however, is that if any acute derangement of MCA function is observed in vivo, then this is likely to have occurred against a background of essentially normal contractile and dilatory *potential*. In this sense, we may therefore conclude—as, indeed, did Bokowski et al[56]—that any *acute* in vivo effects are likely to prove *reversible*: they would not be likely to reflect an inevitable, irreversible loss of function, as implied by aforementioned type I SAH studies[94, 289, 306, 435, 472, 599]. One possible application of this principle derives from the findings of enhanced L-arg E_{max} in the current thesis: in particular, the significantly increased proportion where L-arg E_{max}>100% (Part 6, Section II, 3.8). Thus, should maximal L-arg (i.e. $>10^{-4}$ M) be released after SAH, then this may threaten dysautoregulation in a proportion of MCAs—in a fashion similar to that deduced for HA and PPV (Part 5, Section II, 1.8 & 1.9 respectively). Since, however, such hypothetical 'vasoparalysis' would be purely 'agonist-induced'—and not due to a fundamental cerebrovascular derangement—it would,

potentially, be fully reversible (i.e. with L-arg antagonists, such as L-NAME). 'Vasoparalysis' after acute SAH is then, on this scheme, theoretically pharmacologically reversible.

There may, of course, be several explanations for the disparity between the current findings of preserved overall contractility and those of other studies suggesting otherwise. One piquant explanation, however, relates to the possibility that the results of previous studies may have been distorted by a failure to have compared 'like with like'. The source of such error may include deficiencies in either the SAH model (Part 4, Ch 1 & 2), the vessel study model (Part 4, Ch 4), or in the actual vessel chosen for study (usually the basilar artery: Part 4, 6.8). Some studies have, in fact, suffered from deficiencies in all three domains[94]. However, one other critical factor—and indeed, one not hitherto referred to—may derive from the relationship herein expounded between contractile E_{max} and functional diameter (Part 6, Section II, 1.1). Thus, it may be that many of the effects observed in certain previous studies have, essentially, related to the inter-group comparison of incomparable vessels. As the current study has demonstrated, more than half of the variance in response efficacy may be attributable—at least with some agonists—simply to a variance in vessel functional size. This is important because MCAs demonstrate a wide range of functional diameters, which are frequently asymmetrical. Sampling errors may, therefore, be a problem with small experimental numbers: but, especially, with paired controls. Sampling errors therefore remain a concern in all studies—admittedly the majority—where vessel diameters appear to have gone completely unrecorded.

A rather more prosaic explanation for globally reduced cerebrovascular responses after SAH in other studies[94, 289, 472, 599] may relate to the study of *chronic* VSM per se. In the latter situation, vessels have been exposed to clot in vivo for several days. As a result, the encased and thickened clot (along with a 'fibrous' meningeal reaction[164, 232, 288] [Part 1, 1.3.2.2]) may preclude the careful dissection and manipulation of vessels for in vitro analysis. The possibility of potential **manipulative trauma** is almost never acknowledged in SAH-VSM correspondences: yet it is clearly a likely cause of *global hypofunction* if this is found in such vessels. In contrast, because SAH was terminated within three hours in the current study, most of the peri-vascular clot could still be irrigated away; thus permitting a safer dissection. As a result, MCAs could be manipulated with almost as much vision as those in controls. Therefore, the fact that MCA function was assessed acutely may alone explain the finding of essentially preserved vascular function in the current study: in stark contrast to the findings of chronic studies.

It is certainly possible, however, as aforementioned that preserved cerebrovascular *contractility* in the acute setting might become reversed chronically as a result of the progressive effects of clot lysis. In particular, one must pay especial regard to results obtained in human vessels harvested from patients dying at some time after SAH. For example, in contrast to the present results, Onoue et al demonstrated diminished contractile responses to KCl, PGF2α, NE and to 5HT in one cadaveric study[472]! This suggests a primary dysfunction of VSMCs that may, therefore, only become apparent in the chronic situation. If so, then it would certainly tie in with the finding of a myonecrosis—and a consequent thinning—of the tunica media observed in so many post mortem studies (Part 1, 1.3.1.2). Unfortunately, there are several setbacks with cadaveric studies. A heterogeneous sample of vessels (ACAs, MCAs etc—each of differing diameter) is often obtained from a heterogeneous group of patients (in terms of age, sex, operative status, and time post ictus); whilst a major problem exists with controls. Furthermore, in the aforementioned study, an additional problem related to the use of helical strips—a most unphysiological form of vessel study (Part 4, 4.2.1)[472].

Variable degrees of unilateral CCA clamping were employed in approximately half the animals used in this study. However, prolonged clamping was only employed in 4 animals. The motive for CCA clamping largely stemmed from the findings emphasized in the previous study from this laboratory[625]. In the latter study, prolonged CCA clamping was employed *from the outset* to lessen SAH severity and, a priori, to offset the excess mortality that was inevitably encountered when immediate reperfusion was restored in that study[625]. In the current thesis, CCA clamping was never used electively as a prolonged manoeuvre from the outset: instead, CCA *re*-clamping was only employed whenever an ICP re-rise was encountered with each attempted reperfusion. CCA re-clamping was therefore used in an attempt to offset the confounding effects of a *presumed* secondary haemorrhage. The rationale behind the use of CCA re-clamping in the current study, therefore, radically differed from that of the previous study. Such differences presumably underlie the sometimes radically different findings between the two studies. For example, a 100% mortality was experienced with prolonged CCA clamping (i.e. SAH Group 1) in the current study (Table 21, Appendix); whilst the equivalent group from the previous study yielded the *lowest* mortality (33%) of that study[625]. In similar fashion, the lowest mortality in the current study (29%) was obtained after immediate full, reperfusion; whilst a 100% mortality was experienced in the equivalent group from the previous study[625]. It is interesting that such diametrically opposed results were obtained from each study—a fact that presumably relates to the profound methodological difference that existed between them (i.e. the deliberate ploy of utilizing prolonged clamping *from the outset* in the previous study—Part 4, Ch3.3).

Notwithstanding, varying degrees of CCA clamping appeared to be tolerated without any overt effect in the current thesis. Specifically, no significant right-left side differences were found with any agonist employed in this study, despite even prolonged clamping in SAH Group 1. However, the combined right and left results from SAH Group 1 did significantly differ from SAH Groups 2 & 3 in many agonist responses: in fact, **SAH Group 1 was the most frequently abnormal SAH sub-group that existed in this study.** For example, SAH Group 1 5HT affinity was significantly weaker than either SAH Group 2 or 3; or the affinity of shams and controls. In contrast, SAH Group 1 SNP affinity, as well as SAH Group 1 L-ARG affinity and efficacy—though significantly different from SAH Group 2 and 3 responses—remained equivalent to shams and controls. Thus, in the latter situation, SAH Group 2 and 3 responses were 'abnormal': SAH Group 1 responses were similar to shams and controls. Insignificant side differences in all of these cases argues against the possibility that 'Group 1 effects' related *per se* to unilateral CCA ischaemia. In attempting to explain these findings, one important factor that must be considered is that of the statistically small group number necessarily considered (i.e. n = 4). However, three other factors also distinguish SAH Group 1. Firstly, since CCA re-clamping was only employed after an ICP re-rise, SAH Group1 may be distinguished from the other two groups by the *possibility* that *at least two* re-bleeds may have occurred. Secondly—and perhaps related to the latter—SAH Group 1 animals suffered a 100% mortality rate, with all deaths occurring in the 2nd hour. Thirdly, since 75% of Group 1 animals sustained a type I acute pressor response, the aforementioned 'normality' of this group's findings could also relate to the 'protective effect' of acute hypotension with its necessarily diminished cerebrovascular shears [Part 6, Section I, 1.4.3]. In conclusion, prolonged CCA re-clamping in response to an ICP re-rise with attempted reperfusion probably does not influence ipsilateral vasoreactivity directly: but it may influence bilateral responses as a result of some other factor.

The fact that prolonged unilateral CCA re-clamping may be without significant ipsilateral effect on MCA reactivity is consistent with several fundamental principles and experimental findings. Firstly, the CoW in rats ranks amongst one of the most complete in the animal kingdom. This is such that models employing rats as a substrate for unilateral carotid ischaemia are invariably required to render the animal simultaneously hypoxic for at least 1 hour in addition to persistent CCA clamping[210]. [No additional hypoxia is required, for example, in gerbils because the CoW is characteristically deficient here]. Secondly, the "Sheffield Model" of SAH clearly showed that ipsilateral regional CBF remained unaffected by right carotid sheath manipulation and clamping; the authors concluding that "...perfusion in the distribution of the ipsilateral MCA territory is not

affected by clipping the CCA on the ipsilateral side"[625]. Although bilaterally reduced CBF was recorded after SAH in the latter study, this was largely restricted to their "reperfusion" group—and clearly related to a *global* SCC (i.e. the opposite findings to the current study) [625]. Finally, in one detailed experimental study of pial vasoreactivity after CCA clamping, no significant effect was observed to similar agonists used in the current study—even with clamping up to 6 hours[513]. The latter study was in gerbils where CCA clamping invariably leads to unilateral ischaemia.

More specifically, an absence of statistically significant side differences between right and left MCA reacitivity in the current thesis indicates that EF-SAH models per se do not appear to introduce any significant 'side bias' upon ipsilateral MCA reactivity *by virtue of the potentially confounding combination*: *1)* unilateral CCA manipulation and clamping (of variable duration), *2)* unilateral EF insertion or *3)* potentially ipsilateral intracranial rupture. This proof represented a major preliminary requirement of the current study, since no EF-SAH model to date has subsequently assessed cerebrovascular reactivity. Indeed, as a result of such proof, future studies of delayed (i.e. chronic) VSM using EF-SAH models can be performed with greater re-assurance: in particular, should any side differences be subsequently found in such studies, then these could be more readily attributable to focal factors—such as thickened clot lateralization—with somewhat greater confidence than would otherwise have been the case.

The novel finding of NE response heterogeneity in control MCAs was further endorsed by its incorrigible persistence throughout sham operation and SAH. Although a trend existed toward increased heterogeneity after SAH, this did not reach statistical significance. The ratio of NE-'responders':'subresponders' also remained stable across all SAH sub-groups. Although the current study confirmed that NE responses were significantly weaker than other agonist responses, the NE_{max} in 'responders' remained remarkably invariant across controls, shams, and after SAH. Such uniformity in both heterogeneity and NE_{max} permits a mechanism that is able to account for the inherent deficiencies of Fisher's hypothesis[177]. The latter author implied and stated the 'clot = VSM' axiom: nevertheless, a careful review of the results upon which it was based clearly reveals numerous exceptions. Thus, clot can occur without VSM, and vice versa (Part 6, Section II, 3.5.3). If one assumes that NE acting at α adrenoceptors is instrumental in aetiology[190, 313, 576], then the current results suggest that a maximum only of 75% animals could develop VSM *irrespective of clot site or size* after SAH. Since, however, only bilateral NE-'responders' exist in 43% animals after SAH, only this proportion could sustain VSM *irrespective of clot lateralization*. Large epidemiological studies have indeed shown that clinical VSM only occurs in such a proportion (43-67%) after SAH[134]. Furthermore, decreased NE affinity in some SAH sub-

groups (Part 6, Section II, 3.5.2) would localize VSM still further, because significant responses would only occur in areas of higher concentrations—i.e. within the clot itself.

Such reasoning again provides a mechanism that potentially links acute changes in post-SAH MCA reactivity (as found in this thesis) with the subsequent effects of chronic clot lysis (not analyzed in the current thesis). Nevertheless, whilst entirely possible, it is not—alas— seriously the author's intention to use NE heterogeneity to explain clinical VSM in one fell swoop! Although the current results imply that NE efficacy may continue to increase outside the acute period, the results per se only pertain to the first three hours (i.e. this study cannot extrapolate to the delayed situation of DCI). Furthermore, the evidence that an agonist, or agonists, acting at α adrenoceptors is responsible for clinical VSM is not in abundance in the scientific literature. Nevertheless, the findings with NE permit a generalized mechanism that bases VSM on a dichotomy of response in individual cerebral vessels toward a given agonist. Thus, the ability of some vessels to *respond*—but of others to *fail to respond*—to a given agonist, can clearly explain exceptions to Fisher's hypothesis; and, thus, why clinical VSM does not occur in every patient despite an abundance of agonist within the clot. The principle that VSM may relate to the "individual nature" of cerebral vessels is, in reality, not new[63]: however, to the best of the author's knowledge, no actual mechanism has hitherto been proffered. The persistence of NE heterogeneity after SAH clearly represents *one* possible mechanism—however likely or unlikely it may subsequently prove to be. The hypothesis that responses of cerebral vessels to agonists released as a result of SAH might be discontinuous, and not—as is assumed with most, if not all, studies—continuous, may be referred to as 'Brandt's hypothesis' (although this remained implicit and not explicit in his articles).

Blood in the subarachnoid space is also associated, as a result of its chronic lysis, with the copious release of prostaglandins—including PGF2α—as well as 5HT from platelets. Furthermore, local cell decomposition may be expected to be associated with increased concentrations of K^+ ions. The results from the current study have shown that all of these agonists are potent and efficacious vasoconstrictors of rat MCAs; and, furthermore, that they are all at least as efficacious after SAH as they are in controls. Indeed, responses with PGF2α were significantly more efficacious in 'survivors' after SAH. Furthermore, these agonists also constrict vessels in a predictable and **continuous** fashion, with neither heterogeneity nor discontinuity, and with an efficacy that is largely dependent upon functional vessel size. However, of all the agonists tested, 5HT was also the more potent by several orders of magnitude (Fig 35). Whilst UTP responses were, in contrast, the least potent—and their efficacy weaker, like NE responses (Fig 37)—UTP responses are characteristically prolonged, and do not develop tachyphylaxis. Both UTP and NE are also

present within the clot platelets; whilst NE may also derive from the SNS. In consequence, the current study permits the hypothesis that locally elevated levels of K^+, PGF2α, 5HT, UTP and NE may all mediate VSM: either alone, or in combination. The possible interaction of such agonists with each other—as well as with other agonists(Part 3, Ch 2)—may explain the varying severities of VSM that is ultimately seen[322, 616]. This component of VSM—i.e that resulting from agonists or agonist-interaction—remains perfectly reversible (**'reversible' VSM**): as the results with PPV clearly demonstrate.

It was Nagai's belief that the seeds for VSM and DCI were sewn in the profound physiological disturbance that was associated with the acute ictus. In Nagai's words, "...the initial brain damage (caused) by SAH alters (both) the vasoreactivity and the tolerance of the brain itself for cerebral ischaemia..."[435]. The current study cannot provide any evidence that directly relates to the latter assertion although, should *i*NOS prove to be the cause of L-ARG→NOS→NO→GC→cGMP pathway activation, one could speculate that a similar activation might also arise within the cerebral parenchyma. Putative parenchymal *i*NOS activation could, conceivably, produce a similar degree of metabolic disruption as that previously proposed with VSMCs. **The current results, however, certainly do support Nagai's former assertion that the acute ictus alters cerebral vasoreactivity**. Thus, significant alterations were apparent in receptor-mediated activity to several different agonists within the first three hours of acute SAH in the current study. **The principle hypothesis of the current study— i.e. that events associated with the acute ictus of SAH elicit changes in cerebrovascular physio-pharmacology that are independent of any subsequent effects of chronic clot lysis**—is therefore endorsed. Because such changes might prove crucial to the subsequent development of delayed VSM and DCI they may, in consequence, ultimately explain why currently favoured 'clot-VSM' fail to adequately explain these phenomena (i.e. because demonstrating post-ictal alterations undermines the assumption of 'clot-VSM' models that delayed VSM and DCI merely result from the chronic effects of clot lysis upon *normal* cerebral vessels). Two key factors associated with the acute ictus—seemingly independent of each other—have been identified in this regard: firstly, factors related to the determination of acute mortality; and secondly, factors related to the effects of the acute intracranial and systemic arterial pressor response. It is the current author's belief that the acute ictus may not only be central to *early* mortality (as demonstrated in the current thesis) but also to *delayed* mortality—via DCI—as implied by Nagai. To quote Nagai "...it is with the initial brain damage..." after acute SAH that "...clues to the mechanism..." of DCI may "...ultimately be sought..."[435]. **In addition, the findings of persistent NE heterogeneity endorse 'Brandt's principle'** that it "...is with the individual nature of cerebral vessels why VSM ultimately occurs in some cases, and not in others...". And finally, the secondary hypothesis of the current

study—i.e. that EF-SAH models inherently introduce 'side bias'—is formerly rejected as a result of the results so repeatedly obtained, thus endorsing their use for the analysis of delayed VSM.

PART 7

APPENDIX

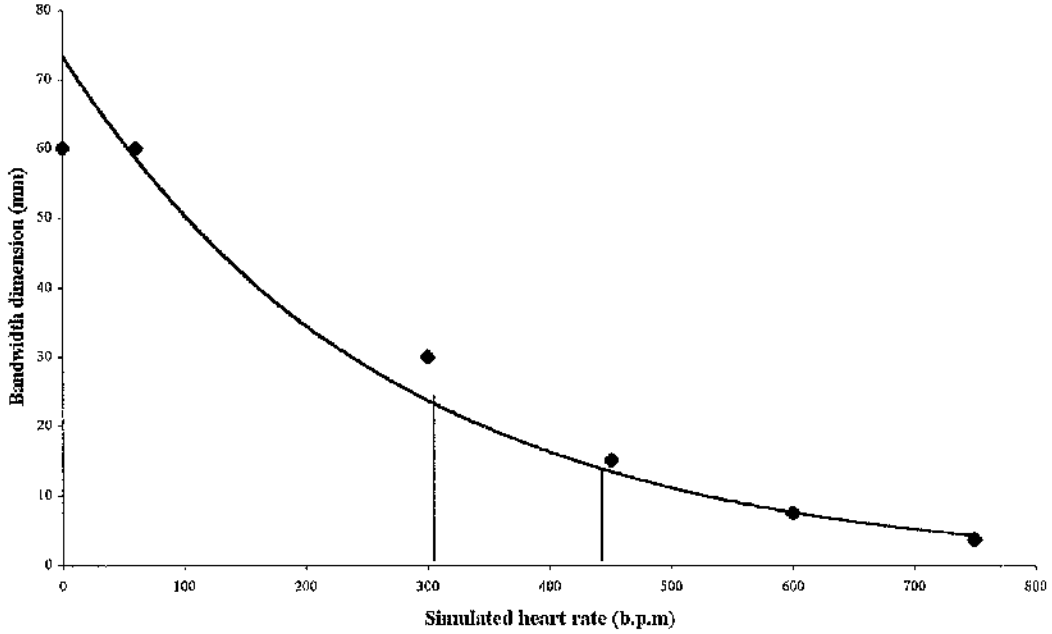


Fig 47. Exponential decay of systolic-diastolic bandwidth with increasing heart rate (HR). The box under the curve denotes the approximate range for the normal rat HR[343, 344]. Thus, in the normal rat HR range, bandwidth dimensions have contracted to approximately 35-60% the statically calibrated scale, as a result of pen inertia artefact. **NOTE**: The graph is presumed to be exponential because 1) a linear relationship would suggest that the bandwidth would become zero at 720 b.p.m., and 2) because of electro-mechanical considerations.

Table 9. FD_{90} (μm) **Non-operative controls**

Right MCA	Left MCA
185	**137**
140	**97**
132	**191**
157	**81**
112	71
164	**103**
191	**147**
126	140
174	157
116	**213**
168	NA
112	**187**
146	128
145	108
206	237
163	**209**
217	214
189	**99**
191	206
158	167

Key: **Bold** type—animals where marked asymmetry (i.e. >x1 SD) was apparent.
NA—non-viable vessel.

Table 10. NE E_{max} responses in Non-operative controls

Animal no.	Right MCA	Left MCA
1.	1.8	2.0
2.	4.3	1.2
3.	3.3	3.8
4.	5.6	1.7
5.	**0.8**	1.2
6.	**0.3**	**0.7**
7.	3.6	4.9
8.	1.9	2.6
9.	1.7	5.8
10.	2.0	4.6
11.	**0**	—
12.	**0.7**	—
13.	1.7	1.8
14.	**0.7**	4.3
15.	4.2	3.3
16.	**0.7**	**0.8**
17.	4.4	4.9
18.	3.4	3.6

Key: **Bold** type NE-'subresponder', (—) non viable MCA (*see* Part 4, 6.5 and 7.4).

Table 11. **NE E_{max} responses in Sham operative controls**

Animal no.	Right MCA	Left MCA
1.	4.6	3.1
2.	4.8	3.8
3.	2.5	2.8
4.	4	**1**
5.	**0.6**	2.1
6.	3.5	5.9
7.	4.7	1.2
8.	2.6	1.1

Key: **Bold** type NE-'subresponder', (—) non viable MCA (*see* Part 4, 6.5 and 7.4).

Table 12. NE E_{max} responses in SAH Group 1

Animal no.	Right MCA	Left MCA
1.	4.0	**1.0**
2.	2.6	4.9
3.	1.9	1.5
4.	**0.4**	**0.5**

Table 13. NE E_{max} responses in SAH Group 2

Animal no.	Right MCA	Left MCA
1.	2.5	3.8
2.	5.2	2.8
3.	**0.8**	**0.5**
4.	2.0	**1.0**
5.	**0**	**0**
6.	2.3	1.8
7.	6.5	**1.0**
8.	2.2	4.6
9.	**0**	1.1
10.	2.2	2.8

Table 14. NE E_{max} responses in SAH Group 3

Animal no.	Right MCA	Left MCA
1.	1.9	2.4
2.	**0**	**0**
3.	1.5	**0.6**
4.	**0**	3.8
5.	4.0	4.4
6.	3.3	6.2
7.	3.4	1.3
8.	**0.5**	1.8
9.	**0.4**	**0.7**
10.	1.1	**0**
11.	**1.0**	**0.5**
12.	**0.5**	2.3
13.	3.3	8.7
14.	—	1.5
15.	**0.3**	**0.6**

Key: **Bold** type NE-'subresponder', (—) non viable MCA (*see* Part 4, 6.5 and 7.4).

Table 15. Arterial blood gases in shams and SAH groups

	Shams (n = 8)	SAH groups (n = 34)
pH	7.4±0.3	7.38±0.24
pO_2 (kPa)	12.2±0.8	12.9±0.7
pCO_2	4.2±0.3	4.3±0.2
BE (mmol/L)	+1±3	+3±2

Key: BE base excess. Values expressed as mean±SE

Table 16. Sham operative pressor responses (mmHg)

No.	MAP	ICP
1.	113	8
2.	142	3
3.	109	3
4.	83	11
5.	125	9
6.	111	4
7.	102	8
8.	111	8
Mean±SE	112±6	7±1

Table 17. SAH Type I acute pressor responses: Hypotension after SAH. All pressures recorded in mmHg. *Key* (—) lost tracing, * SCC (43%). MAP_1/ ICP_1 = pressures *prior* to SAH, MAP_2/ ICP_2 = pressures immediately *after* SAH. [NB ICP_2 = ICP_{max}]

No.	MAP_1	MAP_2	$-\Delta MAP$	ICP_1	ICP_2	ΔICP
1.*	110	72	38	8	33	25
2.*	126	98	28	16	37	21
3.*	79	71	8	4	39	35
4.	84	79	5	13	40	27
5.*	173	125	48	4	24	20
6.*	84	68	16	6	30	24
7.	129	85	44	10	32	22
8.	159	138	21	8	42	34
9.*	139	113	26	2	49	47
10.	183	175	8	10	50	40
11.*	135	65	70	—	—	—
12.	115	81	34	5	25	20
13.*	170	143	27	6	49	43
14.	133	104	29	—	—	—
15.	114	96	18	—	—	—
16.	152	81	71	4	45	41
17.	131	94	37	8	47	39
18.*	104	84	20	—	—	—
19.	119	—	—	—	—	—
20.	154	114	40	12	32	20
21.	88	68	20	10	41	31
Mean±SE	128±6	98±7	30±4	8±1	38±2	31±2

NOTE: In animal no. 19, no quantification of pressor *change* was possible because the original pen recorder tracing was subsequently lost; and because the precise values from the patient monitor could not be stored onto hard disk. Nevertheless, this was faithfully recorded as a hypotension in the notes from the experiment. Note also that the ICP_{max}:MAP_2 ratio is not as simple as dividing the mean values at the column bases, because in five animals there do not exist corresponding ICP and MAP values.

Table 18. SAH Type II acute pressor responses: Invariant. All pressures recorded in mmHg. *Key* (—) lost tracing (see section 3.3), * SCC (40%). ICP_1 = pressure *prior* to SAH, ICP_2 = pressure immediately *after* SAH. [NB $ICP_2 = ICP_{max}$]

No.	MAP	ICP_1	ICP_2	ΔICP
1.*	91	10	86	76
2.	104	5	41	36
3.	84	10	77	67
4.	115	12	72	60
5.	115	15	57	42
6.	140	12	41	29
7.	125	—	—	—
8.*	156	14	54	40
9.*	97	—	—	—
10.*	103	6	54	48
Mean±SE	113±7	11±1	60±6	50±6

Table 19. SAH Type III acute pressor response: Cushing Hypertensive response (CHR). All pressures recorded in mmHg. *Key* (—) lost tracing (see section 3.3), * SCC (50%). MAP_1/ ICP_1 = pressures *prior* to SAH, MAP_2/ ICP_2 = pressures immediately *after* SAH. [NB $ICP_2 = ICP_{max}$]

No.	MAP_1	MAP_2	+ΔMAP	ICP_1	ICP_2	ΔICP
1.*	131	142	11	11	87	76
2.	125	148	23	9	78	69
Mean±SE	128±3	145±3	17±6	10±1	83±5	73±4

Table 20. The distribution of Subacute Cardiovascular Collapse (SCC) with acute pressor response after SAH. Animals developing subacute cardiovascular collapse (SCC: **bold** type) did so in the 1st and 2nd hours of SAH: animals surviving into the 3rd hour did not develop SCC (SCC is equivalent to mortality in this thesis). $\chi^2 = 1.06$ on (3-1)(3-1) degrees of freedom, giving **p»0.2**. No statistical significance is demonstrated.

	SCC occurrence after SAH			
	1st Hour	2nd Hour	3rd Hour	*Total*
Type I	**4**	**5**	12	21
Type II	**2**	**2**	6	10
Type III	**0**	**1**	1	2
Total	**6**	**8**	19	33

NOTE: the total number of animals considered is 33 and not 34: this is because in one animal the *type* of acute pressor response (i.e. hypertension, hypotension or invariant) was not recorded on a pen recorder tracing.

Table 21. Contingency table demonstrating CCA re-clamping effect upon survival within three hours of SAH. $\chi^2 = 6.76$ on (2-1)(3-2) 2 degrees of freedom, giving **p < 0.05**. NB *expected* figures in parenthesis. Prolonged CCA re-clamping is significantly associated with premature mortality in this study. Clearly, Groups 2 and 3 (39% and 29% mortality respectively) were markedly different from Group 1 (100% mortality).

	Group 1	**Group 2**	**Group 3**	*Totals*
Non-survival	4 (1.7)	5 (5.4)	5 (7.0)	14
Survival	0 (2.4)	8 (7.7)	12 (10.0)	19
Totals	4	13	17	34

Key: "Survival" – survival to 3rd hour after SAH, "Non-survival" – SCC within the 3rd hour after SAH. (SCC is equivalent to mortality in this thesis).

Table 22. Contingency table demonstrating the distribution of acute pressor response with CCA re-clamping. $\chi^2 = 4.99$ on (2-1)(3-2) 2 degrees of freedom, giving **p >> 0.05**. No statistical significance is demonstrated.

	Group 1	Group 2	Group 3	*Totals*
Type I	3	7	8	18
Type II	0	3	6	9
Type III	1	1	0	2
Totals	4	11	14	29

NOTE: the total group number is 29 and not 34. Myography was only performed in n = 30 animals (Protocols: Part 4, 7.4). However, in one animal myography was performed without knowledge of the *qualitative* acute pressor response (lost tracing). Nevertheless, BP (patient monitor) and intermittent arterial blood gas values confirmed that the animal was adequately perfused. This Table was only used to provide some explanation for PGF2α and NE EC_{50} values discussed in Part 6, Section II, 3.2 & 3.5.

Table 23. Sham operative E_{max} and EC_{50} values in right and left MCAs. No significant side difference strongly suggests that unilateral CCA-ICA manipulation and thread insertion does not preferentially affect ipsilateral MCA responses.

	RIGHT MCA		n	LEFT MCA		n	p values	
FD_{90}	167±35		8	148±46		8	0.36	
Agonist	**E_{max}**	**EC_{50}**		**E_{max}**	**EC_{50}**		**E_{max}**	**EC_{50}**
KCl	6.3±0.7	—	8	5.4±0.7	—	8	0.39	—
PGF2α	6.3±0.8	5.0±1.0	8	5.4±0.9	7.5±1.8	8	0.46	0.25
PPV	106±11	3.2±0.2	8	118±17	2.3±0.7	8	0.55	0.35
UTP	4.6±0.6	20±1.7	8	4.8±0.9	20.2±2.4	8	0.90	0.95
SNP	70±2	8.1±3.1	8	81±6	9.5±5.9	8	0.10	0.83
L-ARG	74±4	3.3±0.9	8	69±8	6.1±2.6	8	0.60	0.33
5HT	5.4±0.9	0.09±0.02	8	5.4±1.1	0.08±0.04	8	0.98	0.80
NE	3.4±0.5	3.5±1.5	7	2.6±0.6	2.8±1.2	7	0.33	0.69
HA	84±7	6.2±2.4	8	79±6	7.4±2.3	8	0.55	0.72
L-NAME	1.8±0.8	—	3	2.3±0.8	—	3	0.65	—

Key: n = no. MCAs studied, (—) not applicable, Diam± SD measured in μm, E_{max} ±SE values for KCl, PGF2α, UTP, 5HT, NE and **L-NAME** measured in mN, E_{max} ±SE values for PPV, HA, SNP, L-ARG measured in % pre-constricted agonist-induce tone (AT), all EC_{50} ±SE values measured in μM. **NOTE**: NE values refer only to '**responders**'.

Table 24. SAH Group 1 E_{max} and EC_{50} values in right and left MCAs. No significant side differences strongly suggests that prolonged CCA re-clamping does not preferentially affect ipsilateral MCA responses.

	RIGHT MCA		n	LEFT MCA		n	p values	
FD_{90}	152±18		4	157±41		4	0.82	
Agonist	**E_{max}**	**EC_{50}**		**E_{max}**	**EC_{50}**		**E_{max}**	**EC_{50}**
KCl	5.0±1.0	—	4	5.2±0.7	—	4	0.90	—
PGF2α	5.2±0.7	7.3±1.6	4	6.7±1.3	7.6±1.9	4	0.32	0.90
PPV	86±18	3.4±2.3	4	92±4	2.2±0.8	4	0.80	0.63
UTP	2.8±0.6	23.8±1.8	4	4.3±1.1	23.7±3.3	4	0.25	0.97
SNP	55±23	6.1±4.7	3	88±19	9.7±3.9	3	0.32	0.59
L-ARG	95±12	9 .8±4.0	4	91±6	7.0±5.0	4	0.78	0.68
5HT	3.2±0.8	0.45±0.18	4	5.1±1.2	0.99±0.6	4	0.24	0.42
NE	2.8±0.6	—	3	3.2±1.7	—	2	0.82	—
HA	52±3	3.0±1.6	4	64±12	1.9±0.7	4	0.51	0.55

Key: n = no. MCAs studied, (—) not applicable, Diam ± SD measured in μm, E_{max} ±SE values for KCl, PGF2α, UTP, 5HT and NE measured in mN, E_{max} ±SE values for PPV, HA, SNP, L-ARG measured in % pre-constricted agonist-induce tone (AT), all EC_{50} ±SE values measured in μM. **NOTE**: NE values refer only to '**responders**'.

Table 25. SAH Group 2 E_{max} and EC_{50} values in right and left MCAs. No significant side differences strongly suggests that temporary CCA re-clamping for up to 5mins does not singularly affect ipsilateral MCA responses.

	RIGHT MCA		n	LEFT MCA		n	p values	
FD_{90}	163+42		11	156±25		11	0.64	
Agonist	**E_{max}**	**EC_{50}**		**E_{max}**	**EC_{50}**		**E_{max}**	**EC_{50}**
KCl	5.4±0.7	—	10	5.8±0.6	—	10	0.66	—
PGF2α	7.6±0.8	3.5±0.5	10	6.9±0.6	3.8±1.1	10	0.52	0.81
PPV	101±4	2.5±0.4	10	109±7	2.1±0.3	10	0.32	0.43
UTP	4.1±0.5	20.5±2.7	10	3.4±0.6	24.1±3.4	10	0.36	0.43
SNP	93±5	5.1±2.7	10	92±7	3.5±1.3	10	0.92	0.60
L-ARG	91±8	4.0±0.9	10	81+7	2.3±0.7	10	0.39	0.16
5HT	6.3±1.0	0.16±0.05	9	5.5±0.6	0.17±0.03	10	0.47	0.78
NE	3.6±0.7	2.1±0.8	7	2.4±0.4	2.7±1.3	6	0.17	0.69
HA	80±9	5.3±1.3	10	77+5	5.6±0.9	10	0.77	0.84

Key: n = no. MCAs studied, (—) not applicable, Diam ± SD measured in μm, E_{max} ±SE values for KCl, PGF2α, UTP, 5HT and NE measured in mN, E_{max} ±SE values for PPV, HA, SNP, L-ARG measured in % pre-constricted agonist-induce tone (AT), all EC_{50} ±SE values measured in μM. NB There is n = 1 more diameter value than corresponding agonist value for each MCA because in one animal **no** agonist responses were obtained. **NOTE**: NE values refer only to '**responders**'.

Table 26. SAH Group 3 E_{max} and EC_{50} values in right and left MCAs after SAH. No significant side differences strongly suggests that no underlying asymmetry is present in MCA reactivity after SAH. [This group effectively acts as a 'control' for the previous two groups].

	RIGHT MCA		n	LEFT MCA		n	p values	
FD_{90}	163±49		15	163±44		15	0.99	
Agonist	E_{max}	EC_{50}		E_{max}	EC_{50}		E_{max}	EC_{50}
KCl	4.4±0.5	—	14	4.5±0.7	—	15	0.86	—
PGF2α	5.6±0.7	7.9±1.4	13	6.7±0.8	6.3±1.6	14	0.38	0.46
PPV	102±5	2.6±0.5	12	111±4	1.8±0.4	12	0.07	0.21
UTP	3.5±0.5	22.1±2.5	13	3.8±0.7	17.9±2.7	14	0.84	0.26
SNP	105±8	3.6±1.0	13	112±5	1.9±0.5	13	0.42	0.12
L-ARG	92±9	1.5±0.4	12	100±17	2.2±0.7	13	0.68	0.39
5HT	4.5±0.8	0.14±0.04	13	5.0±0.8	0.1±0.02	14	0.67	0.41
NE	2.3±0.4	8.8±4.2	8	3.8±0.8	9.4±3.0	8	0.15	0.90
HA	67±9	5.5±1.3	13	67±7	4.5±0.9	13	0.96	0.56
L-NAME	2.0±0.5	—	7	2.9±0.7	—	6	0.31	—

Key: n = no. MCAs studied, (—) not applicable, Diam ± SD measured in μm, E_{max} ±SE values for KCl, PGF2α, UTP, 5HT , NE and **L-NAME** measured in mN, E_{max} ±SE values for PPV, HA, SNP, L-ARG measured in % pre-constricted agonist-induce tone (AT), all EC_{50} ±SE values measured in μM. **NOTE**: NE values refer only to '**responders**'.

Table 27. Comparison of SAH Group 1, 2 and 3 responses. Statistically significant differences are apparent mainly in EC_{50} values. The 'abnormal' group is mainly SAH III: however, SAH V NE EC_{50} values clearly differ from SAH IV (SAH III values were not amenable to analysis because of their small number).

	SAH 1 (n = 4)		**SAH 2** (n = 11)		**SAH 3** (n = 15)		**p values**	
FD_{90}	154±29		159±34		163±45		0.85	
Agonist	**E_{max}**	**EC_{50}**	**E_{max}**	**EC_{50}**	**E_{max}**	**EC_{50}**	**E_{max}**	**EC_{50}**
KCl	5.1±0.6	—	5.6±0.5	—	4.5±0.4	—	0.52	—
PGF2α	5.9±0.7	7.4±1.5	7.2±0.5	**3.7±0.6**	6.2±0.5	7.1±1.1	0.3	**0.02**
PPV	89±10	2.8±1.1	105±4	2.3±0.3	108±4	2.1±0.3	0.08	0.67
UTP	3.5±0.6	24±2	3.7±0.4	23±2	3.7±0.4	20±2	0.98	0.53
SNP	**72±15**	7.9±2.9	92±4	4.2±1.4	108±3	2.6±0.5	**0.003**	0.08
L-ARG	93±6	**8.4±3.0**	86±5	3.1±0.6	96±10	1.8±0.4	0.66	**0.0006**
5HT	4.1±0.8	**0.72± 0.31**	5.8±0.5	0.17± 0.03	4.8±0.5	0.12± 0.02	0.21	**0.0004**
NE	3.0±0.6	—	3.1±0.4	2.4±0.7	3.2±0.5	**9.2±2.3**	0.97	**0.002**
HA	58±8	2.4±0.8	78±5	5.5±0.8	67±5	5.0±0.8	0.13	0.18

Key: **Bold** type statistical significance, (—) not applicable, n = max. no. animals studied, Diam ± SD measured in μm, E_{max} ±SE values for KCl, PGF2α, UTP, 5HT and NE measured in mN, E_{max} ±SE values for PPV, HA, SNP, L-ARG measured in % pre-constricted agonist-induce tone (AT), all EC_{50} ±SE values measured in μM.
NOTE: NE values refer only to '**responders**'.

Table 28. Comparison of overall (i.e. Groups 1-3) SAH responses with controls and shams.

	Controls (n = 20)		**Shams** (n = 8)		**SAH** (n = 30)		**p values**	
FD_{90}	156±42		157±41		161±39		0.86	
Agonist	**E_{max}**	**EC_{50}**	**E_{max}**	**EC_{50}**	**E_{max}**	**EC_{50}**	**E_{max}**	**EC_{50}**
KCl	5.5±0.3	—	5.9±0.5	—	5.2±0.3	—	0.42	—
PGF2α	5.1±0.4	6.4±1.0	5.9±0.6	6.2±1.1	6.5±0.3	5.8±0.6	0.051	0.88
PPV	106±9	2.1±0.6	107±16	2.7±0.5	104±3	2.3±0.3	0.95	0.61
UTP	3.4±0.4	16.4± 2.8	4.7±0.5	20.1± 1.4	3.7±0.3	21.3± 1.3	0.11	0.17
SNP	72±4	10.7± 4.3	76±3	8.8±3.2	**96±4**	**3.8±0.7**	**0.0003**	**0.04**
L-ARG	72±4	5.0±1.3	72±5	4.7±1.4	**92±5**	3.4±0.6	**0.01**	0.37
5HT	4.4±0.3	0.09± 0.02	5.4±0.7	0.08± 0.02	5.1±0.4	0.21± 0.06	0.39	0.10
NE	3.2±0.3	2.4±1.0	3.3±0.4	3.1±0.9	3.1±0.3	5.9±1.4	0.90	0.08
HA	**102±5**	**2.3±0.6**	82±5	6.8±1.6	70±4	4.9±0.5	**0.0004**	**0.004**
L-NAME	1.3±0.4	—	2.0±0.5	—	2.4±0.4	—	0.20	—

Key: **Bold** type statistical significance, (—) not applicable, n = max. no. animals studied, Diam ± SD measured in μm, E_{max} ±SE values for KCl, PGF2α, UTP, 5HT, NE and **L-NAME** measured in mN, E_{max} ±SE values for PPV, HA, SNP, L-ARG measured in % pre-constricted agonist-induce tone (AT), all EC_{50} ±SE values measured in μM. **NOTE**: NE values refer only to '**responders**'.

Table 29. Survival groups E_{max} and EC_{50} values after SAH.

	1 hour (n = 5)		**2 hours** (n = 8)		**3 hours** (n = 17)		**p values**	
FD_{90}	136±31		160±35		168±41		0.08	
Agonist	**E_{max}**	**EC_{50}**	**E_{max}**	**EC_{50}**	**E_{max}**	**EC_{50}**	**E_{max}**	**EC_{50}**
KCl	5.1±0.8	—	5.0±0.4	—	5.3±0.4	—	0.88	—
PGF2α	**4.6±0.8**	7.0±1.0	6.6±0.7	6.0±0.9	7.2±0.4	5.7±1.0	**0.03**	0.78
PPV	106±8	2.9±0.4	99±6	2.6±0.7	106±3	2.1±0.3	0.52	0.40
UTP	2.6±0.5	21±4	3.8±0.4	24±2	4.0±0.4	20±2	0.18	0.35
SNP	95±9	5.3±3.0	88±9	4.7±1.5	102±4	3.3±0.6	0.30	0.50
L-ARG	76±13	2.3±0.9	91±5	**6.0±1.8**	100±8	2.1±0.3	0.23	**0.01**
5HT	4.4±1.0	0.14± 0.04	4.5±0.6	0.38± 0.17	5.5±0.5	0.14± 0.02	0.35	0.06
NE	2.4±0.5	5.2±3.4	3.1±0.6	4.3±1.4	**4.8±0.6**	7.1±3.5	**0.04**	0.10
HA	87±6	4.1±0.5	77±5	3.9±0.9	67±4	5.6±0.8	0.54	0.31

Key: **Bold** type statistical significance, (—) not applicable, n = max. no. animals studied, Diam ± SD measured in μm, E_{max} ±SE values for KCl, PGF2α, UTP, 5HT and NE measured in mN, E_{max} ±SE values for PPV, HA, SNP, L-ARG measured in % pre-constricted agonist-induce tone (AT), all EC_{50} ±SE values measured in μM.
NOTE: NE values refer only to '**responders**'.

Table 30. E_{max} **and** EC_{50} **values with Type I, II and III pressor responses after SAH.**

	Type I (n = 18)		**Type II** (n = 9)		**Type III** (n = 2)		**p values**	
FD_{90}	161±33		161±52		148±26		0.82	
Agonist	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}	E_{max}	EC_{50}
KCl	5.3±0.4	—	3.7±0.5	—	5±0.7	—	0.05	—
PGF2α	6.8±0.4	6.3±0.9	6.1±0.9	4.9±0.8	4.9±0.7	6.3±2.1	0.29	0.64
PPV	104±3	2.1±0.2	107±7	2.7±0.3	87±14	3.8±2.3	0.23	0.14
UTP	3.6±0.3	20±2	3.7±0.5	22±2	4.2±1.2	24±2	0.83	0.72
SNP	93±5	**7.1±1.3**	99±5	1.7±0.4	90±3	0.6±0.1	0.77	**0.03**
L-ARG	85±4	3.9±0.9	102±6	2.1±0.8	94±13	1.7±0.4	0.06	0.40
5HT	5.1±0.5	0.17± 0.03	4.6±0.7	0.17± 0.05	4.3±1.0	**0.92± 0.64**	0.74	**0.002**
NE	3.3±0.3	4.3±1.3	3.6±1.7	**15.3±5**	2.6±0.7	0.8±0.1	0.67	**0.004**
HA	66±4	5.0±0.7	77±8	5.2±0.8	71±16	1.9±1.1	0.43	0.48

Key: **Bold** type statistical significance, (—) not applicable, n = max. no. animals studied, Diam ± SD measured in μm, E_{max} ±SE values for KCl, PGF2α, UTP, 5HT and NE measured in mN, E_{max} ±SE values for PPV, HA, SNP, L-ARG measured in % pre-constricted agonist-induce tone (AT), all EC_{50} ±SE values measured in μM.
NOTE: the total no. of animals used is one less than in previous tables (i.e. 29 instead of 30). This is because in one animal the acute pressor response was not recorded. Intermittent arterial blood gas sampling and continuous BP readouts from the patient monitor, however, confirmed its suitability for myography. ICP monitoring and post mortem analysis confirmed SAH. **NB**: NE values refer only to '**responders**'.

Table 31. Comparison of E_{max} v FD_{90} correlation coefficients *r* and their statistical significance. Only the *r* value for non-operative controls UTP E_{max} v FD_{90} correlation does not reach statistical significance. NB NE refers only to NE-'responders'

	Non-op Controls		Shams		[overall] SAH	
Agonist	*r*	p value	*r*	p value	*r*	p value
KCl	0.75	<0.01*	0.78	<0.001*	0.52	<0.001
PGF2α	0.45	<0.05*	0.87	<0.001*	0.632	<0.001
5HT	0.523	<0.02*	0.64	<0.01*	0.56	<0.01
NE	0.62	<0.01*	0.72	<0.001*	0.47	<0.001
UTP	0.31	>0.1	0.74	<0.01*	0.58	<0.001

Key * statistical significance

PART 8

REFERENCES

1 Alabadi JA, Salom JB, Torregrossa G, Miranda FJ, Jover T, Alborch E: Changes in the cerebrovascular effects endothelin-1 and nicardipine after experimental subarachnoid hemorrhage. **Neurosurgery 33(4):**707-15, 1993.
2 Alabadi JA, Torregrosa G, Salom JB, Miranda FJ, Barbera MD, Mayordomo F Alborch E.: Changes in the adrenergic mechanisms of cerebral arteries after subarachnoid haemorrhage in goats. **Neurosurgery 34(6)**:1027-33, 1994.
3 Alafaci C, Jansen I, Arbab MA, Shiokawa Y, Svendgaard NA, Edvinsson I: Enhanced vasoconstrictor effect of endothelin in cerebral arteries from rats with subarachnoid haemorrhage. **Acta Physiol Scand 138**:317-319, 1990.
4 Alam M, Smirk FH. Observations in man upon blood pressure raising reflex from voluntary muscles. **J Physiol (Lond.) 89**:372-83, 1937.
5 Alexander III E, Black PM, Liszczak TM et al. Delayed CSF lavage does not prevent arteriographic and morphologic vasospasm after experimental subarachnoid haemorrhage. **J Neurosurg 63**:949-58, 1985.
6 Alexander III E, McL Black P: CBF and arteriographic VSM. Effects of delayed lavage in an experimental canine model. In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York, 1988, pp 395-401.
7 Alksne JF, Greenhoot JH. Experimental catecholamine-induced chronic chronic vasospasm. **J Neurosurg 41**: 440-445, 1974.
8 Alksne JF, Smith RW. Experimental models of spasm. **Clin Neurosurg 24**:216-227, 1977.
9 Allen GS, Gross CJ: Cerebral arterial spasm. Part 5: In vitro contractile activity of vasoactive agents icnluding human CSF on human basilar and anterior cerebral arteries. **J Neurosurg 44**:594-600, 1976.
10 Allen GS: Role of calcium anatagonists in cerebral arterial spasm. **Am J Cardiol 55**:149B-153B, 1985.
11 Allen GS,Henderson LM, Chou SN, French LA. Cerebral arterial spasm , Part 1; In vivo contractile activity of vasoactive agents on canine basilar and middle cerebral arteries. **J Neurosurg 40**: 433-441, 1974
12 Andreoli A, Pasquale GD, Pinelli G, Grazi P, Tognetti F, Testa C. Subarachnoid haemorrhage: frequency and severity of cardiac arrhythmias. **Stroke 18**:558-564, 1987.
13 Antonaccio MJ (ed). Cardiovascular pharmacology. New York, NY Raven press, 1984.
14 Aoki T, Takenaka K, Suzuki S, Kassell NF, Sagher O, Lee KS: The role of hemolysate in the facilitation of oxyhemoglobin-induced contraction in rabbit basilar arteries. **J Neurosurg 81:**261-6, 1994.
15 Arnal JF, Warin L, Michel JB. Determinants of aortic cGMP in hypertension induced by chronic inhibition of nitric oxide synthase. **J Clin Invest. 90**:647-52, 1992.
16 Arnold WP, Longnecker DE, Epstein RM et al. Nitroprusside produces cyanide poisoning via reaction with haemoglobin. **J Pharmacol Exp Ther 191**:557-563, 1974.
17 Arnold WP, Longnecker DE, Epstein RM. Photodegeneration of Sodium nitroprusside biological activity and cyanide release. **Anesthesiology 61**:254-60, 1984.

18 Asano T, Sano K: Pathogenetic role of no-reflow phenomena in experimental subarachnoid haemorrhage in dogs. **J Neurosurg 46**:454-466, 1977.
19 Asano T, Tanishima T, Sasaki T et al. Possible participation of free radical reactions initiated by clot lysis in the pathogenesis of vasospasm after subarachnoid haemorrhage, in *Cerebral Vasospasm*, RH (ed), Raven press, New York, 1988, pp190-201.
20 Asano T, Takakura K, Sano K, Kikachi H, Nagai H, Saito I, Tamura A, Ochiai C, Sasaki T. Effects of a hydroxyl scavenger on delayed ischaemic neurological deficits following aneurysmal subarachnoid haemorrhage: results of a multi-centre placebo-controlled double-blind trial. **J Neurosurg 84**:792-803, 1996.
21 Aschenbrenner R, Bodechtal G. Ueber EKG-veraenderungen bei Hirntumorkvanken. **Klin Wochenschrift 17**: 298-302, 1938.
22 Auer LM, Gallhofer B. Rhythmic activity of cat pial vessels in vivo. **Eur Neurol 20**:448-68, 1981.
23 Badimon L, Badimon JJ, Penny W, Webster MW, Chesebro JH, Fuster V. Endothelium and atherosclerosis. **J Hypertension 10(suppl2)**:S43-50,1992.
24 Baker KM, Booz GW, Dostal DE. Cardiac actions of angiotensin II: role of an intracardiac renin-angiotensin system. **Ann Rev Physiol 54**: 227-41, 1992.
25 Balligand J-L, Kelly RA, Marsden PA, Smith TW, Michel T. Control of cardiac muscle cell function by an endogenous nitric oxide signalling system. **Procl Natl Acad Sci USA 90**:347-351, 1993.
26 Balligand J-L, Ungureanu-Longrois G, Simmons WW, Pimental D, Malinski TA, Kapturczak M, Taha Z, Lowenstein CJ, Davidoff AJ, Kelly RA, Smith TW, Michel T. Cytokine - inducible *i*NOS expression in cardiac myocytes: characterization and regulation of *i*NOS expression and detection of *i*NOS activity single cardiac myocytes *in vitro*. **J Biol Chem 269**: 27580-27588, 1994.
27 Balligand JL, Kelly RA, Marsden PA Smith TW, Michel T. Control of cardiac muscle cell function by an endogenous nitric oxide signalling system. **Proc Natl Acad Sci USA 90**: 347-51, 1993.
28 Balligand J-L, Kobzik L, Han X, Kaye, DM, Belhassen L, O'Hara DS, Kelly RA, Smith TW, Michel T. Nitric oxide dependent parasympathetic signalling is due to activation of constitutive (III) NOS in cardiac myocytes. **J Biol Chem 270**: 14582-6, 1995.
29 Banerjee A, Lock-Winter C, Rogers KB, Mitchell MB, Brew EC, Cairns CB, Bensard DD, Harken AH. Preconditioning against myocardial dysfunction after ischaemia and reperfusion by an α1 adrenergic mechanism. **Circ Res 73**:656-70, 1993.
30 Barry KJ, Goghan MA, Bennett M, Stein MD. Small animal model for investigation of subarachnoid haemorrhage and cerebral vasospasm. **Stroke 10(5)**:538-541, 1979.
31 Bates JN, Baker MT, Guerra R Jr, Harrison DG: Nitric oxide generation from Nitroprusside by vascular tissue: evidence that reduction of the nitroprusside anion and cyanide loss are required. **Biochem Pharmacol 42**:5157-5165, 1991.
32 Baumbach GL, Heistad DD. Heterogeneity of brain blood flow and permeability during acute hypertension. **Am J Physiol 249**: H629-37, 1985.
33 Baumbach GL, Siems JE, Faraci FM, Heistad DD. Mechanics and composition of arterioles in brainstem and cerebrum. **Am J Physiol 256**:H493-501, 1989.

34 Baydown AR, Bogle RG, Pearson JD, Mann GE. Discrimination between citrulline and arginine transport in activated murine macrophages: inefficient synthesis of nitric oxide from recycling of citrulline to arginine. **Br J Pharm 112**:487-492, 1994.
35 Bayliss WM: On the local reactions of the arterial wall to changes of internal pressure. **J Physiol (Lond) 28**: 230-241, 1902.
36 Beard EF, Robertson JW, Robertson RCL. Spontaneous subarachnoid haemorrhage simulating acute myocardial infarction. **Am Heart J 58**:755-9, 1959.
37 Beasely D, Schwartz JH, Brenner BM. Interleukin 1 induces prolonged L-arginine-dependent cGMP and nitrite production in rat vascular smooth muscle cells. **J CLin Invest 87:**602-608, 1991.
38 Beckman DL, Iams SG. Circulating catecholamines in cats before and after lethal head injury. **Proc Soc Exp Biol Med 160**:200-202, 1979.
39 Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. **Proc Natl Acad USA 87**:1620-1624, 1990.
40 Bederson JB, Germano IM, Gaurino L: Cortical blood flow and cerebral perfusion pressure in a new noncraniotomy model of subarachnoid hemorrhage in the rat. **Stroke 26:**1086-92, 1995.
41 Bederson JB, Levy AL, Ding WH, Kahn R, DiPerna C, Jenkins AL, Vallabhajosyula P: Acute vasoconstriction after subarachnoid hemorrhage. **Neurosurgery 42(2):**352-62, 1998.
42 Beitner R, Haberman S, Cycowitz T. The effect of cGMP on phosphofructokinase from rat tissues. **Biochem Biophys Acta 482**:330-340, 1977.
43 Bevan JA, Su C. Effectiveness of adrenergic transmitter concentrations in synaptic clefts of differing dimensions. In *Frontiers in catecholamine research*. by E. Uschin and S.Snyder (Ed), Pergamon press New York, 1973, pp 575-77.
44 Bevan JA, Osher JV. A direct method for recording tension changes in the wall of small blood vessels *in vitro*. **Agents Actions 2**:257-260, 1972.
45 Bevan JA, Duckles SP, Lee TJF. Histamine potentiation of nerve and drug-induced response of rabbit cerebral artery. **Cir Res 36**:647-653, 1975.
46 Bevan JA, Bevan RD, Frazee JG: Functional arterial changes in chronic cerebrovasospasm in monkeys: An in vitro assessment of the contribution to arterial narrowing. **Stroke 18**:472-481, 1987.
47 Bevan RD, Bevan JA: Diltiazem protects against functional changes in chronic cerebrovasospasm in monkeys. **Stroke 19**:73-79, 1988.
48 Bevan JA, Bevan RD, Frazee JG. Increased arterial wall stiffness and abnormal tone contribute to arterial narrowing in chronic cerebral vascular spasm in the monkey: protection by diltiazem pretreatment. In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York, 1988, pp 463-8.
49 Bevan RD, Dodge JT, Wellman T, Walters CL, Bevan JA: Is there a neurogenic influence on the diameter of human small pial arteries? In *The human brain circulation: functional changes in disease*, Bevan RD, Bevan JA (Eds), Humana press, Totowa, New Jersey, 1994, pp 93-107.
50 Bevan RD, Dodge J, Nichols P, Penar PL, Walters CL, Wellman T, Bevan JA: Weakness of sympathetic neural control of human pial compared with superficial

temporal arteries reflects low innervation density and poor sympthetic responsiveness. **Stroke 29**:212-221, 1998.

51 Bill A, Linder J: Sympathetic control of cerebral blood flow in acute arterial hypertension. **Acta Physiol Scand 96**:114-121, 1976.

52 Black P McL. Hydrocephalus and vasospasm after subarachnoid haemorrhage from ruptured intracranial aneurysms. **Neurosurgery 18**:12-16, 1986.

53 Blaine EM, Tallman RD, Frolicher D, Jordan MA, Bluth LL, Howie MB. Vasopressin supplementation in a porcine model of brain dead potential organ donors. **Transplantation 38**: 459-464, 1984.

54 Bobik A, Campbell JH, Carson V, Campbell GR. Mechanism of isoprenaline-induced refractoriness of the β-adrenoceptor-adenyl cyclase system in chick embryo cardiac cells. **J Cardiovasc Pharmacol 3**:541-553, 1981.

55 Bogle RG, Vallance P. Econazole inhibition of the induction of nitric oxide synthase in lipopolysaccharide treated rat aorta. **Br J Pharmacol 114**:130P, 1995

56 Bukowski RD, Wang SN, Bian K, DeWitt DS: Traumatic brain injury does not alter cerebral artery contractility. Am J Physiol 272(Heart Circ. Physiol 41):H1406-H1411, 1997.

57 Boullin DJ, Mohan J, Grahame-Smith DG. Evidence for the presence of a vaso-active substance (possibly involved in the aetiology of cerebral arterial spasm) in cerebrospinal fluid from patients with subarachnoid haemorrhage. **J Neurol Neurosrg Psych 39**:756-66, 1976.

58 Boullin OJ, Brandt T, Ljungren B. Vasoconstrictor activity in cerebrospinal fluid from patients subjected to early surgery for ruptured intracranial aneurysms. **J Neurosurg 55**:237-245, 1981.

59 Brackett CE. Systemic complications of central nervous system trauma. In: CNS trauma research status report, Odom GL ed., 1979, Washington DC: Public health service 1979: pp 232-263.

60 Brady AJB, Warren JB, Poole-Wilson PA, Williams TJ, Harding SE. Nitric oxide attenuates cardiac myocyte contraction. **Am J Physiol 265:**H176-182, 1993.

61 Brady AJB, Wilson PA, Harding SE, Warren JB. Nitric oxide production within cardiac myocytes reduces their contractility in endotoxaemia. **Am J Physiol 263**:H1963-1966, 1992.

62 Brady AJB, Poole-Wilson PA, Harding SE, Warren JB. Nitric oxide production within cardiac myocytes reduces their contractility in endotoxaemia. **AM J Physiol 263**:H1963-1966, 1992.

63 Brandt L, Ljunggren B, Andersson K-E, Hindfelt B: Individual variations in response of human cerebral arterioles to vasoactive substances, human plasma, and CSF from patients with aneurysmal SAH. **J Neurosurg 55**:431-437, 1981.

64 Brandt L, Ljunggren B, Anderson KE et al: Effects of indomethacin and prostacyclin on isolated human pial arteries contracted by cerebrospinal fluid from patients with aneurysmal subarachnoid haemorrhage. **J Neurosurg 55**:877-83, 1981.

65 Bray JJ, Cragg PA, MacKnight ADC, Mills RG, Taylor DW. The cardiovascular system, in *Lecture notes on human physiology*, 1986, pp324-417.

66 Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R, Zera P, Menlove R, Shah P, Jamieson S, Stinson EB. β1 and β2 adrenergic subpopulations in nonfailing and failing human ventricular myocardium: coupling

of both receptor subtypes to muscle contraction and selective β1 receptor down regulation in heart failure. **Cir Res 59:**297-309, 1986.

67 Bristow MR, Anderson FL, Port JD, Skeri L, Hershberger RE, Larrabee P, O'Connell JB, Renlund DG, Volkmana K, Murray J Feldmana AM. Differences in β adrenergic neuroeffector mechanisms in ischaemic versus idiopathic dilated cardiomyopahty. **Circulation 84**:1024-1039,1991.

68 Branston NM, Symon L, Crockard HA et al. Relationship between the cortical evoked potential and local cortical blood flow following acute middle cerebral artery occlusion in the baboon. **Exp Neurol 45**: 195-208, 1974.

69 Browder J, Meyers R. Behaviour of the systemic blood pressure, pulse rate, and spinal fluid pressure associated with acute changes in intracranial pressure artificially produced. **Arch Surg (Chic) 36**:1-19, 1936.

70 Brown JM, White CW, Terada LS, Grosso MA, Shanley PF, Mulvin DW, Banerjee A, Whitman GJR, Harken AH, Repine JE. Interleukin 1 pre-treatmnent decreases ischaemia-reperfusion injury. **Procl Natl Acad Sci USA 87**: 5026-5030, 1990.

71 Bülbring E, Tomita T: Catecholamine action on smooth muscle. **Pharm Rev 29(1)**:49-96, 1987.

72 Bunemann L, Jensen KA, Riisager S, Thomsen LJ. Cerebral blood flow and metabolism during hypotension induced with sodium nitroprusside and metoprolol. **Eur J Anaesthesiology 8**:197-201, 1991

73 Burgen ASV, Mitchell JF. In *Gaddum's Pharmacology*, 8th ed., Oxford university press, New York, Toronto, 1981.

74 Burnstock G, McCullock MW, Story DF, Wright ME. Factors affecting the extraneuronal activation of noradrenaline in cardiac and vascular smooth muscle cells. **Br J Pharm 46**:243-53, 1972

75 Busse R, Mulsch A, Fleming I, Hecker M. Mechanisms of nitric oxide release from the vascular endothelium. **Circulation 87(suppl V):**18-25, 1993.

76 Buus NH, VanBavel E, Mulvany MJ. Differences in sensitivity of rat mesenteric small arteries to agonists when studied as ring preparations or as cannulated preparations. **Br J Pharmacol 112**:579-87, 1994.

77 Bylund DB, Eikenberg DC, Hieble JP, Langer SZ, Lefkowitz RJ, Minneman KP, Molinoff PB, Ruffolo RR Jr, Trendelenberg U. IV. International union of Pharmacology nomenclature of adrenoceptors. **Pharm rev 46(2)**:121-136, 1994.

78 Campbell JH, Campbell GR. Potential role of heparanase in atherosclerosis. **New Phys Sci 4**: 9-12, 1989.

79 Cannon WB, Rosenbleuth A. *The supersensitivity of denervated structures*, MacMillan, New York, 1949.

80 Carpenter DA, Grubb RL Jr, Tempel LW et al. Cerebral oxygen metabolism after aneurysmal subarachnoid haemorrhage. **J Cerebr Blood Flow & Metab 11**:837-842, 1991.

81 Carruthers ME, Patel S. Reduction of stress/catecholamine induced cardiac necrosis by β1 selective blockade. **Lancet 2**:585-9, 1987.

82 Casscelles W, Willesron JT. Endogenous and exogenous nitric oxide protect against intracoronary thrombosis and reocclusion after thrombolysis. **Circualtion 92**:1005-1010, 1995.

83 Casteels R, Kitamura K, Kuriyama H, Suzuki H: The membrane properties of the smooth muscle cells of the rabbit main pulmonary artery. **J Physiol 271**:41-61, 1977.
84 Castellot JJ Jr, Cochrane DL, Karnovsky MJ. Effect of heparin on vascular smooth muscle cells. I Cell metabolism. **J Cell Physiol 124**:21-28, 1985.
85 Centeno JM, Orti M, Salom JB, Sick TJ, Perez-Pinzen MA: Nitric oxide is involved in anoxic preconditioning neuroprotection in rat hippocampal slices. **Brain Res 836(1-2)**:62-9, 1999.
86 Charcot JM: Sur un cas de migraine othalmologique (paralysie oculomotrice periodique). **Progr Med 18**:83, 1890.
87 Chehrazi BB, Youmans JR. Cerebral blood flow in clinical neurosurgery. In *Neurological Surgery* 3rd Ed, Youmans JR (ed)WB Saunders Co, Harcourt Brace Jovanovich Inc, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo, 1990, Vol 2, Ch 24, pp 696-740
88 Chen HI, Sun SC, Chai CY. Pulmonary oedema and haemorrhage resulting from cerebral compression. **Am J Physiol 224**:223-29, 1973.
89 Cheng GC, Libby MD, Grodzinsky AJ, Lee RT. Induction of DNA synthesis by a single transient mechanical stimulus of human vascular smooth muscle cells: role of fibroblast growth factor - 2. **Circulation 93**:99-105 1996.
90 Chertok VM: [Mast cells of the outer coat of the arteries of the basis cerebri] **Arkh Anat Gistol Embriol 79**:72-79, 1980 (Rus)
91 Chi OZ, Wei HM, Weiss HR. Effects of CAS 754, a new nitric oxide donor, on regional cerebral blood flow in focal cerebral ischaemia. **Anaesthesia and analgesia 80(4)**: 703-708, 1995.
92 Cirino G, Tigley AW, Wallace JG, Baydown AR. Inhibition of *i*NOS expression in J774 cells by the novel NSAID Flurbiprofen-nitroxybutylester (FNBE). **Br J Pharmacol 114**:131P, 1995
93 Chung MK, Gulick TS, Rotondo RE, Schreiner GF, Lange LG. Mechanism of cytokine inhibition of β adrenergic agonist stimulation of cyclic AMP in rat cardiac myocytes: impairment of signal transduction. **Cir Res 67**:753-753, 1990.
94 Chyatte D, Sundt TM. Cerebral vasospasm: evidence supporting an inflammatory aetiology. In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York, 1988, pp 357-365.
95 Clarke JA: An X-ray microscopic study of the vasa vasorum of the intracranial arteries. **Ztsch Anat Entwickl 124**:396-400, 1965.
96 Clarkson CW, Eick RE: On the mechanism of lysophosphatidylcholine-induced depolarization of cat ventricular myocardium. **Circ Res 52**:543-556, 1983.
97 Clavier N, Kirsch JR, Hurn PD, traystman RJ. Effect of post-ischaemic hypoperfusion on vasodilatory mechanisms in cats. **Am J Physio 267(5pt2)**:H 2012-2028, 1994.
98 Clouse RE. In *Gastrointestinal disease: pathophysiology, diagnosis, and management*, 4th Ed., Sleisenger MH, Fordtran JS (Eds), WB Saunders Co, Harcourt Brace Jovanovich, Inc., Philadelphia, London, Toronto, Montreal, Sydney, Tokyo, 1989, pp 567-569.
99 Clouston JE, Numaguchi Y, Zoarski GH, Aldrich EF, Simard JM, Zitnay KM. Intra-arterial papaverine infusion for cerebral vasospasm after subarachnoid haemorrhage. **Am J Neuroradiology 16(1)**:27-38, 1995

100 Clower BR, Smith RR, Haining JL, Lockard J: Constrictive endarteropathy following experimental subarachnoid haemorrhage. **Stroke 12(4)**:501-508, 1981.
101 Clower BR, Sullivan DM, Smith RR: Intracranial vessels lack vasa vasorum. **J Neurosurg 61**:44-48, 1984.
102 Clyde BL, Firlik AD, Kaufmann AM, Spearman MP, Yonas H. Paradoxical aggravation of vasospasm with papaverine infusion following aneurysmal subarachnoid haemorrhage. **J Neurosurg 84**:690-695, 1996
103 Cockerham LG, Prell GD, Cerveny TJ, O'Brien M, Hampton JD. Effects of aminoguanidine on pre- and post- irradiation rCBF, sytemic BP and plasma histaminelevels in the primate. **Agents and actions 32 (3-4)**:237-244, 1991.
104 Cohn JN, Levine BT, Olivari MT, Garberg V, Lura D, Francis GS, Simon AB, Rector T. Plasma norepinephrine as a guide to prognosis in patients with congestive cardiac failure. **N Eng J Med 311**:819-823, 1984.
105 Coleman RA, Smith WL, Narumiya S: VIII. International union of pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. **Pharm Rev 46(2)**:205-229, 1994.
106 Colohan ART, Zuccarello M, Kassell NF, Quaqiarello V, Scheld WM, Nazar G, Sasaki T. Permeability changes in the major cerebral arteries in experimental meningitis. In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York, 1988, pp 367-72.
107 Conway LW, McDonald LW. Structural changes of the intradural arteries following subarachnoid haemorrhage. **J Neurosurg 37**:715-723, 1972.
108 Cook DA. The pharmacology of cerebral vasospasm. **Pharmacol 29**:1-16, 1984.
109 Cooper Sir AP. *Lectures on the principles and practice of Surgery*. Vol 1 300 Underwood London 1824.
110 Coyle P. Collateral pial arteries. In: *The human brain circulation: functional changes in disease*, Bevan RD, Bevan JA (Eds), Humana press, Totowa, New Jersey, 1994, pp 237-246.
111 Crompton MR. Cerebral infarction following the rupture of cerebral berry aneurysms. **Brain 87**:491-510, 1964.
112 Crowe SE, Perdue MH. Gastrointestinal food hypersensitivity: basic mechanisms of pathophysiology. **Gastroenterology 103**:1075-95, 1992.
113 Cruikshank JM, Neil-Dwyer G, Degaute JP, Hayes Y, Kuurne T, Kytta J, Vincent JL, Carruthers ME, Patel S. Reduction of stress/catecholamine induced cardiac necrosis by β1 selective blockade. **Lancet 2**:585-589, 1987.
114 Cushing H. Concerning a definite regulatory mechanism of the vasomotor centre which controls blood pressure during cerebral compression. **Bull John Hopkins Hosp 12**:290-2, 1901.
115 Cushing H. Some experimental and clinical observations concerning states of increased intracranial pressure. **Am J Med Sci 124**:375-400, 1902.
116 Cushing H. The blood-pressure reaction of acute cerebral compression, illustrated by cases of intracranial haemorrhage. **Am J Med Sci 125**:1017-1045, 1903.
117 Cushing H. *Studies in intracranial physiology and Surgery*. London Oxford university press 1925
118 Cutler RWP, Page L, Galicich J et al. Formation and absorption of cerebrospinal fluid in man. **Brain 91**:707-720, 1968.

119 D-Alecy L, Feigl EO. Sympathetic control of cerebral blood flow in dogs. **Cir Res 31**:267-283, 1972.
120 Davis MJ, Gore RW. Capillary pressures in rat intestinal muscle and mucosal villi during venous pressure elevation. **Am J Physiol 249**: H174-187, 1985
121 Davson H, Hollingsworth G, Segal MB. The mechanism of drainage of the cerebrospinal fluid. **Brain 93**:665-678, 1970.
122 Debbage PL, Dwyer D, Meyermann R. In *Cerebral vascular spasm* Voth D, Glees P, deGruyter, 1985, pp 41-48.
123 Debdi M, Seylaz J, Sercombe R. Early changes in rabbit cerebral artery reactivity after subarachnoid haemorrhage. **Stroke 23(8)**:1154-1162, 1992.
124 Debdi M, Seylaz J, Sercombe R. Influence of calcium and nicardipine on rabbit basilar artery reactivity after brief subarachnoid haemorrhage. **J Cardiovasc Pharm 21(5)**:754-759, 1993.
125 De Belder AJ, Radomski M, Why HJF, Richardson PJ, Bucknall CA, Salas E, Martin JF, Moncada S. Nitric oxide synthase activities in human myocardium. **Lancet 341**: 84-85, 1993.
126 Delgado TJ, Brismar J, Svengaad NA. Subarachnoid haemorrhage in the rat: angiography and fluorescence microscopy of the major cerebral arteries. **Stroke 16(4)**:595-601, 1985.
127 De Mey JG, Vanhoutte PM. Heterogeneous behaviour of the canine arterial and venous wall. Importance of the endothelium. **Circ Res 51**:439-447, 1982.
128 De Wilt DJ. Etomidate-anaesthesia, with and without fentanyl, compared with urethane-anaesthesia in the rat. **BJPharm 79**:461-9, 1983.
129 Dietrich HH, Dacey RG jr. Effects of extravascular acidification and extravascular alkalinization on constriction and depolarization in rat cerebral arterioles. **J Neurosurg 81**:437-442, 1994.
130 Dinning TAR, Falconer MA. Sudden or unexpected natural death due to ruptured intracranial aneurysm. Survey of 250 forensic cases. **Lancet 2**:799-810, 1953.
131 Docherty JR: Functional changes in the aging cerebrovasculature, In: *The human brain circulation: functional changes in disease*, Bevan RD, Bevan JA (Eds), Humana press, Totowa, New Jersey, 1994, pp 109-116.
132 Dóczi T, Joó F, Sonkodi S, Adám G. Increased vulnerability of the blood brain barrier to experimental subarachnoid haemorrhage in spontaneously hypertensive rats. **Stroke 17(3)**:498-501, 1986.
133 Dóczi T, Joó F, Vecsernyés M, Bodosi M. Increased concentration of atrial natriuretic factor in the cerebrospinal fluid of patients with aneurysmal subarachnoid haemorrhage and raised intracranial pressure. **Neurosurgery 23(1)**:16-19, 1988
134 Dorsch NWC, King MT. A Review of cerebral vasospasm in aneurysmal subarachnoid haemorrhage. **J Clin Neurosci 1**:19-26, 1994.
135 Downey JM. Ischaemic preconditioning: nature's own cardioprotective intervention. **Trends Cardiovasc Med 2**:170-176, 1992.
136 Draper I. *Lecture notes on Neurology*, Sixth ed. Blackwell scientific productions Ch 13, 1984, p 123.
137 Drapier JC, Hibbs JB Jr. Murine cytotoxic macrophage inhibit aconitase in tumour cells. **J Clin Invest 78**:790-797, 1986.

138 Drenth JPH, Nishimura J, Nouailhetas VLA, van Breemen C. Receptor-mediated C-kinase activation contributes to alpha-adrenergic tone in rat mesenteric resistance artery. **J Hypertension 7(S4)**:S41-S45, 1989.
139 Du Boulay GH, Verity PM. In *The cranial arteries of mammals*, Heineman, London 1973.
140 Duckles SP, Bevan JA. Pharmacological characteristics of adrenergic receptors of a rabbit cerebral artery *in vitro*. **J. Pharmacol Exp Ther 197 (2)**:371-378 1976.
141 Duckles SP, Kim J, Bevan RD, Bevan JA. Alteration of innervation and reactivity of monkey cerebral arteries after subarachnoid haemorrhage, **Stroke 8**:10, 1977.
142 Duckles SP, Kim J, Bevan RD: In vitro studies of monkey cerebral arteries after subarachnoid hemorrhage. **Acta Neurol Scand Suppl 64**:314-5, 1977.
White RP: Vasodilator proteins: role in delayed cerebral vasopsasm. **Stroke 17(2)**:207-213, 1986.
143 Duff TA, Feilbach JA, Yusuf Q, McGucken R, Scott G. In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York, 1988, pp 303-9.
144 Duff TA, Feilbach JA, Yusuf Q, McGucken R, Scott G. Bilirubin and the induction of intracranial spasm. **J Neurosurg:69**:593-98, 1988.
145 Eccles R, Eccles KSJ: Asymmetry in the autonomic nervous system with reference to the nasal cycle, migraine, anisocoria, and Menière's syndrome. **Rhinology 19:**121-5, 1981.
146 Ecker A, Riemenschneider PA. Arteriographic demonstration of spasm of the intracranial arteries with special reference to saccular arterial aneurysms. **J Neurosurg 8**:660-667, 1951.
147 Eckly AE, Lugnier C. Role of phosphodiesterase III and IV in the modulation of vascular cAMP content by the nitric oxide/cGMP pathway. **Br J Pharm 113**:445-450, 1994.
148 Echlin F. Experimental vasospasm, acute and chronic, due to blood in the subarachnoid space. **J Neurosurg 35**:646-656, 1971.
149 Edvinsson L, MacKenzie ET. Amine mechanisms in the cerebral circulation. **Pharmacol Rev 28**: 275-348, 1976.
150 Edvinsson L, Owman C, Sjöberg N-O. Autonomic nerves, mast cells and amine receptors in human brain vessels. A histochemical and pharmacological study. **Brain Res 115**:377-393, 1976.
151 Edvinsson L, Owman CH. A Pharmacologic comparison of histamine receptors in isolated extracranial and intracranial arteries *in vitro*. **Neurology (Minneap) 25**:271-276, 1975.
152 Edvinsson L, Jansen JR: Perivascular neuropeptides (NPY, VIP, CGRP, and SP) in human brain vessels after subarachnoid hemorrhage. **Acta Neurol Scand 90**:324-330, 1994.
153 Eisenberg HM, Gray HE Jr, Aldrich EF et al. Initial CT findings in 753 patients with severe head injury: a report from NIH traumatic coma database. **J Neurosurg 73**:688-698, 1990.
154 Edvinsson L, Cervos-Navarro, Larsson LI et al. Regional distribution of mast cells containing histamine, dopamine, or 5HT in the mammalian brain. **Neurology 27**:878-883, 1977.

155 Edwards DH, Byrne JV, Griffifth TM. The effect of chronic subarachnoid haemorrhage on basal endothelial derived relaxing activity in intrathecal cerebral arteries. **J.Neurosurg 76(5)**: 830-837, 1992.
156 Egashira K, Inou T, Hirooka Y, Yamada A, Urabe Y, Takeshita A. Evidence of impaired endothelial derived coronary vasodilation in patients with angina pectoris and normal coronary angiograms. **N Eng J Med 328**:1659-1664, 1993.
157 Egeman N, Turker RK, Sanlidilek U, Zorlutuna A, Bilgic S, Baskaya M, Unlu A, Caglar S, Spetzler RF, McCormick JM. The effect of intrathecal sodium nitroprusside on severe chronic vasospasm. **Neurological Research 15(5)**:310-315 1993.
158 Eichbaum FW, Bisetti PC. Effect of head injury and acute intracranial hypertension the cardiovascular system. In *Third international congress of Neurological surgery*, Copenhagen 1965, Excerpta med (Amst), International congress series, 1965, No 93, pp 163-4.
159 El-Ackad TM, Brody MJ. Fluorescent histochemical localization of non-mast cell histamine, in *Neuropsychopharmacolgy*, Amsterdam: Excerpta Medica, 1974, Series 359, pp551-559.
160 Enevoldsen EM, Cold G, Jensen FT et al. Dynamic changes in rCBF, IVP, CSF pH and lactate levels during acute phase head injury. **Neurosurgery 44**:191-215, 1976.
161 Emery AEH: In *Elements of medical Genetics*, Churchill Livingstone, Edinburgh, London, Melbourne, New York 1983, p156.
162 Emmelin N, Trendelenberg U. Degeneration activity after parasympathetic or sympathetic denervation. **Erberg Physiol 6**:190-211, 1972.
163 Espinosa F, Weir B, Noseworthy T. Non-operative treatment of subarachnoid haemorrhage. In *Neurological Surgery*, 3rd Ed, Vol 3, Youmans JR, WB Saunders Co, 1990 page 1668.
164 Essik CR. Formation of macrophages by the cells lining the subarachnoid cavity in response to the stimulus of particulate matter. Contribution 272, Carnegie Inst Washington, 1920, pp377-388.
165 Evans HG, Lewis JM, Shah AM. IL-1β modulates myocardial contraction via dexamethasone sensitive production of nitric oxide. **CardiovascRes 27**:1486-90, 1993.
166 Faleiro LCM, Machado CRS, Gripp A Jr et al : Cerebral vasospasm: presence of mast cells in human cerebral arteries after aneurysm rupture. Preliminary note: **J Neurosurg 54:**733-735, 1981.
167 Falloon BJ, Stephens N, Tulip JR, Heagerty AM. Comparison of small artery sensitivity and morphology in pressurized and wire-mounted preparations. **Am J Physiol 268(Heart circ physiol 37)**: H670-678, 1995.
168 Faraci FM, Mayhan WG, Heistad DD. Segmental vascular responses to acute hypertension in cerebrum and brainstem. **Am J Physiol 253**:H738-42, 1987.
169 Feelisch M, Noack E. Nitric oxide (NO) formation from nitrovasodilators occurs independently of Hb or non-haeme Fe. **Eur J Pharmacol 142**:465-9, 1987.
170 Fein JM, Flor WJ, Cohan SL, Parkhurst J. Sequential changes of vascular ultrastructure in experimentla vasospasm. Myonecrosis of subarachnoid arteries. **J Neurosurg 41**:49-58, 1974.
171 Ferrendelli JA, Blank AC, Gross RA. Relationships between seizure activity and cyclic nucleotide levels in brain. **Brain Res 200**:93-103, 1980.

172 Feldman AM, Cates AE, Veazey WB, Herschberger RE, Bristow MR, Baughman KL, Baumgartner WA, Van Dop C. Increase of the 40,000 mol-wt pertussis toxin substrate (G protein) in the failing human heart. **J Clin Invest 82**:189-97, 1988.
173 Findlay JM, Weir BKA, Steinke D, Tanabe T, Gordon P, Grace M. Effect of intrathecal thrombolytic therapy on subarachnoid clot and chronic vasospasm in a primate model of subarachnoid haemorrhage. **J Neurosurg 69**:723-735, 1988.
174 Findlay JM, Weir BKA, Kanamura MD, Espinosa F. Arterial wall changes in cerebral vasospasm. **Neurosurgery 25(5)**:736-745, 1989.
175 Fingerle J, Johnson R, Clowes AW, Majesky MW, Reidy MA. Role or platelets in smooth muscle proliferation and migration after vascular injury in rat carotid artery. **Procl Natl Acad Sci USA 86**:8412-8416, 1989.
176 Finkel MS, Oddis CV, Jacob TD, Watkins SC, Hattler BG, Simmons RL. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. **Science 257**: 387-389, 1992.
177 Fisher MD, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid haemorrhage visualized by CT scanning. **Neurosurgery 6**:1-9, 1980.
178 Fishman JA, Ryan GB, Karnovsky MJ. Endothelial regeneration in the rat carotid artery and the significance of endothelial denudation in the pathogenesis of myointimal thickening. **Lab Invest 32**:339-351, 1977.
179 Fitch W, MacKenzie ET, Harper AM. Effects of decreasing arterial blood pressure on cerebral blood flow in the baboon. Influence of the sympathetic nervous system. **Circ Res 37**:550-557, 1975.
180 Fitch W, McDowall DG, Keaney MP, Pickerodt VWA. Systemic vascular responses to increased ICP. 2. The "Cushing" response in the presence of intracranial space occupying lesions; systemic and cerebral heamodynamic studies in the dog and the baboon. **J Neurol Neurosurg Psychiatry 40**:843-852, 1977.
181 Fleming W, Westphal DP: In *Catecholamines vol I, Handbook of experimental pharmacology*, Springer-Verlag, Berlin, Heidelberg, New York, 90,1989, pp 509-559.
182 Foldes FF, Arrowood JG. Changes in cerebrospinal fluid pressure under the influence of continuous subarachnoid infusion of normal saline. **J clin Invest 27**:346-351, 1948.
183 Foley PL, Takenaka K, Kassell NF, Lee KS. Cytotoxic effects of bloody cerebrospinal fluid on cerebral endothelial cells in culture. **J Neurosurg 81**:87-92, 1994.
184 Folkow B. Introduction and historical background. **J Hypertension 7(suppl4)**:S1-S4, 1989.
185 Folkow B, Neil E. In *Circulation*, London , Oxford University press, 1971.
186 Forbus WD. On the origin of miliary ancurysms of the superficial cerebral arteries. **Bull John Hopkins Hosp 47**:239-84, 1930.
187 Forstermann U, Muge A, Alheid U, Haverich A, Frolich JF. Selective attenuation of endothelial-mediated vasodilation in atherosclerotic human coronary arteries. **Circ Res 62**:185-190, 1988.
188 Fox JL, Ko JP. Cerebral vasospasm: a clinical observation. **Surg Neurol 10**:269-275, 1978.
189 Fox JL. In *Intracranial aneurysms*, New York, Springer-Verlag, 1983.

190 Fraser RA, Stein BM, Barret RE, Pool JL: Noradrenergic mediation of experimental cerebrovascular spasm. **Stroke 1(5)**:356-362, 1970.
191 Frazee JG. A primate model of chronic cerebral vasospasm. **Stroke 13**:612-614, 1982.
192 Frazee JG, Bevan JA, Bevan RD, Jones KR: Effect of diltiazem on experimental chronic cerebral vasospasm in the monkey. **J Neurosurg 62:**912-17, 1985.
193 Fredericks KT, Liu Y, Rusch NJ, Lombard JH. Role of endothelium and arterial potassium channels in mediating hypoxic dilation of middle cerebral arteries. **Am J Physiol 267(Heart Circulation physiology 36)**: H580-586, 1994.
194 Freedman R, Taylor D, Seiger A, Olson L, Hoffer B. Seizures and related epileptiform activity in hippocampus transplanted to the anterior chamber in the eye. II Modulation by cholinergic and adrenergic input. **Ann Neurol 6**:281-293, 1979.
195 Froman C, Smith AC. Metabolic acidosis of the cerebrospinal fluid associated with subarachnoid haemorrhage. **Lancet 1**:965-967, 1967.
196 Fujii S, Fujitsu K. Experimental vasospasm using cultured arterial smooth muscle cells. Part 1: contractile and ultrastructural changes caused by oxyhaemoglobin. **J Neurosurg 69**:92-97, 1988.
197 Furchgott RF. The role of endothelium in the response of vascular smooth muscle to drugs. **Annu Rev Pharmacol Toxicol 24**:175-197, 1984.
198 Furlong B, Henderson AH, Lewis MJ, Smith JA. Endothelial derived relaxing factor inhibits *in vitro* platelet aggregation. **Br J Pharmacol 90**:687-692, 1987.
199 Furuya M, Aisaka K, Miyazaki T, Honbou N, Kawashima K, Ohno T, Tanaka S, Minamino N, Kangawa K, Matsuo H. C-type natriuretic peptide inhibits intimal thickening after vascular injury. **Biochem Biophys Res Comm 193(1)**:248-253, 1993.
200 Fyhrquist F, Sirvio ML, Helin K, Saijonmaa O, Metsarinne K, Paakari I, Jarvinen A, Tikkanen I. Endothelin antiserum decreases volume-stimulated and basal plasma concentration of atrial natriuretic peptide. **Circulation 88**:1172-1176, 1993.
201 Gaboury JP, Niu XF, Kubes P. Nitric oxide inhibits numerous features of mast cell-induced inflammation. **Circulation 93**:318-326, 1996.
202 Galli SJ. New concepts about the mast cell. **N Eng J Med.328**:257-265, 1993.
203 Garg UC, Hassid A. Nitric oxide generating vasodilators and 8-bromocyclic GMP inhibits mitogenesis and proliferation of cultured rat vascular smooth muscle cells. **J Clin Invest 83**:1774-1777, 1989.
204 Geng Y-J, Hartwig JH, Hansson GK, Libby P. Cytokine-induced apoptosis of actin cytoskeletons and nucleolysis. **J Cardiovasc Trauma 33(1)**:abstr 111, p29.
205 Geng Y-J, Wu Q, Hansson GK. Protein Kinase C activation inhibits cytokine-induced nitric oxide synthase in vascular smooth muscle cells. **Biochem Biophys Acta 1223(1)**:125-132, 1994.
206 Geng Y-J, Wu Q, Muszynshi M, Hansson GK, Libby P. Apoptosis of vascular smooth muscle cells induced by *in vitro* stimulation with interferon γ, tumour necrosis factor α, and interleukin-1-β. **Arteriosclerosis, thrombosis, and vascular biology 16(1)**:19-27, 1996.
207 Gerzer R, Karrenbrock B, Siess W, Heim JM. Direct comparison of the effects of nitroprusside , SIN 1 and various nitrates on platelet aggregation and soluble guanylate cyclase activity. **Thromb Res 52**:11-21, 1988.

208 Gibbons GH, Dzau VJ. The emerging concept of vascular remodelling. **N Eng J Med 330**:1431-8, 1994.
209 Gibson QH, Roughton FJW. The kinetics and equilibria of the reactions of nitric oxide with sheep haemoglobin. **J Physiol 136**:507-526, 1957.
210 Gilby KL, Armstrong JN, Currie RW, Robertson HA: The effects of hypoxia-ischemia on expression of c-Fos, c-Jun and Hsp70 in the young rat hippocampus. **Brain res 48(1):**87-96, 1997.
211 Giller CA, Purdy P, Giller A, Batjer HH, Kopitnik T. Elevated transcranial doppler ultrasound velocities following therapeutic arterial dilation. **Stroke 26(1)**:123-127, 1995.
212 Goluboff B, Shenkin HA, Haft H. The effects of mannitol and urea on cerebral haemodynamics and cerebrospinal fluid pressure. **Neurology (minneapolis) 14**: 891-898, 1964.
213 Gonzalez NC, Oberman J and Maxwell JA. Circulatory effects of moderately and severely increased intracranial pressure in the dog. **J Neurosurg 6**:721-726, 1972.
214 Graf CJ, Rossi NP. Catecholamine response to intracranial hypertension. **J Neurosurg 49**: 862-868, 1968.
215 Grady MS, Cooper GW, Kassell NF, Login IS: Profound cerebral vasospasm without radiological evidence of subarachnoid haemorrhage; case report. **Neurosurgery 18**:653-659, 1986.
216 Granger DL, Lehninger AL. Sites of inhibition of mitochondrial electron tranpsort in macrophage-injured neoplastic cells. **J Cell Biol 95**:527-535, 1982.
217 Graser T, Leisner H, Tiedt N. Absence of a role of endothelium in the response of isolated porcine coronary arteries to acetylcholine. **Cardiovasc Res 20**:299-302, 1986.
218 Grennhoot JH, Reichenbach DD. Cardiac injury and subarachnoid haemorrhage: a clinical, pathological, and physiological correlation. **J Neurosurg 30**:521-531, 1969.
219 Griffifth TM, Edwards DH, Lewis MJ et al. The nature of the endothelial derived relaxing factor. **Nature 308**:645-647, 1984 (letter).
220 Gross PM. Cerebral histamine; indications for neuronal and vascular regulation. **J Cereb Blood Flow & Metab 2**:3-23, 1982.
221 Grote E, Hassler W. The critical first few minutes after subarachnoid haemorrhage. **Neurosurgery 22**:654-661, 1988.
222 Grubb RL Jr, Phelps ME, Raichle ME et al: The effects of arterial blood pressureon the regional cerebral blood volume by X-Ray fluorescence. **Stroke 4**: 390-399, 1973.
223 Grubb RL Jr, Raichle ME, Phelps ME et al. Effects of increased intracranial pressure on cerebral blood volume, cerebral blood flow and oxygen utilization in monkeys. **J Neurosurg 43**:385-398, 1975.
224 Grubb RL Jr, Raichle ME, Eichling JO Gado MH. Effects of subarachnoid haemorrhage on cerebral blood volume, cerebral blood flow and oxygen utilization in humans. **J Neurosurg 46**:446-453, 1977.
225 Gulick T, Pieper SJ, Murphy MA, Lange LG, Schreiner GF. A new method for assessment of cultured cardiac myocyte contractility detects immune facto-mediated inhibition of β-adrenergic responses. **Circulation 84**:313-321, 1991.

226 Gustafsson H, Mulvany MJ, Nilsson H. Rhythmic contractions of isolated small arteries from rats: influence of the endothelium. **Acta Physiologica Scand 148(2)**:153-163, 1993.
227 Hadfield JM, Yates DW, Kirkman E, Little RA. Regional cerebral blood flow and carbon dioxide reactivity after non-cerebral injury in the rat. **Circ Shock 39(2)**:100-106, 1993.
228 Hagar JM, Hale SL, Kloner RA. Effect of preconditioning ischaemia on reperfusion arrhythmias after coronary artery occclusion and reperfusion in the rat. **Circ Res 68(1)**:61-68, 1991.
229 Hainsworth R. Syncope and fainting, In: *Autonomic failure: a textbook of clinical disorders of the autonomic nervous system*, Bannister R, Mathias CJ (Eds) Oxford university press, Oxford, New York, Tokyo, 1992, 761-781.
230 Halpern W, Kelly M. In vitro methodology for resistance arteries. **Blood vessels28**:245-251, 1991.
231 Hamer J. The significance of cerebral vasospasm with regard to early and delayed aneurysmal surgery; preliminary results of early surgery. **Acta Neurochir (Wien) 63**:209-213, 1982.
232 Hammes EM. Reaction of the meninges to blood. **Arch Neurol Psychiatry 52**: 505-514, 1944.
234 Hamon M, Vallet B, Bauters C, Wernert N, McFadden EP, Lablanche JM, Dupuis B, Bertrand ME. Long term oral administration of L-arginine reduces intimal thickening and enhances neo-endothelial-dependent acetylcholine-induced relaxation after arterial injury. **Circulation 90**:1357-1362, 1994.
235 Handa Y, Kubota T, Kaneko M, Tsuchids A, KObayashi H, Kawano H, Kubota T. Expression of intercellular adhesion molecule 1 (ICAM-1) on the cerebral artery following subarachnoid haemorrhage in rats. **Acta Neurochirurgica 132(1-3)**:92-97, 1995.
236 Harada S, Kamiya K, Masago A, Iwata A, Yamada K: Subarachnoid hemorrhage induces c-fos, c-jun and hsp70 mRNA expression in rat brain. **Neuroreport 8(15):**3399-3404, 1997.
237 Hardebo JE, Owman C: Autonomic receptors in human brain arteries. In: *The human brain circulation: functional changes in disease*, Bevan RD, Bevan JA (Eds), Humana press, Totowa, New Jersey, 1994, pp 109-116.
238 Harder DR, Brann L, Halpern W. Altered membrane electrical properties of smooth muscle cells from small cerebral arteries of hypertensive rats. **Blood Vessels 20**:154-160, 1983.
239 Harder DR. Pressure-dependent depolarization in cat middle cerebral artery. **Circ Res 55**:197-202, 1984.
240 Harder DR, Gilbert R, Lombard JH. Vascular muscle cell depolarization and activation in renal arteries on elevation of transmural pressure. **Am J Physiol 253**:F778-781, 1987.
241 Harder DR. Pressure-induced myogenic activation of cat cerebral arteries is dependent on intact endothelium. **Circ Res 60**:102-107, 1987.
242 Hare JM, Keancy JF Jr, Kelly RA, Loscalzo J, Smith TW, Colucci WS. Inhibition of nitric oxide synthase potentiates the postive inotropic response to β adrenergic stimulation in normal dogs. **Circulation 88(suppl I)**:I 1905 Abstract, 1993.
243 Hare JM, Colucci WS. Role of nitric oxide in the regulation of myocardial function. **Prog Cardiovasc Dis 38**:155-166, 1995.

244 Hare JM, Loh E, Creager MA, Wilson S, Colucci S. Nitric oxide inhibits the positive inotropic response to β adrenergic stimulation in humans with left ventricular dysfunction. **Circulation 92**:2198-2203, 1995.
245 Hare JM, Keaney JF Jr, Balligand J-L, Loscalzo J, Smith TW, Colucci WS. Role of nitric oxide in parasympathetic modulation of β adrenergic myocardial contractility in normal dogs. **J Clin Invest 95**:360-366 1995.
246 Harper AM, Deskmukh VD, Rowan JO et al. The influence of the sympathetic on cerebral blood flow. **Arch Neurol 27**:1-6, 1972.
247 Harrison DG, and Bates JN 1993. The nitrovasodilators. New ideas about old drugs. **Ciculation 87**:1461-1467, 1993.
248 Hart MN. Morphometry of brain parenchymal vessels following subarachnoid haemorrhage. **Stroke 11**:653-655, 1980.
249 Hartmann B, Udenfriend S. The use of dopamine β hydroxylase as a marker for the central noradrenergic nervous system in rat brain. **Proc Natl acad Sci USA 69**:2722-2726, 1972.
250 Hartzell H, Fischmeister R. Opposite effects of cGMP and cAMP on calcium current in single heart cells. **Nature 323**:273-275, 1986.
251 Hasebe N, Shen YT, Vatner SF. Inhibition of endothelial derived relaxing factor enhances myocardial stunning in conscious dogs. **Circulation 64**:1101-1107, 1993.
252 Hashi K, Nin K, Shimotake K. Surgery in the prevasospastic interval. **Acta Neurochir (Wien)63**:141-145, 1982.
253 Hassler O. Morphological studies on the large cerebral arteries, with reference to the aetiology of subarachnoid haemorrhage. **Acta Psychiat Neurol Scand (suppl)154**:1-145, 1961.
254 Hatake K, Wakabayashi I, Kakishita E, Hishida S. Impairment of endothelium-dependent relaxation in human basilar artery after subarachnoid haemorrhage. **Stroke 23(8)**:1116-1117, 1992
255 Hawkins WF, Clower BR. Myocardial damage after trauma and simulated intracranial haemorrhage in mice; the role of the autonomic nervous system. **Cardiovasc Res 5**:524-529, 1971.
256 Hayakawa T, Waltz AG. Experimental subarachnoid haemorrhage from a middle cerebral artery. Neurologic defects, intracranial pressures, blood pressuresand pulse rates. **Stroke 8**:421-426, 1977.
257 Hayashi M, Marukawa S, Fujh H, Kitano T, Kobayashi H, Yamamoto S. Intracranial hypertension in patients with ruptured aneurysms. **J Neurosurg 46**: 584-590, 1977.
258 Hayreh SS, Edwards J. Vascular responses to acute intracranial hypertension. **J Neurol Neurosurg Psych 34**:587-601, 1971.
259 Hergstrand LR, Kanof PD, Greengard P. Histamine-sensitive adenyl cyclase in mamalian brain. **Nature 260**:163-165, 1976.
260 Heistad DD, Marcus ML. Effect of sympathetic stimulation on permeability of the blood brain barrier to albumin during acute hypertension in cats. **Circ Res 45**:331-338, 1979.
261 Heller R, Bussolino F, F, Ghigo D, Garbarino G, Pescarmona G, Till U, Bosia A. Nitrovasodilators inhibit thrombin-induced platelet activating factor synthesis in human endothelial cells. **Biochem Pharmacol 44**:223-229, 1992.

262 Hendrix LE, Dion JE, Jensen ME, Phillips CD, Newman SA. Papaverine-induced mydriasis. **Am J Neuroradiolog 15(4)**:716-718, 1994
263 Hermann L. Ueber die wirkungen des stickoxydulgases auf das blut. **Arch Anat Physiol**, Lpz: 469-481, 1865.
264 Hernandez MJ, Brennan RW, Bowman GS. Cerebral blood flow autoregulation in the rats. **Stroke 9**:150-155, 1978.
265 Heros RC, Zervas NT, Lavyne MH et al. Reversal of experimental cerebral vasospasm by intravenous nitroprusside therapy. **Surg Neurol 6**:227-229, 1976.
266 Herz DA, Baez S, Shulman K. Pial microcirculation in subarachnoid haemorrhage. **Stroke 6**:417-424, 1975.
267 Hillman J, Säveland H, Jakobsson, KE, Edner G, Zygmunt S, Fridriksson S, Brandt L. Overall management outcome of ruptured posterior fossa aneurysms. **J Neurosurg 85**:33-38, 1996.
268 Hirsh LF. Intra-arterial nitroprusside treatment of acute experimental vasospasm. **Stroke 11(6)**:601-605, 1980.
269 Hodgson JS. The relation between increased intracranial and increased interstitial pressure. Changes in the cerebrospinal fluid in increased intracranial pressure. **Res Publ Assoc Res Nerve Ment Dis 8**:182-188, 1927.
270 Hoenpud JD, Campbell SM, Mendelsohn DJ. Interleukin-1 induced myocardial depression in an isolated beating heart preparation. **J Heart Transplant 8**:460-464, 1989.
271 Hogie M, Clozel M, Loffler B-M, Thuillez C. *In Vivo* evidence of an endothelin-induced vasopressor tone after inhibition of nitric oxide synthesis in rats. **Ciculation 91**:771-775, 1995.
272 Hongo K, Nakagomi T, Tsukahara T, Ogawa H, Lehman RM, Sasaki T, Kasselll NF. Inhibition of spontaneously released endothelial derived relaxaing factor after subarachnoid haemorrhage in the rabbit basilar artery. In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York, 1988, pp155-168.
273 Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PPA. VII. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). **Pharm Rev 46(2)**:157-203, 1994.
274 Hubschmann OR, Kornhauser D. Effect of subarachnoid haemorrhage on the extracellular environment. **J Neurosurg 56**:216-221, 1982.
275 Hughes JT, Schianchi PM. Cerebral artery spasm. A histological study at necorpsy of the blood vessels in cases of subarachnoid haeomorrhage. **J Neurosurg 48**:515-525, 1978.
276 Humphrey JH, Toh CC. Absorption of serotonin and histamine by dog platelets. **J Physiol 124**:300-304, 1954.
277 Huszti Z, Deli MA, Joo F. Carrier-mediated uptake and release of histamine by cultrured rat cerebral endothelial cells. **Neuroscience letters 184(3)**:185-188, 1995.
278 Hutcheson IR, Griffifth TM. Release of endothelial derived relaxing factor is modulated both by frequency and amplitude of pulsatile flow. **AmJ Physiol 261**: H257-262, 1991.
279 Hutchings M, Weller RO. Anatomical relationships of the pia mater to cerebral vessels in man. **J Neurosurg 65**:316-325, 1986.
280 Huxley HE. The mechanism of muscular contraction. **Science 164**:1356, 1969.

281 Iadecola C, Pelligrino DA, Moskowitz MA, Lassen NA. Nitric oxide synthase inhibition and cerebrovascular regulation. **J Cereb Blood Flow & Metab 14(2)**:175-192, 1994.
282 Indolfi C, Esposito G, Di LorenzoE, Rapacciuolo A, Feliciello A, Porcellini A, Avvedimento VE, COndorelli M, Chiarello M. Smooth muscle cell proliferation is proportional to the degree of balloon injury in a rat model of angioplasty. **Circulation 92**:1230-1235, 1995
283 Isotani E, Suzuki R, Tomita K, Hokeri M, Monma S, Marumo F, Hirakawa R. Alterations in plasma concentrations of natriuretic peptides and ADH after subarachnoid hacmorrhage. **Stroke 25**: 2198-2203, 1994.
284 Isotani E, Azuma H, Suzuki R, Hamasaki H, Sato J, Hirakawa K: Impaired endothelium-dependent relaxation in rabbit pulmonary artery after subaracnoid hemorrahge. **J Cardiovasc Pharm 28:**639-644, 1996.
285 Ito U, Ohno K, Yamaguchi T, Takei H, Tomita H, Inaba Y. Effect of hypertension on blood brain barrier change after restoration of blood flow in post-ischaemia in gerbil brains. An EM study. **Stroke (11)6**:606-611, 1980.
286 Ito A, Egashira K, Kadokami T, Fukumoto Y, Takanyanagi T, Nakaike R, Kuga T, Sueishi K, Shimokawa H, Takeshita T: Chronic inihibtion of endothelial derived NO synthesis causes coronary microvascular structural changes and hyperreactivity to serotonin in pigs. **Circulation 92**:2636-2644, 1995
287 Iwai A, Sakano T, Uenishi M, Sugimoto H, Yoshioka T, Sugimoto T. The effects of vasopressin and catecholamines on the maintenance of circulatory stability in brain dead patients. **Transplantation 48**:613-617, 1989.
288 Jackson IJ. Aseptic haemogenic meningitis: an experimental study of aseptic meningeal reactions due to blood and its breakdown products. **Arch Neurol Psychiat 65**:572-589, 1949.
289 Jakubowski J, Bell BA, Symon L, Zawirski MB, Francis DM. A primate model of subarachnoid haemorrhage: change in regional cerebral blood flow, autoregulation carbon dioxide reactivity, and central conduction time. **Stroke 13(5)**:601-611, 1982.
290 Jancar S, Schultz R, Krueger CA, Cook DA. Role of cyclo-oxygenase and lipoxygenase products in arachidonic acid-induced contraction of cerebral artery: relevance to cerebral vasospasm. In *Cerebral Vasospasm* Wilkins RH (ed) Raven Press, New York, 1988, pp 259-264.
291 Jawad K, Miller JD, Wyper DJ, Rowan JO: Measurement of CBF and carotid artery pressure compared with cerebral angiography in assessing collateral blood supply after carotid ligation. **J Neurosurgery 46**:185-196, 1977.
292 Jensen PE, Mulvany MJ, Aaljaar C. Endogenous and exogenous agonist-induced changes in the coupling between intracellular calcium and force in rat resistance arteries. **Pflugers Arch-Europ J Physiol 420(5-6)**:536-543, 1992.
293 Jin Y, Sagher O, Thai QA, Kassell NF, Lee KS. The effects of papaverine on phorbol dibutyrate-induced vasocnstriction in brain slice micorvessls. **J Neurosurg 81**:574-578, 1994.
294 Johansson B. Myogenic tone and reactivity: definitions based on muscle physiology. **J Hypertension** 7:(suppl 4):S5-8, 1989.
295 Johnson G, Tsao PS, Lefer AM. Cardioprotective effects of authentic nitric oxide in other control systems. **J Hypertension 7(suppl 4)**: S33-39, 1989.

296 Johnson PC, Wayland H. Regulation of blood flow in single capillaries. **Am J Physiol 212**:1405-1415, 1967.
297 Joly GA, Narayanan K, Griffifth OW, Kilbourn RG. Characterization of the effecst of two new arginine/citrulline analogues on cNOS and iNOS in rat aorta. **Br J Pharm 115**:491-497, 1995.
298 Jones CJH, DeFily DV, Patterson JL, Chilian WM. **Circulation 87(4)**:1264-1274, 1993.
299 Jones CJH, Kuo L, Davis MJ, DeFily DV, Cilian WM. Role of nitric oxide in the coronary microvascular responses to adenosine and increased metabolic demand. **Circulation 91**:1807-1813, 1995.
300 Joris I, Majno G. Endothelial changes induced by arterial spasm. **AM J Pathol 102**:346-352, 1981.
301 Joris I, Zand T, Majno G: Hydrodynamic injury of the endothelium in acute aortic stenosis. **Am J Pathol 106**:394-408, 1982.
302 Kader A, Krauss WE, Onesti ST, Elliot JP, Solomon RA: Chronic cerebral blood flow changes following experimental subarachnoid hemorrhage in rats. **Stroke 21**:577-581, 1990.
303 Kahn AJ. Effects of variations in intracranial pressure. **Arch Neurol Psychiatry 51**:508-527, 1944.
304 Kajita Y, Suzuki Y, Oyama H, Tanazawa T, Takayasu M, Shibuya M, Sugita K. Combined effect of L-arginine and superoxide dismutase on the spastic basilar artery after subarachnoid haemorrhage in dogs. **J Neurosurg 80**:476-483, 1994.
305 Kaku Y, Yonekawa Y, Tsukahara T, Kazekawa K. Superselective intra-arterial infusion of papaverine for the treatment of vasospasm after subarachnoid haemorrhage. **J Neurosurg 77(6)**:842-847, 1992
306 Kamiya K, Kuyama H, Symon L. An experimental study of the acute stage of subarachnoid haemorrhage. **J Neurosurg 59**:917-924, 1983.
307 Kamiya K, Yamashita N, Sugiyama N, Ono M, Nagai H. Does vasospasm alone cause delayed ischaemic neurological deficits after subarachnoid haemorrhage? In *Cerebral vasospasm*, Findlay JM (ed), Amsterdam: Elsevier 1993, pp 63-66.
308 Kanamura K, Waga S, Kojima T, Fujimoto K, Niwa S. Endothelial dependent relaxation of canine basilar arteries. II inhibition by haemoglobin and cerebrospinal fluid from patients with aneurysmal subarachnoid haemorrhage. **Stroke 18**:938-943, 1987.
309 Kanamura K, Weir BKA, Findlay JM, Krueger CA, Cook DA. Pharmacological studies on relaxation of spastic primate cerebral arteries in subarachnoid haemorrhage. **J Neurosurg 71**:909-915, 1989
310 Kanamaru K, Weir BK, Simpson I, Witbeck T, Grace M. Effect of 21-amniosteroid U-74006f on lipid peroxidation in SA clot. **J Neurosurgery 74(3)**: 454-459, 1991.
311 Kanwar S, Kubes P. Mast cells contribute to ischaemia-reperfusion-induced granulocyte infiltraion and intestinal dysfuention. **Am J Physiol 267**:G316-321, 1994.
312 Kanwar S, Kubes P. Ischaemia-reperfusion-induced granulocyte influx is a multistep process mediated by mast cells. **Microcirculation 1**:175-182, 1994.
313 Kapp JP, Neill WR, Neill CL, Hodge LR, Smith RR. The 3 phases of spasm. **Surgical neurol 18**:40-45, 1982.

314 Karliner JS, Simpson PC, Honbo N, Woloszyn W. Mechanisms and time course of β adrenoceptor desensitiaztion in mamalian cardiac myocytes. **Cardiovasc Res 20**:221-228, 1986.
315 Kassel NF, Torner JC. Aneurysmal rebleeding. A preliminary report from the co-operative aneurysm study. **Neurosurgery 13**:479-481, 1983.
316 Kassell NF, Sasaki T, Colohan ART et al. Cerebral vasospasm following aneurysmal subarachnoid haemorrhage. **Stroke 16**:562-572, 1985.
317 Kassell NF, Helm G, Simmons N et al. Treatment of cerebral vasospasm with intra-arterial papaverine. **J Neurosurg 77**:848-852, 1992.
318 Kasuya H, Weir BKA, Nakane M, Pollock JS, Johns L, Marton S, Stefansson K. Nitric oxide synthase and guanylate cyclase levels in canine basilar arteries after subarchnoid haemorrhage. **J Neurosurg 82**:250-255, 1995.
319 Kataoka K, Niiyama K, Uejima T, Kuroda R, Ioka M, Yamada K, Taneda M, Hayakawa T. Treatment of severe localized cerebral vasospasm following recurrent haemorrhage from middle cerebral artery aneurysm. **Neurologica Medico Chirurgica 33(12)**:830-832, 1993
320 Katusic ZS, Shepherd JT, Vanhoutte PM. Endothelial dependent contractions to stretch in canine basilar arteries. **Am J Physiol 252**:H671-673, 1987.
321 Katusic ZS, Shepherd JT, Vanhoutte PM. Endothelial dependent contractions to calcium ionophore A23187, arachidonic acid and acetylacholine in canine basilar arteries. **Stroke 19**:476-479, 1988.
322 Kelly PAT, Ritchie IM. Local cerebral blood flow following repeated exposure to Ng-nitro-L-arginine-Methyl ester. **Br J Pharm 113**:78P, 1994.
323 Keyp JP, Neill WR, Neill CL, Hodge LR, Smith RR. The 3 phases of vasospasm. **Surg Neurol 18**:40-45, 1982.
324 Kikuchi T, Okuda Y, Kaito N, Abe T. Cytokine production in cerebrospinal fluid after subarachnoid haemorrhage. **Neurol Res 17(2)**:106-108, 1995.
325 Kim P, Sundt TM, Vanhoutt PM. Loss of endothelial dependent relaxations and maintenace of endothelial dependent contractions in chronic vasospasm following subarachnoid haemorrhage. In *Cerebral Vasospasm* Wilkins RH (ed) Raven Press, New York, 1988, pp145-154.
326 Kim P, Sundt TM Jr, Vanhoutte PM. Alterations of mechanical properties in canine basilar arteries after subarachnoid haemorrhage. **J Neurosurg 71**:430-436, 1989.
327 Knuckley NW, Fox RA, Surveyor I et al. Early cerebral blood flow and CT in predicting ischaemia after cerebral aneurysm rupture. **J Neurosurg 62**:850-855, 1985.
328 Kobrine AI, Doyle TF, Martins AN. Local spinal cord blood flow in experimental traumatic myelopathy. **J Neurosurg 42**:144-149, 1975.
329 Kohno K, Takeda S. Impairment of vascular reactivity and changes in intracellular calcium and calmodulin levels of smooth muscle cells in canine basilar arteries after subarachnoid haemorrhage. **Neurosurgery 25(5)**:753-761, 1989.
330 Kojima M, Handa H, Hashimoto N, Kim C, Hazama F. Early changes of experimentally induced cerebral aneurysms in rats: scanning electron microscopic study. **Stroke 17(5)**:835-841, 1985.
331 Kolin A, Norris JW. Myocardial damage from acute cerebral lesions. **Stroke 15**: 990-993, 1984.

332 Kontos HA, Wei EP, Navari RM et a. Respnses of cerebral arteries and arterioles to acute hypotension and hypertension. **Am J Physiol 234**:H371-H383, 1978.
333 Kooy NW, Royall JA, Ye YZ, Kelly DR, Beckman JS. Nitration of myocardial protein tyrosines: evidence for peroxynitrate production in myocardial inflammation. **Circulation 90(suppl I)**:I-627 Abstract.
334 Korbut R, Lidbury P, Vane JR. Prolongation of fibrinolytic activity of tissue plasminogen activator by nitrovasodilators. **Lancet 335**:669-675, 1990.
335 Kovách AGB, Szabó C, Faragó M, Lohinai Z, Fehér E. Effect of haemorrhagic hypotension on cerebrovascular reactiovity and ultrastructure in the cat. **Stroke 22**:1541-1547, 1991.
336 Krahn V. The pia mater at the site of entry of blood vessels into the central nervous system. **Anat Embryol 164**:257-263, 1982.
337 Krueger C, Weir B, Nosko M et al. Nimodipine and chronic vasospasm in monkeys. Part2 Pharmacological studies of vessels in spasm. **Neurosurgery 16**: 137-140, 1985.
338 Kronholm V, Lintrup J. Spectrophotometric investigations of the cerebrospinal fluid in the near ultraviolet region: a possible diagnostic aid in diseases of the central nervous system. **Acta Psychiatr Scand 35**:314-329, 1960
339 Ku D, Caufield JB. Human coroanry aortic bypass grafts, but not coronary arteries respond to acetylcholine with endothelial dependent relaxation [Abstract] **Circulation 76 (supplIV)**:382,1987.
340 Kuo JF, Shojh M. Handbook Exp Pharm Ch12 pp 393- 1985
341 Kurose I, Wolf R, Grisham MB, Granger DN. Modualtion of isch/reper induced microvascular dysfunction by nitric oxide. **Circ Res 74**:376-382, 1994.
342 Kuwayama A, Zervas NT, Shintani A. Papaverine hydrochloride and experimental haemorrhagic arterial spasm. **Stroke 3**:27-33, 1972.
343 Lacey PS, Earle AM. A small animal model for ECG abnormalities observed after an experimentla subarachnoid haemorrhage. **Stroke 14**:3; 1983.
344 Lacey PS, Earle AM. Central neural cotnrol of blood pressure and cardiac arrhythmias during subarachnoid haemorrhage in rats. **Stroke 16(6)**:998-1002, 1985.
345 Laher I, Bevan JA. Stretch of vascular smooth muscle activates tone and $45Ca^{2+}$ influx. **J Hypertension 7 (suppl 4)**:S17-20, 1989.
346 Lamontagne D, Pohl U, Busse R. Mechanical deformation of vessel wall and shear stress determine the basal endothelial dependent relaxing factor release in the intact coronary vascular bed. **Circ Res 70**:123-130, 1992.
347 Lang J. In *Clinical anatomy of the posterior fossa and its foramina*, Thieme Verlag, Stuttgart, New York, 1991 p 30.
348 Langer SZ, Shepperson NB. Recent developments in vasospasm pharmacology: the post-synaptic α2 adrenoreceptor. **Trends Pharmacol Sci 3:**440-444, 1982.
349 Langfitt TW, Weinstein JD, Kassell NF, Simeone FA. Transmission of increased intracranial pressure. I Within the craniospinal axis. **J Neurosurg 21**:989-997, 1964.
350 Langfitt TW, Weinstein JD, Kassell NF. Cerebral vasomotor paralysis produced by intracranial hypertension. **Neurology 15**:622-641, 1965.
351 Lanzino G, Kongable GL, Kassell NF. Electrocardiographic abnormalities after nontraumatic subarachnoid haemorrhage. **J Neurosurg Anaesth 6(3)**:156-162, 1994.

352 Lassen NA: Brain extracellular pH: the main factor controlling cerebral blood flow. **Scand J Clin Lab Invest 22**:247-251, 1968
353 Leblanc R. Familial cerebral aneurysms: a bias for women. **Neurosurgery 27**:1050-1054, 1996
354 Lee TJF, McIlhany M, Sarwinski S: Erythrocyte extracts enhance neurogenic vasoconstriction of dog cerebral arteries in vitro. **J Cerebr Blood Flow & Metab 4:**474-6, 1984.
355 Lehman RM, Kassell NF, Nazar GB, Weir BK, Joshita H, Nosko M, Duling BR, Owens GK. Morphometric methods in the study of vasospasm. In *Cerebral Vasospasm* Wilkins RH (ed) Raven Press, New York, 1988, pp113-117.
356 Levy WJ, Bay JW, Sawhny B et al. Aminophylline plus nitroprusside and dopamine for treatment of cerebral vasospasm: a preliminary report. **J Neurosurg 56**:646-649, 1982.
357 Lewis NP, Tsao PS, Rickenbacher PR, Haywood GA, Leyen HVD, Valantine HA, Hunt SA, Gillingham ME, Cooke JP, Fowler MB. Induction of iNOS mRNA in the human cardiac allograft is associated with impaired left ventricular contractile function. **Circulation 90(suppl I)**:I-193 Abstract 1994.
358 Libby P, Schwartz D, Brogi E, Tanaka H, Clinton SK. A cascade model for restenosis: a special case of atherosclerosis progression. **Circulation 86(suppl III)**: III 47-52 1992.
359 Limas CJ, Cohn JN. Stimulation of vascular smooth muscle sodium, potassium-adenosinetriphosphate by vasodilators. **Circ Res 35**:601-607, 1974.
360 Lin SR, O'Connor MJ, King A, Harnish P, Fischer HW. Effect of dextran on cerebral function and blood flow after cardiac arrest. An experimental study in he dog. **Stroke 10(1)**:13-20, 1979
361 Lindpainter K, Ganten D. The cardiac renin-angiotensin system. **Circ Res 68**:905-921, 1991.
362 LiszczakTM, Varsos VG, Black PML et al. Cerebral artreial constriction after experimental subarachnoid haemorrhage is associated with blood components within the arterial wall. **J Neurosurg 58**:18-26, 1983.
363 Livingston K, Guterman LR, Hopkins LN. Intra-arterial papaverine as adjunct to transluminal angioplasty for vasospasm induced by subarachnoid haemorrhage. **Am J Neuroradiol 14(2)**:346-347, 1993.
364 Liu Y, Downey JM. Ischaemic preconditioning protects against in farction in rat heart. **Am J Physiol 263**:H1107-1112, 1992.
365 Ljusegren ME, Axelsson KL. Lactate accumulation in isolated hypoxic rat ventricular myocardium: effect of different modulators of the cGMP system. **Pharmacol Toxicol 72**:56-60, 1993.
366 Ljunggren B, Brandt L, Kagstrom E et al. Results of early operations for rutptured aneurysms. **J Neurosurg 54**:473-479, 1981.
367 Lobato R, Marin J, Salaices M et al. Effect of experimental subarachnoid haemorrhage on the adrenergic innerevation of cerebral artreies. **J Neurosurg 53**:477-479, 1980.
368 Lobato R, Marin J, Salaices M et al. Cerebrovascular reactivity to norepinephrine and 5-hyroxytryptamine following experimental subarachnoid haemorrhage. **J Neurosurg 53**: 480-485, 1980.
369 Locksley HB. Report on the cooperative study of intracranial aneurysms and subarachnoid haemorrhage. Section V, part II. Natural history of subarachnoid

haemorrhage, intracranial aneurysms and arterio-venous malformations based on 6368 cases in the co-operative study. **J Neurosurg 25**:321-368, 1966.
370 Lu GC, Vasquez JA, Gallagher KP, Lucchesi BR. Myocardial protection with pre-conditioning. **Circulation 82**:609-619, 1990.
371 Luscher TF, Boulanger CM, Dohi Y, Yang Z. Endothelial derived contracting factors. **Hypertension 19**:117-130, 1992.
372 Luscher TF, Boulanger CM, Yang Z, Noll G, Dohi Y. Interactions between endothelial derived relaxing and endothelial derived contracting factors in health and cardiovascular disease. **Circulation 87 (suppl V)**:V-36-44, 1993.
373 Luscher TF, Noll G. Endothelial dysfunction in the corornary circulation. **J Cardiovasc Pharmacol 24(suppl3)**:S16-S26, 1994.
374 Lynch P. Soldiers, sport, and sudden death. **Lancet 1**:1235-1237, 1980.
375 MacDonald RL, Wier BKA. A reveiw of haemoglobin and the pathogenesis of cerebral vasospasm. **Stroke 22**:971-982, 1991.
376 Macdonald RL, Weir BK, Grace MG. Mechanism of cerebral vasospasm following subarachnoid haemorrhage in monkeys. **Can J Neurol Sci** 19(4):419-427, 1992.
377 Macdonald RL, Weir BK, Young JD, Grace MG. Cytoskeletal and extracellular matrix proteins in cerebral arteries following subarachnoid hameorrhage in monkeys. **J Neurosurgery 76(1)**:81-90, 1992.
378 MacKenzie ET, Farrar JK, Fitch W, Graham DI, Gregory PC, Harper M. Effects of haemorrhagic hypotension on the cerebral circulation. 1. Cerebral blood flow and pial arteriolar caliber. **Stroke 10(6)**:711-718, 1979
379 Magnoni MS, Kobayashi H, Frattola L, Spano PF, Trabucchi M. Effect of common carotid occlusion on β-adrenergic receptor function in cerebral microvessels. **Stroke 16(3)**:505-509, 1985.
380 Mallov S. Role of calcium and free fatty acids in epipnephrine-induced myocardial necrosis. **Toxicol Appl Pharmacol 71**:280-287, 1983.
381 Mann DL, Kent RL, Parsons B, Cooper G IV. Adrenergic effects on the biology of the adult mamalian cardiocyte. **Circulation 84**:2091-2100, 1991.
382 Marion DW, Segal R, Thompson ME. Subarachnoid hameorrhage and the heart. **Neurosurgery 18 (1)**:101-106, 1986.
383 Marks MP Steinberg GK, Lane B. Intra-arterial papaverine for the treatment of vasospasm. **Am J Neuroradiol 14(4)**:822-826, 1993.
384 Marshman LAG: Elastin degradation in the superficial temporal arteries of patients with intracranial aneurysms reflects changes in plasma elastase. Letter to the Editor. **Neurosurgery 43(4):**982, 1998.
385 Marshman LAG, Ward PJ, Walter PH, Dossetor RS: The progression of an infundibulum to aneurysm formation and rupture. Case report and literature review. **Neurosurgery 43(6):**1445-9, 1998.
386 Martin W, Villani GM, Jothianandan D et al. Selective blockade of endothelial-dependent and GTN-induced relaxation by haemoglobin and by methylene blue in the rabbit aorta. **J Pharmacol Exp Ther 232**:708-716, 1985.
387 Martin NA, Doberstein C, Zane C, Caron MJ, Thomas K, Becker DP. Posttraumatic cerebral arterila spasm: transcranial doppler ultrasound, cerebral blood flow, and angiographic findimgs. **J Neurosurg 77(4)**:575-583, 1992.
388 Martins AN, Doyle TF, Wright SJ, Bass BG. Response of cerebral circulation to topical histamine. **Stroke 11(5)**:469-476, 1980.

389 Masini E, Gambassi F, Di Bello MG, Mugnai L, Raspanti S, Manaioni PF. Nitric oxide modulates cardiac and mast cell anaphylaxis. **Agents Actions (Suppl) 41**:C89-90, 1994.
390 Mathiesen T, Andersson B, Loftenius A, von Holst H. Increased interleukin-6 levels in cerebrospinal fluid following subarachnoid haemorrhage. **J Neurosurg 78**:562-567, 1993.
391 Mathis JM, De Nardo A, Jensen ME, Scott J, Dion JE. Transient neurologic events associated with intra-arterial papaverine infusion for subarachnoid haemorrhage-induced vasospasm. **Am J Neuroradiolog 15(9)**:1671-1674, 1994
392 Mathis JM, De Nardo A, Jensen ME, Scott J, Dion JE. Transient severe brain stem depression during intra-arterial papaverine infusion for cerebral vasospasm. **Am J Neuroradiolog 15(4)**:719-723, 1994.
393 Matsui T, Sugawa M, Johshita H, Takuwa Y, Asano T: Activation of the protein kinase C-mediated contractile system in canine basilar artery undergoing chronic vasospasm. **Stroke 22:**1183-87, 1991.
394 Matta BF, Lam AM, Mayberg TS, Eng CC, Strebel S. Cerebrovascular response to CO_2 during SNP and isoflurane-induced hypotension. **Br J Anaesth 74(3)**: 296-300, 1995.
395 Maulik N, Engelman RM, Wei Z, Lu D, Rousou JA, Das DK. Interleukin 1α preconditioning reduces myocardial ischaemia-reperfusion injruy. **Circulation 88(pt2)**:387-394, 1993.
396 Maurice-Williams RS. In *Subarachnoid haemorrhage. Aneurysms and vascular malformations of the central nervous system*, Wright, Bristol, 1987.
397 Maurice DH, Haslam RJ. Molecular basis of the synergistic inhibition of platelet function by nitrovasodilators and activators of adenylate cyclase: inhibition of cAMP breakdown by cGMP. **Br J Pharm 113**:671-681, 1994
398 Mayberg MR, Liszczack TM, Black PMcL, Zervas NT. Acute structural changes in feline cerebral arteries after subarachnoid haemorrhage. In *Cerebral Vasospasm* Wilkins RH (ed) Raven Press, New York, 1988, pp231-246.
399 Mayberg MR, Okada T, Bark DH. The significance of morphological changes in cerebral arteries after subarachnoid haemorrhage. **J Neurosurg 72**:626-633, 1990.
400 Mc Auliffe W, Townsend M, Eskridge JM , Newel DW, Grady MS, Winn Hr. Intracranial pressure changes induced during papaverine infusion for treatment of vasospasm. **J Neurosurg 83(3)**:430-434, 1995.
401 McCall TB, Feelisch M, Palmer RMJ, Moncada S. Identification of N-iminoethyl-L-ornithine as an irreversible inhibitor of nitric oxide synthase in phagocytic lines. **Br J Pharmacol 102**:234-238, 1991.
402 McCormick JM, McCOrmick PW, Zabramski JM, Spetzler RF. Intracranial pressure reduction by a central α2 adrenoceptor agonist after subarachnoid haemorrhage. **J Autonom Nerv Sys 32(6)**:974-979, 1993.
403 McHenry LC, Jaffe ME, Kawamura J et al. Effect of papaverine on regional blood flow in focal vascular disease of brain. **New Eng J Med 282**:1167-1170, 1970.
404 McNair JL, Clower BR, Sanford RA. The effect of reserpine pre-treatment on myocardial damage associated with simulated intracranial haemorrhage in mice. **Eur J Pharmacol 9(1)**:1-6, 1970

405 Mehta P, Mehta J, Miale TS. Nitroprusside lowers platelet count. **NEJM 299**: 1134, 1978.
406 Mellander S, JohanssonB. Control of resistance, exchange, and capacitance functions in the peripheral circulation. **Pharmacol rev 20**:117-196, 1968
407 Mellander S. Functional aspects of myogenic vascular control. **J Hypertension 7(suppl4)**:S21-S30, 1989.
408 Mendelow AD, Mc Calden TA, Hattingh J, Coull A, Rosendorff C, Eidelman BH. Cerebrovascular reactivity and metabolism after subarachnoid haemorrhage in baboons. **Stroke 12(1)**:58-65, 1981
409 Mendelow AD. Pathophysiology of delayed ischaemic dysfunction after subarachnoid haemorrhage: experimental and clinical data. **Acta Neurochir S45**:7-10, 1988.
410 Mery PF, Pavoine C, Belhassen L, Pecker F, Fischmeister R. Nitric oxide regulates cardiac calcium current: involvement of cGMP-inhibited and cGMP-stimulated phosphodiesterases through guanylyl cyclase activation. **J Biol Chem 268**: 26286-26295, 1993.
411 Meyer JS, Gotoh F. Interaction of cerebral haemodynamics and metabolism. **Neurology 11(2)**:46-65, 1961.
412 Meyer JS, Stoica E, Pascu I, Shimazu K, Hartmann A. Catecholamine concentrations in cerebrospinal fluid and plasma of patients with cerebral infarction and haemorrhage. **Brain 96**:277-288, 1973.
413 Meyer CHA, Lowe D, Meyer M et al. Progressive change in cerebral blood flow during the first three weeks after subarachnoid haemorrhage. **Neurosurgery 12**:58-76, 1983.
414 Meurice T, Bauters C, Auffray JL, Vallet B, Hamon M, Valero F, Van Belle E, Lablanche JM, Bertraud ME. Basic fibroblast growth factor restores endothelial-dependent responses after balloon injury of rabbit arteries. **Circulation 93**:18-22, 1996.
415 Meyers R. Systemic vascular and respiratory effects of experimentally induced alterations in intraventricular pressure. **J Neuropathl Exp Neurol 1**:241-264, 1942.
416 Michenfelder JD, Tinker JH. Cyanide toxicity and thiosulphate protection during chronic administration of sodium nitroprusside in the dog; correlation with a human case. **Anesthesiology 47(5)**:441-448, 1977.
417 Miller CA, Yashon D, Locke G et al. Autonomous activity in the human basilar artery: relationship to cerebral vasospasm. **Neurology 21**:1249-1254, 1971.
418 Miller JA,Cross DT, Moran CJ, Dacey RG Jr,McFarland JG, Diringer MN. Severe thrombocytopenia following intra-arterial papaverine administration for treatment of vasospasm. **J. Neurosurg 83**:435-457, 1995.
419 Miller VM, Komori K, Burnett JC, Vanhoutte PM. Differential sensitivity to endothelin in canine arteries and veins. **AM J Physiol 257**:H1127-1131, 1989.
420 Mine-Golomb D, Tarsafaty I, Schwartz JP. Expression of inducible nitric oxide synthase by neuroines following exposure to endotoxin and cytokine. **Br J Pharm 111**:720-722, 1994.
421 Minneman KP. α-adrenergic receptor subtypes, inositol phosphates, and sources of cell calcium. **Pharm rev 40(2)**:87-117, 1988.

422 Miranda FJ, Alabadi JA, Torregrosa G, Salom JB, Jover T, Barbera MD, Alborch E: Modulatory role of endothelial and nonendothelial nitric oxide in 5-Hydroxytryptamine-induced contraction in cerebral arteries after subarachnoid hemorrhage. **Neurosurgery 39(5)**:998-1004, 1996.
423 Misra HP, Fridovich I. The generation of superoxide radical during the autoxidation of haemoglobin. **J Biol Chem 247**:6960-6962, 1972.
424 Mizukami M, Kim H, Araki G, et al. Is angiographic spasm real? **Acta Neurochir (Wien) 34**:247-257, 1976.
425 Mizukami M, Kawase T, Usami T et al. Prevention of vasospasm by early operation with removal of subarachnoid blood. **Neurosurgery 10**:301-307, 1982.
426 Moncada S, Palmer RMJ, Higgs EA. Nitric oxide;physiology, pathophysiology and pharmacology. **Pharmacol Rev 43**:109-142, 1991.
427 Moss E. Cerebral blood flow during induced hypotension. **Br J Anaesthes 74(6)**:635-637, 1995.
428 Mueller SM, Heistad DD, Marcus ML. Total and regional cerebral blood flow during hypotension, hypertension and hypocapnia. **Circ Res 41**:350-356, 1977.
429 Mulvany MJ, Halpern W. Mechanical properties of vascular smooth muscle cells *in situ*. **Nature 260(5552)**:617-619, 1976.
430 Mulvany MJ, Halpern W. Contractile properties of small arterial resistance arteries in spontaneously hypertensive and normotensive rats. **Circ Res 41(1)**:19-26, 1972.
431 Mulvany MJ, Kelley M. In vitro methodology for resistance arteries. **Blood Vessels 28**:245-251, 1991.
432 Murray CE, Jennings RB, Reimer KA. Preconditioning with ischaemia; a delay of lethal cell injury in ischaemic myocardium. **Circulation 74**:1124-1136, 1986.
433 Muscholl E, Vogt M. The action of reserpine on peripheral sympathetic nervous system. **J Physiol 141**:132-155, 1958.
434 Nagai H, Noda S, Mabe H. Experimental cerebral vasospasm: effects of vasoactive drugs and sympathectomy on early and late spasm. **J Neurosurg 42**: 420-428, 1975.
435 Nagai H, Kamiya K, Sugiyama N, Ueda Y. Change of ischaemic threshold after subarachnoid haemorrhage, In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York, 1988, pp 57-61.
436 Nakagawa Y, Yamamoto YL, Meyer E, Hodge CP, Feindel W. Effects of hypercapnia on enhancement of decreased perfusion flow in non-infarcted brain tissues. **Stroke 12(1)**:85-92, 1981.
437 Nakagomi T, Kassell NF, Sasaki T, Fujiwara S, Lehman RM, Torner JC: Impairment of ednothelium-dependent vasodilation induced by acetylcholine and adeonsine triphosphate following experimental subarachnoid hemorrhage. **Stroke 18**:482-489, 1987.
438 Nakashima T, Takenaka K, Fukazawa S, Yano K, Nishimura Y, Andoh T, Sakai N, Yamada H. Cytosolic free calcium elevation in vascular smooth muscle cells induced by cerebrospinal fluid from patients with subarachnoid haemorrhage - biochemical nature of the calcium-mobilising factor. **Neurologica Medico-Chirurgica 35(1)**:8-12, 1995.
439 Nagata K, Sasaki T, Iwama J Mori, T, Iwamoto S, Nirei H, Kaori H, Kirino T. Failure of FK-506, a new immunosuppressant, to prevent cerebral vasospasm in a canine 2- haem model. **J Neurosurg 79**:710-715, 1993.

440 Nagata K, Nikaido H, Kobayashi E et al. Cisternal talc injection can induce a delayed and prolonged basilar artery constriction resembling vasospasm after subarachnoid haemorrhage in the dog. In *Cerebral vasospasm*, Findlay JM (ed) Amstredam: Elsevier 1993, pp281-284.
441 Nagatani K, Masciopinto JE, Letarte PB, Haworth RA, Duff TA. The effect of haemoglobin and its metabolites on energy metabolism in cultured cerebrovascular smooth-muscle cells. **J Neurosurg 82**:244-249, 1995.
442 Nakane M, Saheki S, Kuno T et al. Molecular cloning of cDNA coding for 70 kilodalton subunit of sol GC from rat lung. **Biochem Biophys Res Comm 157**: 1139-1147, 1988.
443 Nathan C. Nitric oxide as a secretory product of mammalian cells. **FASEB J 6**:3051-3064, 1992.
444 Nazar GB, Kassell NF, Povlishock JT, Lee J, Hudson S. Subarachnoid haemorrhage causes adherence of white blood cells to the cerebral arterial luminal surface. In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York,1988, pp343-356.
445 Neil-Dwyer G, Cruikshank J. The blood leucocyte count and its prognostic significance in subarachnoid haemorrhage. **Brain 97**:79-86, 1974.
446 Neil-Dwyer G, Cruikshank J, Stott A, Brice J. The urinary catecholamine and plasma cortisol levels in patients with subarachnoid haemorrhage. **J Neurol Sci 22**:375-382, 1974.
447 Nielsen KC, Owman CH. Contractile response and amine receptor mechanisms in isolated middle cerebral artery if the cat. **Brain Res 27**:33-42, 1971
448 Nelson PB, Seif S, Gutai J, Robinson AG. Hyponatraemia and natriuresis following subarachnoid haemorrhage in a monkey model. **J Neurosurg 60**:233-237, 1984.
449 Newell DW, Eskridge JM, Mayberg MR et al. Angioplasty for the treatment of symptomatic vasospasm following subarachnoid haemorrhage. **J Neurosurg 71**:654-660 1989.
450 Niroomand F, Bangert M, Beyer T, Rauch B. Reduced adenyl cyclase inhibition by carbachol and GTP during acute myocardial ischaemia. **J Moll Cell Cardiol. 24**:471-475, 1992.
451 Node K, Kitakaze M, Kosaka H, Komamura K, Minamino T, Inoue M, Tada M, Hori M, Kamada T. Increased release of nitric oxide during ischaemia reduces myocardial contractility and improves metabolic dysfunction. **Circulation 93**:356-364, 1996.
452 Nomura T, Ikezaki K, Natori Y, Fukui M. Altered response to histamine in brain tumour vessels: the selective increase of regional cerebral blood flow in transplanted rat brain tumour. **J Neurosurg 79(5)**:722-728, 1993.
453 Nornes H, Magnaes B. Intra cranial pressure in patients with ruptured sacular aneurysms. **J Neurosurg 36**:537-547, 1972.
454 Nornes H. The role of intracranial pressure in the arrest of haemorrhage in patients with ruptured intracranial aneurysm. **J Neurosurg 39**:226-234, 1973.
455 Norris JW. Cardiovascular manifestations of acute neurological lesions, in *Neurology and general medicine*, Aminoff MJ (ed), New York, Churchill Livingstone, 1981, pp159-67.
456 Norris JW. The effects of cardiovascular lesions upon the heart. **Neurol Clin N America 49**:862-868, 1983.

457 Nosko M, Handa Y, Weir B, Grace M, Lunt A, Allen P, Mielke B, Mosewich R, Tsuji T. The effect of subarachnoid clot removal on the development of chronic vasospasm in a primate model. In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York,1988, pp389-94.
458 Novitsky D, Wicomb WN, Cooper DKC, Rose AG, Fraser RC, Barnard CN. ECG, haemodynamic and endocrine changes occurring during experimental brain death in the chacma baboon. **J Heart Transplant 4**:63-69, 1984.
459 Novitsky D, Wicomb WN, Cooper DKC, Reichart B. Haemodynamic changes myocardial injury and pulmonary oedema induced by sympathetic activity during the development of brain death in the baboon. **Transplant Proc 18**:609-612, 1986.
460 Novitsky D, Wicomb WN, Cooper DKC, Tjaalgard MA. Improved cardiac function following hormonal therapy in brain dead pigs. Relevance to organ donation. **Cryobiology 24**:1-10, 1987.
461 Novitsky D, Cooper DKC, Morrell D, Isaacs S. Change from aerobic to anaerobic metabolism after brain death and reversal following triiodothyronine therapy. **Transplantation 45**:32-36, 1988.
462 O'Donnel SR, Saar N. A histochemical study of extraneuronal accumulation of norepinephrine in the gineau pig trachea. **Br J Pharm 49**:267-278, 1973.
463 Ogata M, Marshall BM, Lougheed WM. Observations on the effects of intrathecal papaverine in experimental vasospasm. **J Neurosurg 38**:20-25, 1973.
464 Ohgushi N. Adrenergic fibres to the brain and spinal cord vessels in the dog. **Arcg Jap Chir 37**:294-303, 1968.
465 Ohta H, Ito Z, Yasui N et al. Extensive evacuation of subarachnoid clot for prevention of vasospasm: effective or not? **Acta Neurochir (Wien) 63**:111-116, 1982.
466 Ohlstein EH, Storer BL. Oxyhaemoglobin stimulation of endothelin production in cultured endothelial cells. **J Neurosurg** 77:274-278, 1992.
467 Ohta T, Kuroiwa T, Shimizu K, Shigma M, Yamada K, Yasuda A, Sato G. Exaggerated permeability via the adventitia of the spastic vessel wall in experimental cerebral vasospasm. In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York, 1988, pp 219-230.
468 Ohta T, Satoh G, Kuroiwa T. The permeability change of major cerebral arteries in experimental vasospasm. **Neurosurgery 30(3)**:331-335 discussion 335-6 1992.
469 Okada T, Bark DH, Mayberg MR. Localized release of perivascular heparin inhibits intimal proliferation after endothelial injury without systemic anticoagulation. **Neurosurgery 25(6)**:892-898, 1989.
470 Olesen J, Skinhøj E. The influence of certain vasoactive amines on the rCBF of man. *Proceedings of the international headaches symposium*. Elsinore, Denmark, May 16-18, 1971, Basel Sandoz, 1971, p145-52.
471 Olesen J, Paulson OB. The effect of intra-arterial papaverine on the regional cerebral blood flow in patients with stroke on intracranial tumour. **Stroke 2**:148-159, 1971.
472 Onoue H, Kaito N, Akiyama M, Tomii M, Tokudome S, Abe T. Altered reactivity of human cerebral arteries after subarachnoid haemorrhage. **J Neurosurg 83(3)**:510-515, 1995.

473 Onoue H, Katusic Z: The effect of subarachnoid hemorrhage on mechanisms of vasodilation mediated by cyclic adenosine monophosphate. **J Neurosurg 89:**111-117, 1998.
474 Osol G, Halpern W. Myogenic properties of cerebral blood vessels from normotensive and hypertensive rats. **Am J Physiol 249**:H 914-921, 1985.
475 Osol G, Osol R, Halpern W. Pre-existing level of tone is an important determinant of cerebral artery autoregulatory responsiveness. **J Hypertension 7(suppl4)**:S67-69, 1989.
476 Ostroumoff A. Experiments on the inhibitory nerves of skin blood vessels (inGerman). **Pflugers Arch 12**:219-277, 1868
477 Pakarinen S. Incidence, actiology, and prognosis of primary subarachnoid haemorrhage. **Acta Neurol Scand 43**:S29:1967.
478 Palmer RM, Ferridge AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelial derived relaxing factor. **Nature 327**:524-526, 1987.
479 Parfenova H, Shibata M, Leffler CW. Subarachnoid haemorrhage causes pial arteriolar constriction in newborn pigs. **Stroke 24(11)**:1729-1734, 1993
480 Pasqualin A, DaPian R. An analysis of vasospasm following early surgery for intracranial aneurysms. **Acat Neurochir(Wien) 63**:153-159, 1982.
481 Pearce WJ, D'Alecy LG. Haemorrhage-induced cerebral vasoconstriction in dogs. **Stroke 11(2)**:190-196, 1980.
482 Pearce WJ, Ashwal S, Cuevas J. Direct effects of graded hypoxia on intact and denuded rabbit cranial arteries. **Am J Physiol (Heart Circ Physiol 26)**:H824-H833, 1989.
483 Peerless SJ, Yasargil MG. Adrenergic innervation of the cerebral blood vessels in the rabbit. **J Neurosurg 35**:148-154, 1971.
484 Peerless SJ, Kendall MJ. The significance of the perivascular innervation of the brain, in *The cerebral vessel wall*, Cervas-Navarro J, Betz E, Matakas F, et al (eds), New York, Raven press, 1975, pp175-82.
485 Peerless SJ, Griffifths JC. Plasma catecholamines following subarachnoid haemorrhage. In *Subarachnoid haemorrhage and cerebral vasospasm*, Smith RR, Robertson JT (eds), Springfield III, Charles C Thomas, 1975, pp148-156.
486 Pelligrino DA. Saying NO to cerebral ischaemia. **J Neurosurg Anaesth 5(4)**:221-231, 1993.
487 Persson L, Valtysson J, Enblad P, Wärme P-E, Cesarini K, Lewén A, Hillered L. Neurochemical monitoring using intracerebral microdialysis in patients with subarachnoid haemorrhage. **J Neurosurg 84**: 606-616, 1996.
488 Peterson EW, Cardoso ER. The blood-brain barrier following experimental subarachnoid haemorrhage. Part 1 and 2: **J Neurosurg 58**:338-351, 1983
489 Pfister H-W, Feiden W, Einhäupl K-M. Spectrum of complications during bacterial meningitis in adults. Results of a prospective study. **Arch Neur0l 50**: 575-581, 1993.
490 Phillips SF. In *Gastrointestinal disease: pathophysiology, diagnosis, and management, 4th Ed.*, Sleisenger MH, Fordtran JS (Eds) WB Saunders Co, Harcourt Brace Jovanovich, Inc., Philadelphia, London, Toronto, Montreal, Sydney, Tokyo, 1989, pp 1391-1392.

491 Pickard JD, Lovick AHJ, Read DH. Evidence for brain engorgement as the initial cause of brain swelling following intraoperative aneurysm rupture in man after subarachnoid haemorrhage. **Acta Physiol Scan d Suppl 552**:94-95, 1986.
492 Pickard JD, Murray GD, Illingworth R et al: Effect of oral nimodipine on cerebral infarction and outcome after subarachnoid hemorrhage: British aneurysm nimodipine trial. **BMJ 298(6674):**636-42, 1989.
493 Pilati CF, Clark RS, Gilloteaux J, Bosso FJ, Holcomb P, Maron MB. Excessive sympathetic nervous system activity decreases myocardial contractility. **Proc Soc Exp Biol Med 193**:225-231, 1990.
494 Pluta RM, Zauner A, Morgan JK, Muraszko KM, Oldfield EH. Is vasospasm related to proliferative arteriopathy? **J Neurosurg** 77:740-748, 1992.
495 Pohl U, Holtz J, Busse R, Bassenge E. Crucial role of endothelium in the vasodilator response to increased flow *in vivo*. **Hypertension 8**:37-44, 1986.
496 Polikar R, Burger AG, Scherrer U, Nicod P. The thyroid and the heart. **Circulation 87(5)**:1435-1441, 1993.
497 Pool JL, Potts DG. In *Aneurysms and arterio-venous anomalies of the brain: diagnosis and treatment*, New York, Harper & Row, 1965, p201
498 Prasad MR, Tzigaret CM, Smith D et al . Decreased α1 adrenergic receptors after experimental brain injury. **J Neurotrauma 9**:269-279, 1992.
499 Quigley MR, Bailes JE, Kwaan HC, Cerullo LJ, Brown JT. Aneurysm formation after low power carbon dioxide laser-assisted vascular anastomosis. **Neurosurgery 18(3)**:292-298, 1986.
500 Radomski MW, Palmer RMJ, Moncada S. The anti-aggregating properties of vascular endothelium; interactions between prostacyclin and nitric oxide. **Br J Pharmacol 92**:639-646, 1987 .
501 Raper AJ, Kontos EP, Wei P, Patterson JL. Unresponsiveness of pial pre-capillary vessels to catecholamines and sympathetic nervous stimulation. **Circ Res 31**:257-266, 1972.
502 Rees DD, Celler S, Palmer RMJ, Moncada S. Dexamethasone inhibits the induction by endotoxin of a nitric oxide synthase and the associated effects on vascular tone. An insight into endotoxic shock. **Biochem Biophys Res Commun** 173:541-547, 1990.
503 Rees DD, Palmer RMJ, Schultz R, Hodson HF, Moncada S. Characterization of three inhibitors of endothelial nitric oxide synthase *in vitro* and *in vivo*. **Br J Pharmacol 336**:897-900, 1990.
504 Rennels ML, Nelson E. Capillary innervation of the mammalian central nervous system: an electron microscopic demonstration. **Am J Anat 144**:233-234, 1975.
505 Revest PA, Abbott NJ, Gillespie JI. RM changes in intracellular calcium in cultured rat brain capillary endothelial cells. **Brain Research 549(1)**:159-161 1991.
506 Rhodin JAG. Architecture of the vessel wall. In *Handbook of physiology, section 2, The cardiovascular system, vol III, Vascular Smooth Muscle*, Bohr DF, Somlyo AP, Sparks HV Jr, Geiger SR (eds) Betheseda, Maryland: American physiological society, 1980, pp 1-31.
507 Roberts AB, Roche NS, Winokur TS, Burmester JK, Sporn MB. Role of transforming growth factor-β in maintenance of function of cultured neonatal cardiac myocytes. **J Clin Invest 90**: 2056-2062, 1992.

508 Roberts A, Vodovotz BY, Roche NS, Sorn MB, Nathan CF. Role of nitric oxide in antagonistic effects of TGFβ and IL-1β on the beating rate of cultured cardiac myocytes. **Mol Endocrinol 6**:1921-1930, 1992.
509 Rodriguez Y, Baena R, Graetani P et al. Cisternal lumbar cerebrospinal fluid concentration of arachidonate metabolism in vasospasm following subarachnoid haemorrhage from ruptured aneurysm: biochemical and clinical considerations, **Surg Neurol 24**:428-432, 1985.
510 Rona G. Editorial review: catecholamine cardiotoxocity. **J Mol Cell Cardiol 17**: 291-306, 1985.
511 Rosenblum WI. Cerebral arteriolar spasm inhibit by adrenergic blocking agenst. **Arch Neurol 21**:296-302, 1969.
512 Rosenblum WI. Pial arteriiolar responses in mouse brain revisited. **Stroke** 7:283-286, 1976
513 Rosenblum WI: Contractile responses of pial arterioles in gerbils with unilateral carotid ligation. **Stroke 12(1)**:83-85, 1981.
514 Rosenblum WI. Endothelial dependent relaxation demonstrated *in vivo* in cerebral arterioles. **Stroke 17**:494-497, 1986.
515 Rosenblum WI, Nelson GH, Povlishock. Laser induced endothelial damage inhibits endothelial dependent relaxation in the cerebral microcircualtion of the mouse. **Circ Res 60(2)**:169-176, 1987.
516 Rosner MJ, Newsome HH, Becker DP. Mechanical brain injury: the sympathoadrenal response. **J Neurosurg 61**:76-86, 1984.
517 Rothwell NJ, Hopkins SJ. Cytokines and the nervous system II: actions and mechanisms of action. **Trends Neurosci 18**:130-136, 1995.
518 Rousseaux P, Scherpereel B, Bernard MH, et al. Fever and cerebral vasospasm in ruptured intracranial aneurysms. Surg Neurol 14:459-65, 1980.
519 Rüegg JC. In *Human Physiology 20th Ed,* Schmidt RF, Thews G (eds) Springer-Verlag, Berlin, Heidelberg, New York, 1983, Ch 2, pp32-51.
520 Ruskoaho H, Lang RE, Toth M, Ganten D, Unger T. Release and regulation of atrial natriuretic peptide. **Eur Heart J 8(SB)**:99-109, 1987.
521 Sagher O, Zhang XQ, Szeto W et al. Live computerized videomicroscopy of cerebral microvessels in brain slices. **J Cereb Blood Flow & Metab 13**:676-682, 1993.
522 Sahar A. The effect of pressure on the production of cerebrospinal fluid by the choroid plexus. **J Neurol Sci 16**:49-58, 1972.
523 Sahlin CH, Owman CH, Chang JY, Delgado T, Salford LG, Svendgaard NA: Changes in contractile response and effect of a calcium antagonist, nimodipine, in isolated intracranial arteries of baboon following experimental subarachnoid haemorrhage. **Brain Res Bull 24(3):**355-361, 1990.
524 Sakaki S, Ohue S, Kohno K, Takeda S. Impairment of vascular reactivity and changes in intracellular calcium and calmodulin levels of smooth muscle cells in canine basilar arteries after subarachnoid haemorrhage. **Neurosurgery 25(5)**:753-761, 1989.
525 Sakakibara Y, Fugiwara M, Muramatsu I. Pharmacological characterization of the α adrenoreceptors dog basilar artery. **Naunyn-Schmiederbergs Arch Pharmacol 319**:1-7, 1981

526 Sano K, saito I. Early operation and washout of blood clots for prevention of cerebral vasospasm. In *Cerebral arterial spasm*, Wilkins RH (ed), Baltimore, Williams and Wilkins, 1980, pp 510-513.
527 Sano K. Cerebral vasospasm as a deficiency syndrome: In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York, 1988, pp 285-295.
528 Sasaki T, Asano T, takakura K, et al. Nature of the vasoactive substance in cerebrospinal fluid from patients with subarachnoid haemorrhage. **J Neurosurg 60**:1186-1191, 1984.
529 Sasaki S, Ohue S, Kohno K, Takeda S. Impairment of vascular reactivity and changes in intracellullar calcium and calmodulin levels of smooth muscle cells in canine arteries after subarachnoid haemorrhage. **Neurosurgery 25(5)**:753-761, 1989.
530 Schaller C, Rohde V, Meyer B, Hassler W: Amount of subarachnoid blood and vasospasm: current aspects. A transcranial Doppler study. **Acta Neurochir** 136(1-2):67-71, 1995.
531 Schievink WI, Schaid DJ, Michels W, Piepgras DG. Familial aneurysmal subarachnoid haemorrhage. A community based study. **J Neurosurg 83(3)**:426-429, 1995.
532 Schrader H, Krogness K, Aakvaag A, Sortland O, Purvis K. Changes of pituitary hormones in brain death. **Acta Neurochir (Wien) 52**:239-248, 1980.
533 Schrader H, Hall C, Zetnow NN. Effects of prolonged supratentorial mass expansion on regional blood flow and cardiovascular parameters during the Cushing response. **Acta Neurol Scand 72**:283-294, 1985.
534 Schrader H, Zetnow NN, Morkid L. Regional cerebral blood flow and cerebrospinal fluid pressures during Cushing response induced by a supratentorial expanding mass. **Acta Neurol Scand 71**:453-463, 1985.
535 Schulz R, Nava E, Moncada S. Induction and potential biological relevance of a calcium independent nitric oxide synthase in the myocardium. **Br J Pharmacol 105**:575-80, 1992.
536 Segal MB, Zlokovic BV. In *The blood-brain barrier, amino acids and peptides*, Kluwer academic publishers, Dordrecht, Boston, London, 1990.
537 Segawa H, Saito I, Okada T, Nagayama I, Kitamura K, Takakura K, Sano K. Efficacy of intracisternal papaverine on symptomatic vasospasm. In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York, 1988, pp 433-441.
538 Seiler RW, Grolimund P, Auslid R et al. Relation of cerebral vasospasm evaluated by trans-cranial doppler ultrasound to clinical grade and CT-visualized subarachnoid haemorrhage. **J Neurosurg 64**:594-600, 1986.
539 Seifert V, Stolke D, Kuntz U, Resch K. Influence of blood volume on cerebrospinal fluid levels of arachidonic acid metabolites after subarachnoid haemorrhage: experimental study on the pathogenesis of cerebral vasospasm. **Neurosurgery 23(3)**:313-321, 1988.
540 Seifert V, Eisert WG, Stolke D, Goetz C. Efficacy of single intracisternal bolus injection of recombinant tissue plasminogen activator to prevent delayed cerebral vasospasm after experimental subarachnoid haemorrhage. **Neurosurgery 25(4)**:590-598, 1989.
541 Seiro V. Über die Messung des intraduralen reserveraums. **Acta Chir Scand 89**: 139, 1943.

542 Sellke FW, Shafique T, Johnson RG, Daj H-B, Banitt PF, Schoen FJ, Weintraub RM. Blood- and albumin-cardioplegia preserve endothelial dependent microvascular responses. **Ann Thorac Surg 55**:977-985, 1993.
543 Shabo AL, Maxwell DS. Electron microscopic observations of particulate matter in the cerebrospinal fluid. **J Neurosurg 29**:464-474, 1968.
544 Shah AM, Spurgeon H, Sollot SJ, Talo A, Lakatta EG. 8-Bromo cGMP reduces the myofilament response to calcium in intact cardiac myocytes. **Circ Res 74**:970-978, 1994.
545 Shigeno T. Norepinephrine in cerebrospinal fluid patients with cerebral vasospasm. **J Neurosurg 56**:344-349, 1982.
546 Shigeno T, Fritschka E, Brock M, Schramm J, Shigeno S, Cervos-Navarro J: Cerebral oedema following experimental subarachnoid haemorrhage. **Stroke 13 (3)**:368-379, 1982.
547 Shigeno T, Mori K, Saito I, Sano K, BrockM. Early operation of ruptured cerebral aneurysms: the role of norepinephrine in subarachnoid haemorrhage and experimental vasospasm. In *Advances in Neurosurgery vol 9*, Schiefer M. Klinger M BrockM (eds) Berlin-Heidelberg-New York, Springer, 1981, pp189-96.
548 Shiguma M. Change in the ionic environment of cerebral arteries after subarachnoid haemorrhage. **Neurol Med Chir (Tokyo)22**:805-812, 1982.
549 Shiki K, Hearse DJ. Preconditioning of ischaemic myocardium-reperfusion-induced arrhythmias. **Am J Physiol 253**:H1470-1476, 1987.
550 Shimokawa H, Flavahan NA, Lorenz RR, Vanhutte PM. Prostacyclin releases endothelial derived relaxaing factor and potentiates its action in the coronary arteries of the pig. **Br J Pharmacol 95**:1197-1203, 1988.
551 Shirahase H, Fujiwara M, Usui H, Kurahashi K. A possible role of thromboxane A2 in endothelium in maintaining resting tone and producing contractile response to acetylcholine and arachidonic acid in canine cerebral arteries. **Blood Vessels 24**:117-119, 1987.
552 Shivalkar B, Van Loon J, Wieland W, Tjandra-Maga TB, Borgers M, Piets C, Flameng W. Variable effects of explosive or gradual increase of intracranial pressure on myocardial structure and function. **Circulation 87**:230-239, 1993.
553 Sivakumar V, Rajshekhar V, Chandy MJ. Management of neurosurgical patients with hyponatraemia and natriuresis. **Neurosurgery 34(2)**:269-273, 1994.
554 Smith RR, Clower BR, Grotendorst GM, Yabuno N, Cruse JM. Arterial wall changes in early human vasospasm. **Neurosurgery 16(2)**:171-176, 1985.
555 Smith RR, Clower BR, Honma Y, Cruse JM. The constrictive angiopathy of subarachnoid haemorrhage: an immunopathological approach. In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York, 1988, pp 247-252.
556 Smith RR, Miller JD. Pathophysiology and clinical evaluation of subarachnoid haemorrhage. In *Neurological Surgery 3rd Ed*, Youmans JR (ed), WB Saunders Co, Harcourt Brace Jovanovich, Inc, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo,1990 Vol 2, Ch 53, pp 1644-60.
557 Solokoff L. The action of drugs on the cerebral circulation. **Pharm Rev 11**:1-85, 1959.
558 Solomon RA, Antunes JL, Chen RYZ, Bland L, Chien S. Decrease in cerebral blood flow in rats after experimental subarachnoid haemorrhage: a new animal model. **Stroke 16**:1,1985.

559 Soloman RA, Post KD, Mc Murty JG. Depression of circulating blood volume in patients after subarachnoid haemorrhage. Implications for the management of symptomatic vasospasm. **Neurosurgery 15**:354-365, 1984.
560 Stehbens WE: The role of plasma elastase in elastin degeneration in the superficial temporal arteries and cerebral aneurysms. Letter to the Editor. **Neurosurgery** (Unpublished).
561 Steiner L, Lofgren J, ZwetnowNM. Characteristics and limits of tolerance in repeated subarachnoid haemorrhage in dogs. **Acta Neurol Scand 52**:241-267, 1975.
562 Stoner HB. A role for the central nervous system in the response to trauma. In *The scientific basis for the care of the critically ill*, Little RA and Frayn KN (eds) Biddles Ltd, Guildford and Kings Lynn, 1986 Ch 14 pp 215-229.
563 Stromberg DD, Fox JR. Pressures in the pial arterial microcirculation of the cat during changes in systemic arterial pressure. **Circ Res 31**:229-239, 1972.
564 Stryer L. In *Biochemistry, 2nd Ed*, W.H. Freeman and Co., San Fransisco,
565 Summerville LE, Hartsthorne DJ. Intracellular calcium and smooth muscle contraction. Cell calcium 7:353-364, 1986.
566 Sun II, Kanamura K, Ito M, Suzuki H, Kojima T, Waga S, Kureishi Y, Nakano T: Mysoin light chain phosphorylation and contractile proteins in a canine two-hemorrhage model of subarachnoid hemorrhage. **Stroke 29**:2149-2154, 1998.
567 Sunbarget G, Ponten U. Intracranial pressure and cerebrospinal fluid absorption impairment after subarachnoid haemorrhage. In *Intracranial pressure III* Becks JWF, Bosch DA, Brock M(eds), Springer Berlin, Heidelberg New York, 1976, pp 139-146.
568 Sundt TM Jr, Winkelmann RK. Humoral responses of smooth muscle from rabbit subarachnoid arteries compared to kidney, mesenteric, lung, heart and skin vasospasm. **Stroke 3**:717-725, 1972.
569 Sutter B, Suzuki S, Arthur AS, Kassell NF, Lee KS. Effects of subarachnoid haemorrhage on vascular responses to calcitonin gene-related peptide and its second messengers. **J Neurosurg 83(3)**:516-521, 1995.
570 Suzuki J, Onuma T, Yoshimoto T. Results or early operations on cerebral aneurysms. **Surg Neurol 11**:407-412, 1979.
571 Suzuki Y, McMaster D, Lederis K et al. Characterization of the relaxant effects of vasoactive intestinal peptide (VIP); and PHI on isolated brain arteries. **Brain Res 322**:9-16, 1984.
572 Suzuki Y, Satoh S, Lederis K, Rorstad O. Cardiovascular effects of peptide histidine methionine in canine and bovine species. **Pharmacolcogy 42(5)**:241-245 1991.
573 Suzuki S, Takenaka K, Kassell NF, Lee KS. Haemoglobin augmentation of interleukin-1β-induced production of nitric oxide in smooth-muscle cells. **J Neurosurg 81**:895-901, 1994.
574 Svengaad NA, Edvinsson L, Owman CH, Sahlin CH. Increased sensitivity of the basilar artery to norepinephrine and 5Hydroxytryptamine following experimental subarachnoid haemorrhage. **Surg Neurol 8**:191-195, 1977.
575 Svendgaard NA, Brismar J, Delgado TJ, Rosengren E. Subarachnoid haemorrhage in the rat: effect on the development of vasospasm of selective lesions of the catecholamine systems in the lower brain stem. **Stroke 16(4)**:602-608, 1985.

576 Symon L. An experimental study of traumatic cerebral vasospasm. **J Neurol Neurosurg Psy 30**:497-505, 1967

577 Symon L. Experimental features and therapeutic implications of "intracerebral steal". In *Research on the cerebral circulation*, Meyer JA, Reivich M, Lechner H, Eichorn O (Eds). Springfield, Ill, Charles C thomas, 1970, p 80-5.

578 Szabo C, Emilsson K, Hardebo JE, Nystedt S, Owman C. Uptake and release of serotonin in rat cerebrovascular nerves after subarachnoid haemorrhage. **Stroke 23(10)**:54-61, 1992.

579 Tagashira Y, Matsuda M, Welch KMA, Chabi E, Meyer JS. Effects of cyclic AMP and dibutyryl cyclic AMP on cerebral haemodynamics and metabolism in the baboon. **J Neurosurg 46**:484-493, 1977.

580 Takahashi I, Abumiya T, Imamura H, Saitoh H, Nomura M, Hokin K,Abe H. A case of posttraumatic delayed cerebral arterial spasm: case report and review of the literature on the pathogenesis. No Shink Geka. **Neurological surgery 20(2)**: 161-164, 1992.

581 Takayasu M, Dacey RG Jr. Spontaneous tone of cerebral parenchymal arterioles: a role in cerebral hyperemic phenomena. **J Neurosurg 71**:711-717, 1989.

582 Takenaka K, Kishino J, Yamada H, Sakai N, Arita H, Okano Y, Nozawa Y. DNA synthesis and intracellular calcium elevation in porcine cerebral arterial smooth muscle cells by cerebrospinal fluid from patients with subarachnoid haemorrhage. **Neurol res 14(4)**:330-334, 1992.

583 Talacchi A. Sequential measurements of cerebral blood flow in the acute phase of subarachnoid haemorrhage. **J Neurol Sci 37(1)**:9-18, 1993.

584 Tanabe Y, Sakata K, Yamada H, Ito T, Takada M. Cerebral vasospasm and ultrastructural changes in cerebral arterial wall. **J Neurosurg 49**:229-238, 1978.

585 Tanaka Y, Kassel NF, Machi T, Toshima M, Dougherty DA. Effect of bilirubin on rabbit cerebral arteries *in vivo* and *in vitro*. **Neurosurgery 30(2)**:195-201 1992

586 Tanaka Y, Kassell NF, Machi T, Toshima M, Dougherty DA. Effect of bilirubin on rabbit cerebral arteries *in vivo* and *in vitro*. **Neurosurgery 30(2)**:195-201, 1992.

587 Tani E, Yamagata S, Ito Y. Intercellular granules and vesicle in prolonged cerebral vasospasm. **JNeurosurg 48**:179-189, 1978.

588 Tanishima T. Cerebral vasopsasm: comparison of contractile responses in isolated human and canine basilar arteries. **Brain and nerve 35**:323-329, 1983.

589 Tatemichi TK, Mohr JP. In: *Stroke: pathophysiology, diagnosis, and management*, Barnet HJM, Mohr JP, Stein BM, Yatsu FM (Eds) Churchill Livingstone, New York, Edinburgh, London, Melbourne, Tokyo, 1992, pp 761-785.

590 Tenjin H, Hirakawa K, Mizukawa N, Yano I, Ohta T, UchiboriM, Hino A. Dysautoregulation in patients with ruptured aneurysms: CBF measurements obtained during surgery by a temp-controlled thermo-electric method. **Neurosurgery 23(6)**:705-709, 1988.

591 Thelen KI, Dembinska-Kiec A, Pallapics D, Simmet T, Peskar BA. Effect of 3-morphlinosydnonimine (SIN-1) and Ng-nitro-L-argining (NNA) in isolated perfused anaphylactic guinea pig hearts. **Nauny Scmiedebergs Arch Pharmcol 345(1)**:93-99, 1992.

592 Thomas D, Crockard A. In *Neurosurgery: the scientific basis of clinical practice*, Crockard A, Hayward R, Hoff JT, Blackwell scientific publications, Oxford, London, Edinburgh, Boston, Palo Alto, Melbourne, 1985, Ch 13: pp 223-308..

593 Thomas VH, Hopkins IJ. Arteriographic demonstration of vascular lesions in the study of neurological deficit in advanced Haemohpilus influenzae meningitis. **Develop Med Child Neurol 14**:783-787, 1972.

594 Thornton JD, Daly JF, Cohen MV, Yang XM, Downey JM. Catecholamines can induce adenosine receptor-mediated protection of the myocardium but do not participate in ischaemic preconditioning in the rabbit. **Circ Res 73**:649-655, 1993.

595 Tindall GG, Greenfield JC. The effects of intra arterial histamine on blood flow in the internal and external carotid artery of man. **Stroke 4**:46-49, 1973.

596 Toda N, Fujita Y. Responsiveness of isolated cerebral and peripheral arteries to serotonin, NE, and transmural electrical stimulation. **Cir Res 33**:98-104, 1973.

597 Toda N. The action of vasodilatory drugs on isolated basilar, coronary and mesenteries arteries of the dog. **J Pharm Exp Ther 191**:139-146, 1974.

598 Toda N, Ozaki T, Ohta T. Cerebrovascular sensitivity to vasoconstricting agents induced by subarachnoid haemorrhage and vasospasms in dogs. **J Neurosurg 46**: 296-303, 1977.

599 Toda N, Ozaki T, Ohta T. Cerebrovascular sensitivity to vasoconstricting agents induced by subarachnoid haemorrhage and vasospasm in dogs. **J Neurosurg 46**: 296-303, 1977.

600 Toda N. Alpha adrenergic receptor subtypes in human, monkey and dog cerebral arteries. **J Pharmacol Exp Ther 226**:861-868, 1983.

601 Toda N. Mechanisms of contracting action of oxyhaemoglobin in isolated monkey and dog cerebral arteries. **AM J Physiol 258**:H57-63, 1990

602 Todd GL, Baroldi G, Pieper GM, Clayton FC, Eliot RS. Experimental catecholamine induced myocardial necrosis. I Morphological, quantification and regional distribution of acute contraction band lesions. **J Mol Cell Cardiol 7**:317-338,1985.

603 Torner JC, Kassell NF, Wallace RB, Adams HP Jr. Pre-operative prognostic factors for rebleeding and survival in aneurysm patients receiving antifibrinolytic therapy: report of the cooperative aneurysm study. **Neurosurgery 9**:506-513, 1981.

604 Tsuji T, Cook DA. Mechanism of vascular responses to 5HT in isolated and perfused canine basilar arteries during the early stage of experimental subarachnoid haemorrhage. In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York, 1988, pp 105- 111.

605 Tsuji T, Cook DA, Weir BKA, Handa Y: Effect of clot removal on cerebrovascular contraction after subarachnoid hemorrhage in the monkey: pharmacological study. **Heart & Vessels 11**:69-79, 1996.

606 Tsujino M, Hirata Y, Imai T, Kanno K, Eguchi S, Ito H, Marumo F. Induction of NOS gene by Il-1β in cultured rat cardiocytes. **Circulation 90**:375-383, 1994.

607 Tsukahara T, Taniguchi T, Fujiwara M, Handa H, Nishikawa M. Alterations in Alpha adrenergic receptors in human cerebral arteries after subarachnoid haemorrhage. **Stroke 16(1)**:53-58, 1985.

608 Tsukahara T, Yoshimura S, Kazekawa K, Hashimoto N. Intra-arterial papaverine for the treatment of cerebral vasospasm after subarachnoid haemorrhage. **J Auton Nerv Sys 49(suppl s)**:163-166, 1994.
609 Tsutsui T, Maekawa T, Goodchild C, Jones JG. Cerebral blood flow distribution during hypotension with haemorrhage, trimethaphan or nitroprusside in rats. **Br J Anaesth 74(6)**:686-690, 1995.
610 Udenfriend S, Weissbach H. Studies in serotonin (5HT) in platelets. **Fed Proc 13**:412-413, 1954.
611 Ueno M, Lee TJ-F. Endotoxin decreases the contractile responses of the porcine basilar artery to vasoactive substances. **J Cerebr Blood Flow & Metab 13**:712-719, 1993.
612 Urabe K, Miura TM, Iwamoto TI, Ogawa T, Goto M, Sakamoto J, Limura O. Preconditioning enhances myocardial resistance to post-ischaemic myocardial stunning via adenosine receptor activation. **Cardiovasc Res 27**:657-662, 1993.
613 Urquilla PR, Marco EJ, Lluch S. Pharmacological receptors of the cerebral arteries of the goat. **Blood Vessels 12**:53-67, 1975.
614 Urquilla PR. Prolonged contraction of isolated human and canine cerebral arteries induced by UTP. **Stroke 9**:133-136, 1978.
615 Ursino M. Regulation of the circulation of the brain, in: *The human brain circulation: functional changes in disease*, Bevan RD, Bevan JA (Eds), Humana press, Totowa, New Jersey, 1994, pp 291-318.
616 Usui H, Kurahashi K, Shirahase H, Fukui K, Fujiwara M. Endothelial dependent vasoconstriction in response to NE in the canine cerebral artery. **Jpn J Pharmacol 44**:228-231, 1987.
617 van Gelderen EM, De Bruijne EL, Agteresch HJ, Saxena PR. The effect of NO donors on haemodynamics and blood flow distribution in the porcine carotid circulation. **Br J Pharm 114(6)**:1303-1309, 1995
618 Vanhoutte PM, Rubanyi GM, Miller VM et al . Modulation of vascular smooth muscle contraction by the endothelium. **Ann Rev Physiol 48**:307-320, 1986.
619 Vanhoutte PM, Houston DS. Platelets, endothelium and vasospasm. **Circulation 72**:728-734,1985.
620 Van Riper DA. Norepinephrine and neuropeptide Y as neurotransmitters to cerebral arteries. In: *The human brain circulation: functional changes in disease*, Bevan RD, Bevan JA (Eds), Humana press, Totowa, New Jersey, 1994, pp 61-72.
621 Van Vliet PD, Burchell HB, Titus JL. Focal myocarditis associated with phaeochromocytoma. **N Eng J Med 274**:1102-1108, 1966.
622 Vardiman AB, Kopitnik TA, Purdy PD, Batjer HH, Samson DS. Treatment of traumatic arterial vasospasm with intra-arterial papaverine infusion. **AM J Neuroradiology. 16(2)**:319-321, 1995
623 Varsos VG, Liszczak TM, Han DH, et al. Delayed cerebral vasospasm is not reversible by aminophylline nifedipine or papaverine in a "two haemorrhage" canine model. **J Neurosurg 58**:11-17, 1983.
624 Vatner DE, Vatner SF, Nejima J, Uemura N, Susanni EE, Hintze TH, Homcy CJ. Chronic NE elicits desensitization by uncoupling the β receptor. **J Clin Invest 84**:1741-1748, 1989.
625 Veelken JA, Laing RJ, Jakubowski J. The Sheffield Model of subarachnoid haemorrhage in rats. **Stroke 26(7)**:1279-1283, 1995.

626 Verstraete M. New thrombolytic drugs in acute myocardial infarction: theoretical and practical considerations. **Circulation 76(suppl II)**:31-38, 1987
627 Vicaut E. Hypertension and the microcirculation: a brief overview of experimental studies. **J Hypertension 10(S5)**:S59-S68, 1992.
628 Villamor E, Perez-Vizcaino F, Ruiz T, Leza JC, Moro M, Tamargo J. Group B streptococcus and E. Coli lipopolysaccharide-induced NO-dependent hyporesponsiveness to norepinephrine in isolated intrapulmonary arteries of neonatal piglets. **Br J Pharm 115**:261-266, 1995.
629 Vinall PE, Simeone FA. A pressurized cannulated *in vitro* model for the study of cerebral arterial mechanics. In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York, 1988, pp 449-445.
630 Vincent FM. Hypoglycorrhachia after subarachnoid haemorrhage. **Neurosurgery 8**:7-9, 1981.
631 Viquerat CE, Daly P, Swedberg K, Evers C, Curran D, Parmely WW, Chaterjee K. Endogenous catecholamine levels in chronic heart failure Relation to the severity of haemodynamic abnormalities. **Am J Med 78**:455-460, 1985.
632 Voldby B. Pathophysiology of subarachnoid haemorrhage. Experimental and Clinical data. **Acta Neurochir suppl 45**:1-6, 1988.
633 Vollrath BAM, Weir BKA, MacDonald RL, Cook DA. Intracellular mechanisms involved in the responses of cerebrovascular smooth-muscle cells to haemoglobin. **J Neurosurg 80**:261-268, 1994.
634 Vollmer DG, Jane S, Torner JC, Kassell NF. Intracisternal papavevrine reverses experimental cerebral vasospasm in a rabbit model. In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York, 1988, pp425-431.
635 Vollmer DG, Takayasu M, Dacey RG Jr. An *in vitro* comparative study of conducting vessels and penetrating arterioles after experimental subarachnoid haemorrhage in the rabbit. **J Neurosurg 77(1)**:113-119, 1992.
636 von Bergmann E. In *Die Lehre von de Kopfuerletzungen* 1880. F. Erike Stuttgart.
637 Vorkapic P, Bevan JA, Bevan RD: Clentiazem protects against chronic cerebral vasospasm in rabbit basilar artery. **Stroke 22:**1409-1413, 1991.
638 Vorkapic P, Bevan JA, Bevan RD. Two indicies of functional damage of the artery wall parallell the time course of irreversible narrowing in experimental vasospasm in the rabbit. **Blood Vessels 28(1-3)**:179-182, 1991
639 Walmsley JG, Campling MR, Chertkow HM. Interrelationships among wall structure, smooth muscle orientation, and contraction in human major cerebral arteries. **Stroke 14(5)**:781-790, 1983.
640 Wang J, Ohta S, Sasaki S, Araki N, Matsuda S, Sakanaka M. Changes in Ca^{++}-ATPase activity in smooth-muscle cell membranes of the canine basilar artery with experimental subarachnoid haemorrhage. **J Neurosurg 80**:269-275, 1994.
641 Waters A, Harder DR. Altered membrane properties of cerebral vascular smooth muscle following subarachnoid haemorrhage: an electrophysiological study. I Changes in resting membrane potential (Em) and effect on the electrogenic pump potential contribution to Em. **Stroke 16(6)**:990-997, 1985.
642 Waters VE, O'Connor PJ. Prevalence of migraine. **J Neurol Neurosurg Psych 38**:613, 1975.
643 Watters JW. The effects of bradykinin and histamine on cerebral arteries of monkeys. **Radiology 98**:299-304, 1971

644 Weaver DD, Winn HR, Jane JA. Differential intracranial pressure in patients with unilateral mass lesions. **J Neurosurg 56**:660-665, 1982.
645 Weinberg JB, Hibbs JB Jr. Endocytosis of red cells or haemoglobin by activated macrophages inhibited their tumoricidal effect. **Nature (Lond) 269**:245-247, 1977.
646 Weinand ME, O'Boynick PL, Goetz KL. A study of serum anti-diuretic hormone and atrial natriuretic peptide levels in a series of patients with intracranial disease and hyponatraemia. **Neurosurgery 25(5)**:781-785, 1989.
647 Weir B, Grace M, Hansen J et al. Time course of vasospasm in man. **J Neurosurg 48**:173-8, 1978.
648 Weisensee D, Berciter-Hahn J, Schoeppe W, Low-Friedrich I. Effects of cytokines on the contractility of cultured cardiac myocytes. **Int J Immunopharmacol 51**:581-587, 1993.
649 Weissbach H, Bogdanski DF, Undenfriend S. Binding of serotonin and other amines by blood platelets. **Arch Biochem Biophys 73**:492-499, 1958.
650 Wellum GR, Irvine TW, Zervas NT. Dose responses of cerebral arteries of the dog, rabbit and man to human haemoglobin *in vitro*. **J Neurosurg 53**:486-490, 1980.
651 West CGH: Intra-operative brain swelling: letter to the Editor. **Br J Neurosurg 4(4)**:358, 1990.
652 White RP, Hagen AA, Morgan H et al. Experimental study on the genesis of cerebral vasospasm. **Stroke 6**:52-57, 1975.
653 White RP. Vasospasm II. Clinical considerations. In *Intracranial aneurysms*, Fox JL (ed) New York, Springer-Verlag, 1983, pp250-271.
654 White RP, MacLeod RM, Muhlbauer MS. Evaluation of the role haemoglobin in cerebrospinal fluid plays in producing contraction of cerebral arteries. **Surg Neurol 27**:237-242, 1987.
655 White RP. Pharmacodynamic features of delayed cerebral vasospasm. In *Cerebral Vasospasm*, Wilkins RH (ed), Raven press, New York, pp373-382, 1988.
656 White M, Wiechmann RJ, Roden RL, Hagan MB, Wollmering MM, Port JD, Hammond E, Abraham WT, Wolfel EE, Lindenfield J, Fullerton D, Bristow MR. Cardiac β adrenergic neuroeffector systems in acute myocardial dysfunction related to brain injury. Evidence for catecholamine mediated myocardial damage. **Circulation 92**:2183-2189, 1995.
657 Wiebers DO: Unruptured intracranial aneurysms – risk of rupture and risks of surgical intervention. The international study of unruptured intracranial aneurysms investigators. **NEJM 339(24)**:1725-33, 1998.
658 Wilkins RH, Levitt P. Potassium and the pathogenesis of cerebral arterial spasm in dog and man. **J Neurosurg 35**:45-50, 1971.
659 Wilkinson HA. In *Neurological Surgery 3rd Ed*, Youmans JR (ed) 1990, WB Saunders Co, Harcourt Brace Jovanovich, Inc, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo Vol 2 Ch 23 pp 661-695, 1990.
660 Wilkonson HA, Wilson RB, Patel PP et al. Corticosteroid therapy of experimental hydrocephalus after intraventricular subarachnoid haemorrhage. J Neurol Neurosurg **Psychiatry 37**:224-229, 1974.

661 Willette RN, Zhang H, Mitchell MP: Nonpeptide endothelin antagonist. Cerebrovascular characterization and effects on delayed cerebral vasospasm. **Stroke 25**:2450-6, 1994.
662 Willette RN, Shiloh AO, Sauermelch CF: Identification, characterization, and functional role of phosphodiesterase type IV in cerebral vessels: effects of selective phosphodiesterase inhibitors. **J Cereb Blood Flow & Metab** 17:210-219, 1997.
663 Witzleb E; In Schmidt RF, Thews G (eds). In *Human Physiology 20th Ed.* Ch 18 p 404, 1984.
664 Wolfe BB, Molinoff PB. In *Catecholamines vol I,* Handbook of experimental pharmacology, Springer-Verlag, Berlin, Heidelberg, New York, 90 , pp 376-392,1989.
665 Woolf PD, Hamill RW, Lee LA, Cox C, MacDonald JV. The predictive value of catecholamines in assessing outcome in traumatic brain injury. **J Neurosurg 66**:872-882, 1987.
666 Wright IK, Armichetty-Rao S, Kendall DA. Forskolin potentiates both SNP and ANP-stimulated cGMP accumulation in porcine isolated palmar lateral veins. **Br J Pharm 112**:542P, 1994.
667 Yakubu MA, Shibata M, Leffler CW. Subarachnoid haematoma attenuates vasodilation and potentiates vasoconstriction induced by vasoactive agents in new-born pigs. **Paediatric research 36(5)**:589-594, 1994.
668 Yamamoto Y, Bernanke DH, Smith RR. Accelerated non-muscle contraction after subarachnoid haemorrhage: cerebrospinal fluid testing in a culture model. **Neurosurgery 27(6)**:921-928, 1990
669 Yamashima T, Yamamoto S. Cerebral arterial pathology in experimental subarachnoid haemorrhage. **J Neurosurg 58**:843-850, 1983.
670 Yamashima T, Kashihara K, Kawano H et al. Myonecrosis following cerebral arterial spasm in meningitis: Case report. **Neurol Med Chir (Tokyo) 25**:818-825, 1985.
671 Yanigasawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yatsaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. **Nature 332**:411-415, 1988.
672 Yao S-K, Akhtar S, Scott-Burden T, Ober JC, Golino P, Buja M, Casscelles W, Willesron JT. Endogenous and Exogenous NO protect against intracoronary thrombosis and reocclusion after thrombolysis. **Circulation 92**:1005-1010, 1995.
673 Yoshimoto H, Uozumi T. Anterior pituitary dysfunction in cerebrovascular disease. Ruptured cerebral aneurysm cases. **Neurol Med Chir (Tokyo)**:25:433-439, 1985.
674 Yoshimoto Y, Kim P, Sasaki T, Kirino T, Takakura K: Functional changes in cultured strips of canine cerebral arteries after prolonged exposure to oxyhemoglobin. J Neurosurg 83:867-74, 1995.
675 Zabramski JM, Spetzler RF, Bonstelle C. Chronic cerebral vasospasm: effect of volume and timing of haemorrhage in a canine model. **Neurosurgery 18(1)**:1-6, 1986.
676 Zervas NT, Liszczak TM, Mayberg MR et al. Cerebrospinal fluid may nourish cerebral vessels through pathways in the adventitia that may be analogous to systemic vasa vasorum. **J Neurosurg 56**:475-481, 1982.

677 Zhang F, White JG, Iadecola C. NO donors increase blood flow and reduce brain damage in focal ischaemia: evidence that NO is beneficial in the early stages of cerebral ischaemia. **J Cerebr Blood Flow & Metab 14**:217-226, 1994

678 Ziche M, Morbidelli L, Parenti S, Amorini HJ, Granger and Ledda P. Nitric oxide modulates substance P-induced mobilization of coronary capillary endothelial cells. **Br J Pharm 111**:103P, 1994.

679 Zweifach BW. In *The scientific basis for the care of the critically ill*, Little RA, Frayn KN (Eds), Biddles Ltd, Guildford and King's Lynn, 1986, Ch 3, pp 15-32.

680 Zwetnow NN. Effects of increased intracranial pressure on the blood flow and energy metabolism of the brain. **Acta physiol Scand 79(suppl 339)**:1-31, 1970

681 Zuccarello M, Bonasso CL, Sperelakis N, Rappaport RM. Role of membrane potential in cerebral vasospasm after subarachnoid haemorrhage. In *Cerebral vasospasm*, Findlay JM (ed), Amstredam: Elsevier 1993, pp 229-233.

682 Zuccarello M, Romano A, Passalacqua M, Rapoport RM: Decreased endothelium-dependent relaxation in subarachnoid hemorrhage-induced vasospasm: role of ET-1. **Am J Physiol 269(Heart circulation 38)**:H1009-H1015, 1995.

PUBLICATIONS FROM THIS THESIS

Marshman LAG: Cushing's variant response (acute hypotension) following subarachnoid haemorrhage. Association with moderate intracranial tensions and subacute cardiovascular collapse in rats. **Stroke 28(7):**1445-1450, 1997.

Marshman LAG, Thompson JS, Morice AH: Increased efficacy of Sodium nitroprusside in middle cerebral arteries following acute subarachnoid haemorrhage. **J Neurosurg Anaesth 10(3):** 171-177, 1998.

Marshman LAG: Preserved Contractility without Side Bias in Endovascular Filament Models for Subarachnoid Haemorrhage. **J Neurosci Meth** 117:193-200, 2002