AN INVESTIGATION OF THE NATURE OF THE NEURAL AND LOCAL MECHANISMS THAT REGULATE VASCULAR FUNCTIONING

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by

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SUMMARY

1. In the pithed rat, stimulation of the autonomic outflow at different levels between C_1 and L_3 produced characteristic increases in blood pressure and heart rate. Stimulation between C_6 and T_1 and between T_2 and T_4 produced large increases in heart rate with only small increases in blood pressure. Stimulation at T_{8-9} produced the largest increase in blood pressure and a large increase in heart rate.

2. Spinal nerve stimulation at T_{8-9} characteristically produced pressor responses during stimulation and a secondary, pressor response that persisted after stimulation ceased. These responses were frequency-dependent so that the response at 20 Hz (supramaximal voltage, 0.4 ms pulse-width, 100 pulses) was larger than that at 2 Hz (supramaximal voltage, 0.4 ms pulse-width, 100 pulses).

3. The pressor response to stimulation at T_{8-9} to 2 Hz and 20 Hz probably resulted from stimulation of nerve fibres to blood vessels and also to the adrenal glands, which contributed both to the component of the pressor response that occurred during stimulation and to the secondary component that persisted after stimulation ceased. This was indicated by experiments in which adrenalectomy reduced the first component of the pressor response that occurred during stimulation at T_{8-9} and abolished the second component that persisted after stimulation.

4. The effect of propranolol (2 mg kg^{-1}) on both components of the pressor response to spinal nerve stimulation at T_{8-9} was complex and dependent on the basal blood pressure of the pithed rat. In most pithed rats, propranolol enhanced both components of the pressor response to nerve stimulation.

This was most marked at 2 Hz and most obvious in the secondary post-stimulation component, which probably resulted from adrenaline released from the adrenals.

5. In some experiments where the blood pressure was low (<18-22 mm Hg) propranolol did not enhance the pressor response to spinal nerve stimulation. This may have been because in these few rats propranolol impaired cardiac output and as a result the ability of the heart to pump blood through the constricted blood vessels diminished, so that despite nerve stimulation-induced peripheral vasoconstriction, blood pressure did not increase.

6. α,β-methylene ATP (α,β-mATP,2x0.05 and 5x0.1 mg kg^{-1}) produced pressor responses that showed tachyphylaxis. There was no evidence that α,β-mATP desensitized the pressor response to spinal nerve stimulation at T_{8-9}.

7. Captopril inhibited pressor responses to nerve stimulation and to exogenous NA (9 μg kg^{-1}). This result suggests that angiotensin contributes to the pressor response to spinal nerve stimulation. An alternative explanation of this effect is that bradykinin breakdown might be inhibited by captopril so that more nitric oxide (NO), released by bradykinin, might be available to act as a physiological antagonist of NA.

8. There was no evidence of a prazosin-resistant pressor response to spinal nerve stimulation at T_{8-9} in the pithed rat.

9. L-NAME (45 mg kg^{-1}) increased the basal blood pressure in the anaesthetized rat and also, but to a lesser extent, in the pithed rat in the

presence of propranolol (2 mg kg^{-1}). There was little difference in the extent to which L-NAME increased the basal blood pressure in the anaesthetized and the pithed rats in the absence of propranolol. In pithed rats but not in anaesthetised rats, the increment in the basal blood pressure caused by L-NAME was proportional to the level of the basal blood pressure. It appears that endogenous NO was continuously produced in both anaesthetized and pithed rats and had a role in keeping the basal blood pressure low. The greater increase in the basal blood pressure produced by L-NAME (45 mg kg^{-1}) in anaesthetized rats was probably due to the withdrawal of the inhibitory effect of NO on the sympathetic tone, which was thereafter able to operate unopposed and raise the blood pressure. Such a mechanism would not occur in the pithed rat.

10. In most experiments L-NAME did not affect pressor responses to spinal nerve stimulation at T_{8-9}. This lack of effect of L-NAME on pressor responses to spinal nerve stimulation might be due to the fact that all the experiments were performed in the presence of propranolol (2 mg kg^{-1}), unlike those of MacLean et al. (1994), who showed that L-NAME increased both components but especially the second component of the pressor response to the spinal nerve stimulation. It may be that pressor responses to spinal nerve stimulation can be enhanced either by propranolol or L-NAME but not by both simultaneously, suggesting that activation of ß-adrenoceptors may be linked eventually to activation of NO synthase so that blockade of one step (eg. the ß-adrenoceptor) in this pathway renders subsequent inhibition of a later step (eg. NO synthase) irrelevant. An alternative explanation of the lack of effect of L-NAME on the pressor responses to spinal nerve stimulation in the presence of propranolol is that blockade of $ß_1$-adrenoceptors in the heart may have reduced cardiac output so that the ability of the heart to maintain an elevated blood pressure was

impaired. Despite this, nerve stimulation-induced increases in blood pressure were enhanced by propranolol, probably as a result of blocking vascular $ß_2$-adrenoceptors. However, further vasoconstriction due to inhibition of the synthesis of NO might not be reflected in a further increase in blood pressure if L-NAME-induced vasoconstriction caused increased afterload on the heart and further diminished cardiac output. Another factor to be considered is that when the blood pressure is low, perhaps because of a propranolol-induced reduction in cardiac output, shear stress-induced production of NO will also be low and so the opportunity for L-NAME to enhance basal blood pressure is reduced. In some experiments, L-NAME apparently did enhance the pressor response to spinal nerve stimulation but this was only after exogenous NA (2 mg kg^{-1}) had inhibited this response. In this experiment, the apparent ability of L-NAME to potentiate the nerve-mediated pressor response may merely have been due to recovery from the inhibitory effect of NA.

11. L-NAME (45 mg kg^{-1}) increased the toxicity of α,ß-methylene ATP (0.05 mg kg^{-1}) in pithed rats.

12. Intravenous bolus injections of NA (0.09, 2 and 15 μg kg^{-1}) produced dose-related pressor responses that were unaffected by L-NAME(45 $mgkg^{-1}$) in the propranolol (2 mg kg^{-1})-treated pithed rats.

13. Intravenous bolus injections of 5-HT (6, 12, 25 and 50 μg kg^{-1}) produced dose-related pressor responses in the presence of propranolol (2 mg kg^{-1}) in pithed rats. In some rats, 5-HT produced a slight secondary vasodepressor response, which was most marked in pithed rats that had higher blood pressures (50-60 mm Hg^{-1}). In propranolol-treated anaesthetised rats, 5-HT produced a triphasic response in blood pressure,

which consisted of an initial brief depressor component, an intermediate pressor component followed by a final, prolonged depressor component. L-NAME (45 mg kg^{-1}) enhanced and prolonged the pressor responses to 5-HT (6, 12 and 25 μg kg^{-1}) in the pithed rat and in the anaesthetised rat L-NAME also enhanced and prolonged the secondary pressor component to 5-HT and reduced but did not abolish the depressor components to 5-HT.

14. In the pithed rat, a single dose of 5-HT (6 μg kg^{-1}) inhibited both components of the pressor response to spinal nerve stimulation at 2 Hz and 20 Hz. This effect occurred in the presence of L-NAME (45 mg kg^{-1}) and propranolol (2 mg kg^{-1}).

15. The ability of L-NAME (45 mg kg^{-1}) to prolong responses to 5-HT may have arisen because of diminished inactivation of 5-HT in the pulmonary circulation, where L-NAME has profound effects.

16. These results indicate that the pressor response to pre-ganglionic nerve stimulation in the pithed rat at T_{8-9} is very complex. They also identify some of the problems likely to be encountered in studying complex phenomena such as co-transmission at neuro-effector junctions in the vasculature of the pithed rat.

17. Rat isolated lungs were perfused through the pulmonary artery with Krebs-buffer (35 ^{o}C) at a rate of 5 ml min^{-1}.

18. The responsiveness of the pulmonary circulation to vasoactive agents was investigated by administering each drug in the Krebs-buffer and perfusing it until an equilibrium response was obtained. In some experiments when an equilibrium response was obtained, drug administration was

discontinued and the preparation allowed to recover before another concentration of the drug was administered. In contrast to these "single concentration" experiments, in other studies the drugs were administered cumulatively, with the response to each concentration being allowed to reach equilibrium before the next higher concentration was administered.

19. In the perfused pulmonary circulation the drugs examined included the following vasopressor agents: adrenaline (Adr), 5-hydroxytryptamine (5-HT), noradrenaline (NA), phenylephrine (PE) and potassium chloride (KCl). The vasorelaxants included, isoprenaline (Isop) and sodium nitroprusside (SNP).

20. Adrenaline (0.1-100 μM), NA (0.1-100 μM) and PE (0.1-100 μM), all produced small concentration-dependent pressor responses in the perfused lungs. Responses to 5-HT (0.1-1000 μM) were especially small. In contrast, KCl (3-80 mM) produced large well-maintained pressor responses.

21. Pressor responses to 5-HT (0.1-1000 μM), PE (0.01-10 μM) and KCl (30 mM) were enhanced by L-NAME (400 μM).

22. Pressor responses to NA (0.1-100 μM) were enhanced by propranolol (10 μM) but were unaffected by flurbiprofen (2 μM).

23. Pressor responses to PE (0.01-10 μM) were enhanced by L-NAME (400 μM). The addition of propranolol (10 μM) produced no further enhancement.

24. The responses to PE (0.01-100 μM) and to 5-HT (0.1-100 μM) in "single concentration" experiments, where each concentration was perfused until an equilibrium response was obtained, after which administration was discontinued and the preparation allowed to recover, were much larger than

those obtained in experiments where cumulative concentration-response curves were obtained.

25. 5-HT (0.1-100 μM) administered cumulatively produced very small pressor responses even at the high concentration (100 μM). The addition of L-NAME (400 μM) produced a slight increase in the basal perfusion pressure and also increased the size and rate of development of the pressor responses to 5-HT. Responses to high concentrations were enhanced to a greater extent than those to lower concentrations. These results suggest that there is a basal release of NO in the pulmonary circulation and that 5-HT may also release NO which then acts as its physiological antagonist.

26. In the presence of L-NAME, pressor responses to 5-HT (10-100 μM) were enhanced by prior administration of a submaximal concentration of KCl (40 mM).

27. Flurbiprofen (2 μM) produced a small increase in the basal perfusion pressure and potentiated the presssor response to 5-HT (20 μM) in the presence of L-NAME (400 μM), suggesting that endogenous prostaglandins (perhaps prostacyclin, PGI_2) may act as a physiological antagonist of 5-HT.

28. Ketanserin (1 nM) a selective 5-HT_2-receptor antagonist had little or no effect on the basal perfusion pressure but inhibited pressor responses to increasing doses of 5-HT in the presence of L-NAME (400 μM).

29. Isoprenaline (0.01-10 μM) administered cumulatively produced a concentration-dependent vasorelaxation in perfused pulmonary vasculature that had been constricted with either a submaximal concentration of 5-HT (50 μM) or PE (100 μM). Since the vasorelaxant effect of isoprenaline was

unaffected by L-NAME, it appears that this inhibitory action of isoprenaline does not involve the synthesis of NO.

30. Isoprenaline (0.1-100 μM) was less effective in relaxing the pulmonary vasculature when it had been constricted with KCl (50 mM) than when it had been constricted with 5-HT (50 μM) or PE (100 μM).

31. SNP (0.1 nM-100 μM) produced small concentration-dependent vasodepressor responses in pulmonary vasculature that had been constricted with PE (50 μM) or KCl (40 mM). L-NAME (400 μM) potentiated the vasodepressor responses to SNP (0.1 nM-100 μM) in preparations in which the vasculature had been constricted with PE (50 μM) or KCl (40 mM).

32. These results indicate that endogenous NO has an important role in the pulmonary circulation in attenuating pressor responses to vasoconstrictors and perhaps also in modifying the responses to vasodilators such as SNP.

33. The effects of vasoactive agents were investigated in the vasculature of the rat hindquarters perfused with Krebs-buffer (35^{o}C, 5 ml min^{-1}).

34. The vasopressor agents; 5-HT, PE and KCl and the vasodepressor agents carbachol and SNP were perfused in Krebs-buffer and cumulative dose-response curves were obtained for each drug.

35. Pressor responses to 5-HT (0.01-100 μM) were apparently potentiated by L-NAME (400 μM), but when comparison was made with appropriate time-controls, which were not exposed to L-NAME, 5-HT induced pressor responses were found to be unaffected by L-NAME.

36. Pressor responses to 5-HT (0.01-100 μM) were also unaffected by indomethacin (10 μM).

37. SNP (0.001-100 μM) produced dose-related inhibition of submaximal pressor responses to 5-HT (1 μM), PE (10 μM) and KCl (60 mM).

38. SNP (0.001-100 μM) were more effective in inhibiting a standard submaximal pressor response to PE ($EC_{70} = 10$ μM) in rat hindquarters from Wistar rats than in hindquarters from Spontaneously hypertensive rats (SHR) or the Wistar Kyoto controls (WKY).

39. Carbachol (0.001-100 μM) produced complex effects when administered in combination with PE (10 μM) which was used to raise the tone in the perfused hindquarters. At low doses carbachol increased the perfusion pressure but at higher doses carbachol inhibited the tone in a dose-dependent manner.

40. During experiments in which dose-response curves to PE, 5-HT or KCl were repeated in the same hindquarters preparation, the baseline tone rose during the experiments so that there was a tendency for the maximum response to be increased in the second dose-response curve.

Abbreviations Used in the Text and Figures

AC	Adenylate cyclase
ACE	Angiotensin converting enzyme
ACh	Acetylcholine
ADP	Adenosine diphosphate
Adr	Adrenaline
AI	Angiotensin I
AII	Angiotensin II
AIII	Angiotensin III
AMP	Adenosine monophosphate
5'AMP	5'Adenosine monophosphate
ANAPP3	Arylazido amino propionyl ATP
ANS	Autonomic nervous system
AP	Action potential
APUD	Amino-precursor-uptake-decarboxylation
ATP	Adenosine triphosphate
ATPase	ATPase enzyme
AV-node	Atrio-ventricular node
AV-shunts	Arterio-venous shunts
α	Alpha-adrenoceptor
α,ß-mATP	Alpha,beta-methylene adenosine triphosphate
B_2	Bradykinin$_2$-receptor
ß	Beta-adrenoceptor
bBP	Basal blood pressure
Bk	Bradykinin
BP	Blood pressure

bPP	Basal perfusion pressure
C	Captopril
C_{1-6}	First to sixth cervical vertebrae
Ca^{2+}	Calcium ion
$[Ca^{2+}]_i$	Intracellular calcium concentration
$CaCl_2$	Calcium chloride
Carb	Carbachol
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CGRP	Calcitonin gene related peptide
CCK	Cholecystokinin
CNS	Central nervous system
CO_2	Carbon dioxide
DAG	Diacyl glycerol
DNA	Deoxyribonucleic acid
EC_{70}	Effective concentration (70% of the population data)
EDCF	Endothelium derived contracting factor
EDV	End diastolic volume
Flur	Flurbiprofen
5'GMP	5'Guanosine monophosphate
EDRF	Endothelium derived relaxing factor
GABA	γ amino butyric acid
GC	Guanylate cyclase
G_i	Inhibitory G protein
GIT	Gastrointestinal tract
G_p	G protein
G_s	Stimulatory G protein
GTP	Guanosine triphosphate

Gu	Guanethidine
H_1	H_1-receptor (Histamine)
H_2	H_2-receptor (Histamine)
Hb	Haemoglobin
HbO_2	Oxyhaemoglobin
5-HIAA	5-hydroxyindoleacetic acid
HR	Heart rate
5-HT	5-Hydroxytryptamine
Hz	Hertz
IP_3	Inositol trisphosphate
ip	intraperitoneal
Isop	Isoprenaline
i.u. kg^{-1}	International unit per kilogram
iv	Intravenous
K^+	Potassium ion
KCl	Potassium Chloride
KH_2PO_4	Potassium dihydrogen ortophosphate
L_{1-3}	First to third lumbar vertebrae
L-arg	L-arginine
LHRH	Luteinising hormone releasing hormone
L-NAME	L-N^W-Nitro-Arginine Methyl Ester
LSD	Lysergic acid diethylamide
M	Muscarinic receptor
MetHb	Methaemoglobin
$MgSO_4.7H_2O$	Hydrated magnesium sulphate
MLCK	Myosin light-chain kinase
n	Number of observations
N_2	Nitrogen gas
Na^+	Sodium ion

NA	Noradrenaline
NaCl	Sodium chloride
$NaHCO_3$	Sodium hydrogen carbonate
NANC	Non-adrenergic non-cholinergic nerves
NO	Nitric oxide
NPY	Neuropeptide Y
NS	Nerve stimulation of spinal sympathetic outflow
O_2	Oxygen gas
O_2^-	Superoxide anion
P	Probability
P_1	P_1-purinoceptor
P_2	P_2-purinoceptor
P_{2t}	P_{2t}-purinoceptor
P_{2x}	P_{2x}-purinoceptor
P_{2y}	P_{2y}-purinoceptor
P_{2z}	P_{2z}-purinoceptor
PA	Pulmonary artery
P_{CO_2}	Partial pressure of CO_2
PDE	Phosphodiesterase
PDGF	Platelet derived growth factor
PE	Phenylephrine
PGE_2	Prostagladin E_2
$PGF_{2\alpha}$	Prostagladin $F_{2\alpha}$
PGI_2	Prostacyclin
PGs	Prostaglandins
PI-turnover	Phosphatidyl inositol hydrolysis
PKC	Protein kinase C
Pl	Pulses

PLA_2	Phospholipase A_2
PLC	Phospholipase C
P_{O_2}	Partial pressure of O_2
Pr	Prazosin
Prop	Propranolol
Ptd Ins (4,5) P_2	Phosphatidyl inositol (4,5) bisphosphate
R	Correlation coefficient
S_1	Electrical stimulation of the spinal sympathetic outflow at 2 Hz
S_2	Electrical stimulation of the spinal sympathetic outflow at 20 Hz
SA-node	Sino-atrial node
SDR	Sprague Dawley rat
SEM	Standard error of mean
SHR	Spontaneously hypertensive rat
SNP	Sodium nitroprusside
T_{1-12}	First to twelvth thoracic vertebrae
TXA_2	Thromboxane A_2
VIP	Vasoactive intestinal peptide
VOC	Voltage operated calcium ion channels
WKY	Wistar Kyoto rat
Y	Yohimbine

INTRODUCTION

THE CARDIOVASCULAR SYSTEM

The Function of Cardiovascular System

The cardiovascular system has several important functions, which include transport, communication and immunological protection. Many of the substances required for cellular activity and the waste products of metabolism are also transported in the blood stream. Some of these such as oxygen and carbon dioxide, are transported bound to the red blood corpuscles, whilst others, such as nutrients and ions, are transported in solution in the plasma. The cardiovascular system also distributes hormones and other regulatory molecules from their sites of origin to remote target organs whose functioning is controlled by these humoral signals. The cardiovascular system also protects against microorganisms and toxins, whose presence trigger complex immune mechanisms involving lymphocytes and soluble antibodies that are distributed within the circulatory system.

The Organisation of the Cardiovascular System

The cardiovascular system consists of the heart and blood vessels, which together with the lymphatic vessels, constitute the circulatory system. The heart is a four-chambered pump, that pushes the blood into the vessels, which form a closed network of tubes, through which the blood flows to all

the organs of the body and then returns to the heart (Malseed, 1990). The blood vessels that carry blood away from the heart are the arteries, which branch successively, to form arterioles and ultimately capillaries, which in turn lead to venules and finally to veins, through which the blood returns to the heart.

THE HEART

Venous blood returns to the heart via the right atrium, from which it is pumped to the right ventricle, which pumps blood through the pulmonary artery to the lungs. Blood that has been oxygenated and partly depleted of carbon dioxide, returns from the lungs through the pulmonary vein to the left atrium, from which it is pumped to the left ventricle, which then pumps oxygenated blood to the entire body.

The functioning of the heart is regulated by the autonomic nervous system, which supplies both sympathetic and parasympathetic fibres, that regulate heart rate and cardiac output. In the absence of any neural influence the heart will continue to beat according to the rhythm determined by the sino-atrial node (SA-node). Normally, the spontaneous rate of depolarization of the SA-node is modified by the activity of the sympathetic and parasympathetic (vagus) nerves, which respectively release noradrenaline (NA), which increases the rate of firing of the SA-node, and acetylcholine (ACh), which hyperpolarizes the SA-node (Fozzard et al, 1986; Gravanis, 1987; Guyton, 1951 and Mazzanti and De Felice, 1987). Sympathetic and parasympathetic fibres also innervate the atrio-ventricular node (AV-node), whose conduction rate is increased by sympathetic activity and decreased by parasympathetic activity. Sympathetic fibres also innervate the

musculature of the atria and ventricles and activation of these nerves increases the force of contraction and decreases the duration of systole. Because the terminals of the functionally-opposed sympathetic and parasympathetic nerves at the SA-node are close together, transmitter released by one type of nerve can inhibit the release of the other so that these nerves not only regulate the functioning of the heart directly but also influences it indirectly by inhibiting the activity of the opposing nerve (Boyle, 1988).

The functioning of the heart is also regulated by an intrinsic non-neuronal mechanism, that involves stretching of the heart when the end diastolic volume (EDV) increases within the physiological range. There is a direct relationship between the EDV, the contraction strength and the stroke volume, both of which increases when the heart muscle is stretched and the EDV increases. This important characteristic, which is known as the Frank-Starling Law of the heart, explains how the heart normally adjusts to an increase in the total peripheral resistance (Weatherall et. al., 1987).

The functioning of the heart is also influenced by hormones, such as the thyroid hormones (thyroxine and triiodothyronine), which increase the sensitivity of the beta-adrenoceptors in the heart to NA and to circulating adrenaline from the adrenal medulla (Hohl et al, 1989; Tsujimoto et al, 1987; Tse et al, 1980).

BLOOD VESSELS

Blood vessels have characteristic anatomical features such as smooth muscle and endothelial cells (Fig-1). The vascular endothelium modulates

the reactivity of the smooth muscle (Robertson and Rosen, 1978; Vanhoutte et al, 1986) by releasing vasoactve agents in response to various stimuli (Vanhoutte et al, 1986; Vanhoutte and Luscher, 1986; Greenberg and Diecke, 1988). The vascular smooth muscle in the systemic and pulmonary circulations are controlled by the sympathetic branch of the autonomic nervous system unlike many organs such as the heart and gastrointestinal tract (GIT) which are innervated by both branches of the autonomic nervous system. In mammals, including man, the density of the adrenergic nerves to blood vessels decreases with age (Frewin et al, 1971; Waterson et al, 1974; Gerke et al, 1975).

Structure of Blood Vessels

Arteries are divided into three types according to their diameters, structure and function:

a) large elastic arteries b) muscular arteries c) arterioles

Arteries

Histologically all arteries have three distinctive layers: The intima in the luminal side of the blood vessels is composed of the endothelial cells, basement membrane and different amounts of connective tissues. The media is the middle layer which is composed of smooth muscle and finally the adventitia which is the outermost layer. These three layers are less easily seen as the diameter of the arteries reduces (Clark and Glagov, 1985; Rao et. al., 1983; Ross et. al., 1976-1977, 1977 and Moran, 1975).

In both elastic and muscular arteries, the intima and the inner part of the media get nutrition from the vascular lumen by perfusion whereas the vasa vasorum is for nourishing the outer media and hence there is a nutritional boundary in the vascular media which is susceptible to damage by changes in the thickness of the intima, mural thrombosis, impaired oxygenation and obstruction of the vasa vasorum (Castleman and Smithwick, 1948; Gamble, 1986; Haust, 1978).

A large proportion of the elastic arteries is the media consist of concentrically arranged, fenestrated layers of elastic tissue and intervening smooth muscle cells. The large number of elastic layers in the media of elastic arteries enable them to absorb and transmit the pulsatile force of the left ventricular systole and maintain intra-arterial pressure during diastole, creating a relatively constant pressure and flow of blood.

The muscular arteries have almost no elastic layers in their media and the intima of these arteries is thinner but similar in composition compared to the elastic arteries. The media of the muscular arteries is also thicker than the adventitia (Neufeld and Blieden, 1978).

In large blood vessels, because of the proportion of smooth muscle to connective tissue, it is harder for the neurotransmitters and vasoactive agents to reach their receptor sites in smooth muscle (Vanhoutte, 1978). Arterioles are the smallest arteries and in the smaller arterioles there are only one or two layers of smooth muscles. The adventitia consists mainly of collagen and elastic fibres.

Capillaries

The intima is the major part of the capillaries and consists mainly of endothelium. At the point of branching of a capillary from an arteriole, there is a precapillary sphincter to regulate blood flow into the capillary.

Veins

Veins are different from arteries in that they have thinner walls and are less well demarcated into three structural layers when two blood vessels of similar size are compared (Hirsh et. al., 1986; Lie et. al., 1977; Smith and Geer, 1983; Strandness and Thiele, 1981). Veins have scanty elastic layers and fewer smooth muscle cells in their media. These are mainly separated by collagen fibres, arranged in both circular and longitudinal fashions. All veins have valves in their lumens except the vena cavae and the common iliac veins.

In veins, nerves usually penetrate into the media, whereas in most arteries they penetrate into the adventitia-media border (Vanhoutte and Luscher, 1986). Vascular smooth muscle has been reviewed by (Furchgott, 1955; Bohr, 1964; Somlyo and Somlyo, 1968 and Gabella, 1981).

History of Sympathetic Nerves

The discovery of the communication between the nerve terminals and the effector cells goes back over a century ago when it was suggested by Du Bois Reymond in 1877 that a chemical mediator might be the biological link.

Oliver and Schafer (1895) were among the first to show that extracts from the adrenal medulla can increase the arterial blood pressure. The possibility that the chemical agent might be the same in both cases was postulated by Elliott (1905). Some years later Langley (1910) demonstrated that sympathetic nerve stimulation could also increase the arterial blood pressure.

Loewi in 1921 concluded from his famous experiment on frog heart that a physiologically-active substance was released from the sympathetic nerves onto cardiac muscle and accelerated its pumping activity. He set up two frog hearts in series and the physiological solution from the first heart was allowed to come into contact with the second heart. When the sympathetic nerve to the first heart was stimulated its cardiac activity accelerated and after a while the activity of the second heart was also increased. Therefore a chemical, released from the nerve terminals in the first heart, activated the first heart and also activated the second heart. He named this neuronally-released chemical 'acceleranstoff'.

Barger and Dale (1910) showed that there is a great similarity between the actions of noradrenaline and the effect of sympathetic nerve stimulation in the peripheral tissues of mammals. Cannon and Uridil (1921) named this active substance 'sympathin' after the sympathetic nerves from which it was released. The sympathetic innervation proved not to be exclusive for the cardiovascular system and other tissues were also found to be innervated by these important nerves. Finkleman in 1930 showed that sympathetic nerve stimulation of a piece of rabbit intestine brought about its relaxation and also the relaxation of the second piece of rabbit intestine which was in communicaton with the first one through perfusion. Adrenaline also produced a similar kind of relaxation in this tissue. It was

not until 1946 when von Euler showed that the main sympathetic transmitter was noradrenaline and not adrenaline, since the concentration of noradrenaline was much higher than adrenaline in the sympathetically innervated tissues. Von Euler also demonstrated that sympathectomy led to a decrease in the concentration of noradrenaline and hence relaxation of sympathetically-innervated blood vessels. In Cannon's experiment the sympathin activity was very similar to noradrenaline (Cannon and Rosenbleuth, 1933).

Adrenoceptors

In early experiments adrenaline was found to produce a vasopressor effect which consisted of two components, a dominant pressor component, caused mainly by vasoconstriction and a secondary depressor component caused by vasodilatation, which was mostly covered by the pressor component. The vasodilatation was more pronounced when the first component was inhibited by ergot extracts (Dale, 1906). Ahlquist (1948) noted that the order of potency of six sympathomimetics were different in a variety of tissues and postulated that different receptors might exist and hence the concept of α- and ß-adrenoceptors on the effector tissues. α-Adrenoceptors were also found on the nerve terminals and these were classified as α_2-adrenoceptors. Activation of these presynaptic neuronal α_2-adrenoceptors inhibited the release of neurotransmitter(s) into the synaptic cleft (Langer, 1974; Starke, 1977; Westfall, 1977 and Vizi, 1980). Both kinds of α-adrenoceptors (ie. α_1- and α_2-adrenoceptors) were shown to be present post-synaptically (Langer, 1974). The post-junctional α_2-adrenoceptors may not be homogeneously distributed and could vary in their location at synaptic and extrasynaptic sites (McGrath, 1982) and

among species (Alabaster et al, 1986). There is now evidence that noradrenaline may not be the only neurotransmitter released from the sympathetic nerves (Hokfelt et al, 1986; Furness et al, 1989).It is possible that other substances such as adenosine triphosphate (ATP) may be released together with NA as a cotransmitter (Stjarne, 1989).

There is evidence that in the pithed rat, the vasopressor response to sympathetic nerve stimulation may not be mediated exclusively by NA since there was a component in the response that was resistant to α-adrenoceptor blockade (Flavahan et al, 1985). This non α-adrenoceptor component could be reduced in size by α,β-methylene ATP which is a stable analogue of ATP and is a desensitizing agent for purinoceptors. This phenomenon was only seen in the presence of an α-adrenoceptor antagonist (Flavahan et al, 1985; Grant et al, 1985). This observation suggests that purines may be involved in cotransmission in the sympathetic nerves in the rat and may contribute to the pressor responses resulting from activation of these nerves.

Adenosine triphosphate (ATP)

Paradoxically, ATP also has vasodilator activity in many vascular beds including the coronary circulation (Wolf and Berne, 1956; Moir and Downs, 1973), skeletal muscles (Boyd and Forrester, 1968; Berne et al, 1975, Haddy and Scott, 1968), adipose tissue (Sollevi and Fredholm, 1981) and cerebral blood vessels (Rubio et al, 1975). In addition ATP was also demonstrated to be present in the perfusate of hypoxic hearts (Paddle and Burnstock, 1974).Holton and Holton (1954) related the vasodilator activity

of ATP to nerves when it was released during antidromic activity from sensory nerves.

There are two main types of peripheral purine receptors: P_1- and P_2-purinoceptors (Burnstock, 1978). P_2-purinoceptors are more sensitive to ATP and can be selectively blocked by arylazido amino propionyl ATP (ANAPP3) (Hogaboom et al, 1980) or desensitised by α,β-mATP (Kasakov and Burnstock, 1983). P_2-purinoceptors was further subdivided into P_{2x}-, P_{2y}-, P_{2z}- and P_{2t}-purinoceptors, the latter being platelet specific and responding to ADP (Burns, 1988; Daly, 1982; Daly, 1985). Activation of P_{2x}-purinoceptors causes vascular smooth muscle contraction (Burnstock and Warland, 1987a) and P_{2y}-purinoceptors causes vascular smooth muscle relaxation (Burnstock and Warland, 1987b). All P_2-purinoceptors have some role in regulating Ca^{2+}, K^+ and Na^+ movements and prostaglandin (PG) formation (Burnstock, 1988).

Evidences for ATP as a neurotransmitter

I) There are biological mechanisms for the synthesis and storage of ATP in nerves (Burnstock; 1985, 1988).

II) There are post-synaptic receptors for ATP and activation of them by exogenous ATP produces complex physiological effects. For instance ATP causes relaxation of the intestine (Ambache and Aboo Zar, 1970; Dean and Downie, 1978) but causes a transient contraction in the bladder (Burnstock et al, 1972). These effects are similar to those obtained following stimulation of non-adrenergic non-cholinergic (NANC) nerves in these tissues.

III) Efficient uptake and enzymic degradation mechanisms are present for the inactivation of ATP. ATPase which is the enzyme responsible for the degradation of ATP is located on the cell plasma membrane (Manery and Dryden, 1979) and is abundant in the rat heart and lungs (Baer and Drummond, 1968).

IV) ATP is released during nerve stimulation in tissues such as taeni coli and bladder and can be detected using the sensitive ATP-specific firefly luciferin-luciferinase assay (Burnstock et al, 1978a,b). However, this evidence does not conclusively prove that ATP is a neurotransmitter since most excitable tissues including muscle cells can release ATP.

ATP Cotransmission

In adrenergic nerves, NA is stored with ATP, chromogranin and dopamine ß-hydroxylase (Smith, 1972). ATP was also shown to be released from adrenal chromaffin cells (Douglas and Poisner, 1966; Douglas, 1968; Stevens et al, 1972) and released with NA from adrenergic nerves (Su et al, 1971).

Vascular Smooth Muscle Contraction

Smooth muscle cells are contracted by many pharmacologically-active substances. The underlying cause of the contraction is a rise in the cytosolic concentration of Ca^{2+} ions (Bolton, 1979; Jones, 1981; Karaki, 1989) which bind to calmodulin. The Ca^{2+}-calmodulin complex activates

the myosin light-chain kinase (MLCK) which is an enzyme that phosphorylates one of the constituents of myosin (Kamm and Stull, 1985). The phosphorylated myosin is then capable of interacting with its counterpart, actin, to initiate the smooth muscle contraction (Bulbring and Tomita, 1987; Bulbring et al, 1982). However, smooth muscle differs from cardiac and striated muscles in having calmodulin rather than troponin for binding Ca^{2+} ions and the consequent interaction of myosin with actin in the contractile mechanism.

The Effect of Vasoconstrictor Agents Causing Vascular Contraction

There are number of ways by which pharmacological agents can cause smooth muscle to contract by increasing cytosolic free Ca^{2+} ions $[Ca^{2+}]_i$:

a) Phosphatidyl inositol hydrolysis (PI-turn over). Activation of receptors coupled to cell membrane phospholipase C (PLC) leads to the production of inositol trisphosphate (IP_3) and diacyl glycerol (DAG) (Berridge and Irvine, 1984; Abdel-Latif, 1986; Berridge, 1987; Litosch, 1990). IP_3 subsequently acts on its specific receptors (Furuichi et al, 1989) on the intracellular stores of Ca^{2+} ions in the endoplasmic reticulum (Carafoli, 1987), to release Ca^{2+} ions into the cytosol (Michel, 1986; Berridge, 1987; Fink et al, 1988) and cause the contraction (Rasmussen, 1983).

b) Activation of receptor-operated channels which open and allow the influx of Ca^{2+} ions and also cause depolarisation.

c) Depolarisation of the cell membrane allows further entry of Ca^{2+} through the voltage-gated Ca^{2+} ion channels. These voltage-gated Ca^{2+}

ion channels may also be facilitated by agents such as endothelin which is a potent vasoconstrictor released from the endothelium and, indirectly, by agonists acting on the α-adrenoceptors.

Vasodilator Agents Causing Vascular Relaxation

Vasodilator agents cause vascular relaxation by reducing $[Ca^{2+}]_i$ or directly by acting on the contractile mechanisms (Furchgott, 1981; Furchgott, Vanhoutte, 1989; Ignarro, Kadouitz, 1985).

a) Inhibit Ca^{2+} entry through voltage-gated Ca^{2+} channels. Hyperpolarisation through increased permeability of intracellular ATP-sensitive K^+ channels (Nayler, 1988; Cook, 1988; Quast and Cook, 1989).

b) Increase the synthesis of cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP) production, or they may inhibit the breakdown of these nucleotides by phosphodiesterases.

The vascular plasma membrane controls Ca^{2+} flux mainly through a specific ATPase that extrudes Ca^{2+} from the cell and Na^+/Ca^{2+} exchange by which three Na^+ ions are exchanged for a Ca^{2+} ion (Van Breemen and Saida, 1989). In addition, the cell membrane adenylate cyclase which is the enzyme that catalyses the biosynthesis of cAMP from ATP has an important role in regulating $[Ca^{2+}]_i$. cAMP activates protein kinase A which inhibits myosin light-chain kinase and hence cause relaxation. cAMP is also required for the pumping of cytosolic Ca^{2+} out of the cell (Conti and Adelstein, 1981; Eggermont et al, 1988; Van Breemen and Saida, 1989). The inhibitory effect of cAMP on voltage operated

calcium (VOC) ion channels has been confirmed by Ousterhout and Sperelakis (1984). Guanylate cyclase catalyses the synthesis of cGMP from GTP. This enzyme is activated by nitric oxide (NO) derived from the vascular endothelial cells or from nitrovasodilators that release NO intracellularly. Nitrovasodilators and endogenous NO act by stimulating soluble guanylate cyclase (Murad et al, 1988). cGMP in turn activates protein kinase G which inhibits the contraction.

The Role of the Vascular Endothelium and its Endogenous Vasoactive Agents

The endothelial cells lining the vasculature act as a passive barrier to plasma proteins and release various vasoactive substances which include endothelium-derived relaxing factor (EDRF) which is characterised as nitric oxide (NO) (Moncada et al, 1987; Furchgott, 1988; Ignarro et. al., 1987a,b; Palmer et. al., 1987), and prostacyclin (PGI_2) and the vasoconstrictor endothelins. PGI_2 and NO also have inhibitory effects on platelets and therefore reduce their tendency to aggregate and cause vascular thrombotic occlusion (Radomski et al, 1987). The endothelial cells also produce the important vasoconstrictor, thromboxane A_2 (TXA_2) which opposes the vasodilator effect of prostacyclin. In addition, endothelial cells also release endothelium derived contracting factor (EDCF) in response to distension and anoxia (De May and Vanhoutte, 1983; Rubanyi and Vanhoutte, 1985) and stretch (Katusic et al, 1986).

Endogenous NO and its Vasodilator Activity

Many vasodilator substances such as acetylcholine (ACh), bradykinin (Bk), 5-hydroxytryptamine (5-HT) and histamine act through the release, via a Ca^{2+}-dependent mechanism, of vascular endothelial NO, which mediates the vascular relaxation (Furchgott and Vanhoutte, 1989). Vasopressin causes endothelium-dependent relaxation of basilar arteries but causes vasoconstriction in systemic blood vessels (Katusic et al, 1984). Various receptors are located on the endothelial cell membrane and the mediators which act on these receptors activate the PI-mechanism and hence cause an increase in the production of IP_3 and thus elevate intracellular Ca^{2+} ions. The amino acid L-arginine is the precursor for NO biosynthesis which is catalyzed by the enzyme NO synthase (Ignarro et al, 1987a,b; Collier and Vallance, 1989; Palmer et al, 1988). The released endothelial NO passes through the cell membrane and activates the smooth muscle guanylate cyclase. The consequent increase in the cGMP production causes a series of protein phosphorylations associated with reduced release of Ca^{2+} ions from endoplasmic reticulum or reduced permeability to extracellar Ca^{2+} ions and consequently causes smooth muscle relaxation (Moncada et. al., 1991).

NO has also been suggested to act as a mediator of cytotoxicity in macrophages (Moncada et al, 1991; Hibbs et al, 1990) and in the central nervous system (CNS) it has been associated with the short-term effects of excitatory amino acids and in their long-term effects on brain development, learning and memory (Moncada et al, 1991) and other tissues (Collier and Vallance, 1989).

Endogenous Vasoconstrictor Agents

These are long-acting potent vasoconstrictor peptides which are released from vascular endothelial cells in response to chemical and physical stimuli. They were first discovered by Yanagisawa et al (1988) and later, it was realized that there is a family of endothelin peptides designated endothelin 1, 2 and 3. These were identified by analyzing human genomic DNA which revealed the existence of distinct loci for each endothelin subtype (Inoue et al, 1989). There are many endogenous vasoconstrictor substances these include sympathomimetic amines (eg. NA, adrenaline), peptides such as angiotensin II, vasopressin, thromboxanes (eg. TXA_2) and platelet derived growth factor (PDGF). In some pathological states the superoxide anion (O_2^-) may accummulate and cause the endothelium-dependent contraction in blood vessels such as the dog basilar artery (Vanhoutte and Katusic, 1988).

Generally vasoactive substances can affect the resistance of vascular beds and hence alter the blood flow to and the distribution of blood within different organs. Such effects are involved in disorders such as angina, migraine and hypovolaemic shock.

Angiotensin and its Vasoconstrictor Activity

Angiotensin is an endogenous peptide and has a role in hypertension. Angiotensin II (AII) is a potent vasoconstrictor that acts through the PI-mechanism. It mainly affects cutaneous, splanchnic and renal blood flow with less effect on the cerebral and skelatal muscle blood flow. It also affects coronary blood flow and increases the rate and force of contraction

of the heart, secondary to the release of NA from the sympathetic nerve terminals (Khairallah, 1972; McCubbin, 1974). AII has less effect on the venous system than α-adrenoceptor agonists. It also releases aldosterone from the adrenal cortex. Its significance can be revealed by drug such as captopril, which inhibits the synthesis of AII (Fig-2) and therefore has a profound effect on the cardiovascular system (Zhu et al, 1993; Valloton, 1987; Katzung, 1989).

Angiotensin converting enzyme inhibitors (ACE inhibitors) such as captopril and enalapril, are vasodilators that exert their effects through inhibition of the conversion of AI to the potent vasoconstrictor AII. These ACE inhibitors have their most powerful effect when the production of renin in the kidneys has been increased through sympathetic nerve activity (Laurence, Bennett, 1992b).

The General Mechanisms of Action of Vasodilator Drugs

Vasodilator drugs exert their effects in different ways. Some cause vasodilatation indirectly by releasing an endogenous vasodilator such as NO. Others inhibit the release or effect of an endogenous vasoconstrictor. For example, vasodilators may disrupt the sympathetic pathway to the vasculature, either by acting in the CNS (eg. clonidine, guanfacine), the autonomic ganglia (eg. hexamethonium, trimetaphan), the sympathetic nerve terminal (eg. guanethidine, reserpine) or on α-adrenoceptors (eg.prazosin, doxazosin) (Goodman et al, 1990; Brown, 1980).

Other vasodilator agents including $ß_2$-adrenoceptor agonists, increase the intracellular level of cAMP through activation of cell membrane adenylate cyclase (Fig-3).

There are many clinical uses of the vasodilator drugs which are used to lower the blood pressure by reducing the resistance of blood vessels to perfusion such as in hypertension and cardiac failure.

BLOOD

Blood, which can also be considered as a fluid tissue, circulates throughout the body in the blood vessels. It carries O_2 and nutrients as well as other necessary substances to the tissues. Haemoglobin (Hb), which is the oxygen-carrying pigment of the erythrocytes, is capable of binding avidly to NO and preventing its vasodilator activity (Gillespie and Sheng, 1988). Whether or not this property of Hb is significant in vivo in regulating the effects of endogenous NO remains to be determined.

Haemostasis and Prevention of Bleeding

In the event of vascular injuries, haemostasis is the mechanism for the prevention of blood lose and ischaemia which can have fatal consequences. The three main phenomena of haemostasis are:

a) vascular contraction

b) adhesion, activation and aggregation of platelets

b) fibrin clot formation (blood coagulation)

The latter two phenomena are mainly involved in the formation of a haemostatic plug which blocks the vascular damaged area and stops the lose of blood. Each of these processes has its own specific role related to the size of the blood vessels involved (arterial, venous or capillary).

LUNGS

The differential amounts of structural smooth muscles in the systemic and pulmonary vasculatures play a major role in the wide differences in the blood pressure and blood flow in these two distinct blood circulatory systems that are well adapted to their functional purposes.

The pulmonary artery and arterioles are more distensible and have greater compliance than the systemic vessels. The wall thickness of the pulmonary vasculature is less than that of the systemic vasculature for arteries of similar diameter. The pulmonary arteries can accommodate about two thirds of the right ventricular output after each heart beat. However, the distensibility of pulmonary veins and systemic veins are not very different. Therefore, the pulmonary vascular bed is a low-resistance, high-flow circulation (Bhattacharya, Staub, 1980; Nagasaka et al, 1984; Dock, 1946). Flow in the microcirculation is pulsatile because of the low arterial resistance (Lee, Du Bois, 1955).

The systolic pressure in the right ventricle is about 25 mm Hg and the diastolic pressure is about 0 to 1 mm Hg. These values are approximately one fifth of those for the left ventricle in a normal human (Ganong, 1989b).

The pulmonary arterial systolic blood pressure (25 mm Hg) matches the systolic pressure in the right ventricle. The pulmonary arterial diastolic blood pressure is only about 8 mm Hg, which is more than the diastolic pressure in the right ventricle. This is due to the steady fall in the pulmonary arterial blood pressure as blood flows through the capillaries after closure of the pulmonary valve. The mean pulmonary arterial pressure is arround 15 mm Hg.

The mean pulmonary capillary pressure is approximately 7 mm Hg when measured by indirect methods and this low pressure helps in the gas exchange between these capillaries and the alveoli. The capillaries in the lungs are very close to each other and often in close contact with one another. This makes the pulmonary capillaries look like a 'sheet' rather than a separated network of fragile blood vessels in the lungs. The pulmonary capillaries are large and have multiple anastomoses.

The mean pressure in the left atrium and in the pulmonary veins is about 2 mm Hg when measured in humans in a supine position (Taylor et al, 1989).

The chief function of this vital circulation is in the regulation of O_2 supply to the blood circulation and hence to the all tissues of the body as well as removing CO_2 from the blood. Water evaporation which reduces the temperature of the body also occurs while the blood is being conveyed in this fragile vasculature (Souhami and Moxham, 1990).

The lungs also contain a fibrinolytic system that lyses blood clots in the vasculature. Filtration of small blood clots out of the blood circulation is another function of the lungs. This occurs when some branches of the pulmonary arteries are obstructed by blood clots. As a result, there is an increase in the pulmonary arterial pressure, caused by a reflex sympathetic nerve-mediated response. In addition, there is a reflex, rapid, small increase in respiration (ie. tachypnea). This latter response is due to activation of vagally-innervated pulmonary deflation receptors close to the vessel walls. There is evidence that 5-HT, released from platelets at the site of vascular embolization, can sensitize these deflation receptors (Ganong, 1989c).

Vasoactive regulation plays an important part in the local regulation of blood flow (Grover et al, 1984; Dawson, 1984). Blood P_{O2} and certain metabolic by-products such as P_{CO2}, pH, adenosine, lactate, K^+ and inorganic phosphate can alter blood flow.

Low local alveolar O_2 causes a vasoconstriction in the pulmonary blood vessels in that area. This is the opposite of what happens in the systemic vasculature (ie. vasodilatation in response to hypoxia). This property of pulmonary vasculature to hypoxia diverts blood flow to better ventilated regions of the lungs and hence cause more effective oxygenation of blood. This mechanism, which is not throughly understood, might be due to the release of a vasoconstrictor or a class of vasoconstrictors from the alveolar epithelial cells, hence causing an increase in the vascular resistance. Higher than usual levels of CO_2 also reduce pH in the area and may cause pulmonary vasoconstriction.

There is a difference in the blood pressure between the top and lower portions of the lungs due to gravity or hydrostatic pressure arising from the

weight of the blood itself. This pressure gradient, which is about 23 mm Hg in humans, may be another factor in keeping the pulmonary vasculature open in the lower part of the lungs and in maintaining a higher blood flow there. In addition, more blood vessels are recruited to allow increased blood flow through the lungs when the cardiac output is increased (eg. in exercise) and then the pressure difference between the top and lower part of the lung is also reduced in proportion (West et al, 1964; West, 1977; Murray, Nadel, 1988a). The overall pulmonary blood pressure remains approximately the same although the cardiac output increases during heavy exercise in a normal subject. That is achieved through higher blood flow in the already-contributing pulmonary capillaries and also through recruiting inactive capillaries (Murray, Nadel, 1988b).

Pulmonary oedema normally occurs when the blood pressure increases in the pulmonary vasculature and therefore causes a shift of intravascular fluid out into the pulmonary interstitial space. When the left atrial pressure increases due to a disorder then the blood pressure in the pulmonary veins and capillaries are increased, hence causing an increase in the pulmonary arterial pressure (Guyton and Lindsey, 1959; Guyton et al, 1979).

The pulmonary capillaries are more leaky to blood proteins than the systemic capillaries and this makes the osmotic pressure in the pulmonary interstitial space higher than the interstitial space in the systemic capillaries (Staub, 1974). The epithelial cells lining the alveolar walls are very thin and can easily be ruptured by a positive pulmonary interstitial pressure (ie. a pressure more than the atmospheric pressure) (Staub, 1983; Tucker et al, 1973; Mason and Effros, 1983).

In diseases such as pneumonia and during inhalation of noxious gases, such as sulphur dioxide and chlorine, the pulmonary capillary membranes are damaged and this accelerates the leakage of plasma fluid and proteins into the interstitial space. Pulmonary alveolar oedema occurs secondary to an excess in pulmonary interstitial oedema due to a rupture in the alveolar epithelial cells. This interferes with the gas exchange mechanism and leads to suffocation (Guyton and Lindsey, 1959). There is a slight net mean filtration pressure (ie. 1 mm Hg) in the lungs which filters pulmonary intravascular fluid into the interstitial space. The lymphatic system helps to remove the excessive fluid in the pulmonary interstitial space.

Under normal conditions the alveoli are kept dry even though there are openings in the alveolar lining epithelium and protein molecules as well as electrolytes and water can get through. The higher osmotic pressure in the pulmonary interstitial space can be the underlying force that attracts the protein molecules and fluids from the alveoli into the interstitial space (Effros, 1984).

Contrary to the heart, which is innervated by both branches of the autonomic nervous system, the pulmonary vasculature is only innervated by the sympathetic nerves (Nadel and Barnes, 1984). Stimulation of the cervical sympathetic ganglia where the sympathetic nerves innervating the pulmonary vasculature originate, increases the resistance of the blood vessels in the lungs and causes a reduction in blood flow. This pulmonary vasoconstriction is mediated through α-adrenoceptors but vasodilatation of this vascular bed can be produced by circulating adrenaline (Adr) released from the adrenal medulla. This effect of circulating adrenaline is mediated via $ß_2$-adrenoceptors (Su, Bevan, 1976).

The pulmonary vasculature responds to a number of vasoactive substances. For example, pulmonary arterioles are constricted by NA, Adr., AII, thromboxanes and $PGF_{2\alpha}$ and are dilated by isoprenaline, ACh and PGI_2. Pulmonary venules are constricted by 5-HT, histamine and Escherichia coli endotoxins (Murray and Nadel, 1986).

Various physiologically-active substances are synthesized, stored and released into the pulmonary blood circulation from the pulmonary arteries (eg. prostaglandins, histamine, kallikrein, etc.). Other substances are removed by the pulmonary veins (eg. prostaglandins, bradykinin, the adenine nucleotides (ATP, ADP, AMP and adenosine), 5-HT, NA, ACh (Said, 1982; Furchgott, 1984). A number of other vasoactive hormones pass through the pulmonary vasculature without being metabolized (eg. Adr., dopamine, oxytocin, vasopressin and AII) (Ganong, 1989c).

Some nerve fibres and amine-precursor-uptake-decarboxylation (APUD) cells in the lungs contain biologically-active peptides (eg. vasoactive intestinal peptide (VIP), NO, substance P, opioid peptides, CCK and somatostatin). The exact functions of most of the peptides is still poorly understood but it appears that some of them such as VIP and NO may be involved as non-adrenergic non-cholinergic (NANC) bronchodilator mediators (Burnstock, 1969).

The Pulmonary Angiotensin Converting Enzyme (ACE)

The lungs are also a reservoir for ACE. This enzyme is located in the luminal surface of the pulmonary capillaries (ie. on the surface of endothelial cells and particularly in pits called caveolae). Its main function is the conversion

of AI to AII. The vasodilator, bradykinin which acts through releasing endogenous NO is also inactivated by ACE (Erdos et. al., 1978; Erdos, 1990).

Autacoids

Autacoids are physiologically-active, endogenous substances such as histamine, 5-HT, angiotensin, nitric oxide (NO), prostaglandins, endothelins, etc. (Laurence, Bennett, 1992a; Pohl, Kaas, 1994) which have their own effects in the lungs. Histamine is found in all body tissues, particularly in the mast cells and their related blood basophils. The lungs have the highest concentration of histamine. Histamine causes dilatation of blood vessels through endothelial H_1-receptor activation and release of NO to vascular smooth muscle causing vasodilatation. Histamine, increases also the permeability of venules, reduces the blood pressure and contracts most smooth muscles, including the bronchial smooth muscle of the lungs. Histamine also accelerates the heart rate via H_2-receptors (Dale and Foreman, 1989; Ganellin and Parsons, 1982; Hill, 1987 and Black et al, 1972).

Pulmonary Chemoreceptors as a Storage Site for 5-HT

Hormones or neurotransmitters such as 5-HT can also affect the pulmonary vascular bed. Chemoreceptors in the neuro-epithelial bodies within the lungs, have stores of 5-HT. These neuro-epithelial bodies are located in the lower respiratory system (ie. small airways and alveoli). The specific physiological role of 5-HT in the lungs and in many other organs is unclear.

Neuronal or chemical stimuli such as hypoxia releases 5-HT, which could have a role in regulating regional blood flow in the lungs.

Normally, a large fraction of the pulmonary vasculature is open to perfusion but there are also some high resistance blood vessels that are closed. These can open and reduce the perfusion pressure perhaps in response to an increase in the production of NO. N^{W}-Nitro-L-Arginine Methyl Ester (L-NAME) is the inhibitor for the biosynthesis of NO (Ignarro, 1989; Palmer et. al., 1988).

Endogenous NO appears to have some regulatory effect on pressor responses to agonists such as 5-HT, phenylephrine (PE) and potassium chloride (KCl). This was suggested by experiments in which NO synthase inhibitors enhanced pressor responses to these agonists (Shaw et al, 1992a,b,c).

The biosynthesis and release of NO in the pulmonary vasculature is likely to be regulated by many factors that are mostly unclear. NO may be produced and released by pressor agonists that cause vasoconstriction and hence increase intraluminal pressure and shear stress which also releases PGI_2 from the endothelial cells (Bhagyalakshmi and Frango, 1989). However, the basal release of NO may be responsible for some or all of its inhibitory actions (Archer et al, 1989). In addition to these factors, endogenous NO may also be produced and released through receptor interactions of some agonists such as 5-HT, which acts on endothelial receptors (Houston, Vanhoutte, 1988; Martin et al, 1987).

THE AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system (ANS) consists of the sympathetic and parasympathetic divisions. They both have their preganglionic cell bodies in the CNS and the postganglionic cell bodies in the autonomic ganglia. In addition, the enteric nervous system (Gershon, 1981) which innervates the intramural plexus of the gastrointestinal tract (GIT) is in close association with the sympathetic and parasympathetic divisions of the ANS (Furness and Costa, 1987).

The root of the sympathetic nerves originate in the thoracic and lumbar vertebral column. There are two paravertebral chains with some sparse midline ganglia. The postgangionic nerves leave these sympathetic ganglia and innervate tissues such as the cardiovascular system, the gastrointestinal tract, the bladder and the genitalia. The parasympathetic nerves leave CNS in cranial nerve outflow (the vagus) and in the sacral outflow. The parasympathetic ganglia contrary to the sympathetic ganglia usually lie near to the target organs. Transmitters of the autonomic nervous system are mainly ACh and NA although cotransmission with other transmitters such as 5-HT, ATP, dopamine, u amino butyric acid (GABA) and several other neuropeptides are also possible.

The autonomic preganglionic neurons are cholinergic and release ACh which acts on the nicotinic cholinoceptors that are located on the cell bodies of the postganglionic nerves, although muscarinic cholinoceptors are also present and also play an excitatory role. Postganglionic sympathetic neurons are commonly noradrenergic though in some cases, as in the sweat glands, they are cholinergic. The postganglionic parasympathetic neurons are mainly cholinergic and release ACh which acts on the muscarinic

cholinoceptors of tissues such as salivary glands to increase their secretions (Ganong, 1989a).

Neuromodulation

Neuromodulation in the autonomic nervous system is a common phenomenon and consists of the ability of chemical mediators to increase or decrease the amount of transmitter released (Kaczmarek and Levitan, 1987; Rand et al, 1987 and Burnstock, 1987).

When the action potential is propagated down the axon and reaches the nerve terminal, it releases neurotransmitters such as NA from the sympathetic nerve terminals. Neurotransmitters as well as having stimulatory effects on the effector cells, such as smooth muscles for NA and exocrine glands for ACh, can also inhibit their own release from the nerve terminal through a negative feedback mechanism, commonly called an auto-inhibitory feedback (Rand et al, 1982; Starke et al, 1989). The effect of neurotransmitters on their own nerve terminals are not always inhibitory but may also be excitatory in some occasions, though the inhibitory effect is usually predominent (Vizi, 1980; Starke et. al. 1989). The auto-inhibitory or excitatory feedback mechanisms involve the usual second messenger regulation and cell membrane ion channels. Many endogenous mediators such as 5-HT, prostaglandins (PGs), adrenaline, AII, adenosine, histamine, dopamine, GABA, enkephalin, opioids and other peptides as well as the transmitters themselves exert presynaptic modulatory control over autonomic transmitter release (Rang and Dale, 1991).

Cotransmission

Cotransmission involves the release of more than one transmitter from the same nerve (Hokfelt et. al. 1986; Furness et. al. 1989). Cotransmission may result in postsynaptic synergism between two transmitters in some blood vessels. For example, there is a synergistic effect of neuropeptide Y (NPY) on the vasoconstrictor action of NA in addition to its ability to inhibit NA release (Edvinsson et al, 1987). The release of NPY may also be frequency dependent and more NPY might be released at higher frequencies (Stjarne, 1989). Another example is the NA/ATP cotransmission that occurs in some blood vessels and in the vas deferens. Whereas ATP has a fast depolarizing vasoconstrictor activity, NA produces more slowly developing response (Stjarne, 1989). The synergistic effect of ACh and Leuitenizing hormone releasing hormone (LHRH) in the sympathetic ganglia, where LHRH also has a depolarizing effect and is a cotransmitter with ACh (Jan and Jan, 1983).

In cotransmission, one transmitter may be inactivated earlier than the other one(s). One cotransmitter may have its site of action further away from the site of release and hence have a delayed or a longer lasting effect. There are other NANC transmitters and cotransmitters in the peripheral nervous system (PNS) such as substance P and calcitonin gene related peptide (CGRP). Substance P in sympathetic ganglia has a slow depolarizing effect (Otsuka and Konishi, 1983) and in non-myelinated sensory neurons causes vasodilatation (Foreman, 1987). CGRP, another NANC transmitter, causes vascular leakage and neurogenic inflammation again in the non-myelinated sensory neurons (Saria et al, 1989).

HYPERTENSION

Hypertension is a sustained increase in the blood pressure which if it is not properly treated would lead to other life threatening diseases such as coronary thrombosis (Eich et.al, 1966), renal failure (Guyton et. al., 1990) and strokes (Lund-Johanson and Omvik, 1990) (Kannel, 1974). There are some recognisable causes of hypertension such as phaeochromocytoma that can be cured. However, most types of hypertension are less easily treatable and are classified as essential hypertension.

The causes of elevated blood pressure are usually multifactorial (Rose, 1986, Benowitz and Bourne, 1989) and include genetic and environmental factors. The severity and duration of hypertension also have serious consequences for the arterial wall.

In sustained hypertension, the increase in the peripheral vascular resistance can be caused by:

a) An increase in sympathetic neurotransmitter discharge

b) The presence of vasoconstrictor agents in the circulation

c) An increase in the level of sodium and thus in the extracellular fluid

d) An increase in the production and release of renin from the kidneys, hence more biosynthsis of the potent vasoconstrictor AII

e) Excessive responsiveness to other key factors

Or a combination of these factors.

In hypertension, there is also an elevation of the magnitude of the pressor response to a standard pressor stimulus. This could be the result of the vascular structural changes. Resistance vessels have walls that are thicker than normal in hypertension. This characteristic not only increases vascular resistance but also amplifies all pressor responses caused by vascular smooth muscle contraction (Folkow, 1982; Owen and Schwartz, 1983; Mulvany et. al., 1985).

In hypertension there may be an increased sensitivity to various agonists such as 5-HT or a deficiency in the plasma membrane binding of Ca^{2+} ions or an increased membrane permeability to Na^{+}, K^{+} and Ca^{2+} ions. There may also be a fault in the contractile process of vascular smooth muscle in the hypertension and this may be related to the Ca^{2+} binding protein that regulates contraction (Hatton et. al., 1987; McCarron, 1989). Generally, an increase in the cytosolic concentration of Ca^{2+} is associated with increased vascular tone in hypertension (Cohen, 1983).

The Role of Major Physiological Control Systems in the Maintenance of the Blood Prssure

Several physiological systems are involved in the control of blood pressure: these are the sympathetic nervous system, the renin-angiotensin-aldosterone system, which has a crucial role in the control of Na^{+} ion excretion and body fluid volume, (Valloton, 1987) and nitric oxide (Moncada et al, 1991).

In hypertension, the baroreceptors and the renal blood pressure control are apparently set at a higher blood pressure (Benowitz and Bourne, 1989). The secretion of renin in the kidneys from the cells of juxtaglomerular apparatus can be increased by an abnormality in the vascular smooth muscle cell membrane that causes renal artery sclerosis and hence a fall in the renal blood flow. The secondary effect of the renal blood flow reduction is a further increase in the blood pressure. Therefore it is necessary to determine the original cause of the hypertension to increase life expectancy.

Pulmonary Hypertension

Normally, pulmonary blood vessels are very distensible and can accommodate an increase in the blood flow without a marked change in the pulmonary blood pressure. However, pulmonary hypertension can develop when:

a) The pulmonary vascular bed is constricted

b) The pulmonary vasculature is distended too much with a high flow

c) There are pathological changes, especially in the inner layer of the pulmonary blood vessels including the intima and/or the media layers of the muscular arteries and the arterioles

A defect in the ventricular septum can also give rise to pulmonary hypertension. Less commonly in atrial septal defect or in the patent ductus arteriosus, pulmonary hypertension occurs. The defective pumping activity of the heart due to the anatomical abnormalities mentioned, can be the

underling causes for the development of the pulmonary hypertension, since more blood is accumulated in the pulmonary vasculature in relation to lowered cardiac output (Kissane, 1990).

Some Drugs in the Treatment of Hypertension

ß-adrenoceptor blocking drugs alone or in combination with diuretics are used to treat hypertension. This is not the best choice since ß-blockers have their own specific side effects. ACE inhibitors and Ca^{2+} channel antagonists have been used widely because of their relative lack of side effects and metabolic effects. Other effective vasodilators include nitroprusside, which releases NO in the vascular smooth muscle, and diazoxide which is a K^{+} channel stimulant and therefore causes vascular hyperpolarization (Quast and Cook, 1989) have been used increasingly.

The general use of vasodilators is mainly to augment local tissue blood flow, to attenuate the central venous pressure and reduce arterial pressure. The cardiac work is reduced when there is a reduction in the cardiac pre-load and after-load. When there is a reduction in the cardiac pre-load, the filling pressure is reduced. A reduction in the cardiac after-load would reduce the vascular resistance.

5-HT IN THE PERIPHERAL TISSUES

5-hydroxytryptamine which can be considered as a local hormone and a neurotransmitter because of its various physiological actions and locations

was first found in 1948. It is abundant in platelets, in the GIT (chromaffin cells and enteric neurones) and in CNS (Green, 1985; Fozard, 1989).

5-HT is synthesized from dietary tryptophan and its metabolism closely resembles that of NA (tyrosine is the precursor for the production of NA). Tryptophan is converted to 5-hydroxytryptophan in the presence of the enzyme tryptophan hydroxylase and then decarboxylated to 5-HT by a non-specific decarboxylase (L-aromatic acid decarboxylase). The metabolism of 5-HT is through monoamine oxidase and an aldehyde dehydregenase that converts 5-HT to an aldehyde and then to the main degradative product 5-HIAA (5-hydroxyindoleacetic acid) which is readily excreated in the urine. 5-HT is taken up or transported to the 5-HT-containing cells by a spcific transport system.

Various Functions of 5-HT

5-HT has many actions and functions. These include:

a) Vasoconstriction in large arteries and veins, though the sensitivities of these large blood vessels differ

b) In the microvasculature, 5-HT acts on 5-HT receptors to inhibit transmitter release from the adrenergic nerve terminals. It also dilates arterioles, increases capillary permeability and constricts venules (Rang and Dale, 1991)

c) In platelets, which act as a 5-HT reservoir, 5-HT causes platelet aggregation, leading to thrombosis when the vascular wall is injured. The

accumulation of platelets also encourages further release of 5-HT from platelets. This is an important process in haemostasis (Houston and Vanhoutte, 1986)

d) Increases the motility of the GIT. This action of 5-HT is either due to direct excitation of the GIT smooth muscles or to an indirect effect on the enteric neurons

e) 5-HT also has the ability to contract smooth muscle in the bronchi and in the uterus

f) In the CNS, 5-HT has a variety of effects which stem from both excitatory and inhibitory actions

g) 5-HT initiates pain (Richardson, 1990; Le Bars, 1988) by acting on the peripheral sensory nociceptive nerve endings that also causes flare and wheal

5-HT and its Receptors

5-HT receptors are classified into different subtypes. These include 5-HT_1, 5-HT_2 and 5-HT_3 receptors (Bradley et al, 1986; Peroutka, 1988). 5-HT_1 receptors which are mainly in the CNS are further subdivided into four categories. These are A,B,C and D. In addition to the CNS location of the 5-HT_1 receptors, other 5-HT_1 receptor sites in the choroid plexus, the stomach, the enteric neurons and in the blood vessels are alse involved. The effects of 5-HT in nerves are mainly to inhibit transmitter release, chiefly by

inhibiting adenylate cyclase and in the case of 5-HT_{1C}, by stimulating PI-turnover (Hoyer, 1988 and Julius et al, 1988).

5-HT_2 receptors occur in the smooth muscle and in platelets, as well as in the CNS. They mainly have excitatory effects on smooth muscle (Roth et al, 1986; Cory et al, 1986) and nerves (Hide et al, 1989; Conn and Sanders-Bush, 1984, 1986). Ketanserin and mianserin are specific antagonists for these receptors. Activation of which is associated with increased PI-turnover.

5-HT_3 receptors are found in the peripheral nervous system (PNS) including nociceptive sensory nerves, autonomic nerves and enteric nerves. These have excitatory activity which is mediated through an action on plasma membrane ion channels, that are linked to the 5-HT_3 receptors. In inflammation; Pain, flare and wheal, are also mediated via this 5-HT_3 receptors. Subtypes of 5-HT_3 might also exist but this is still uncertain (Richardson and Engel, 1986). 5-HT also plays a role in migraine (D'Andrea et al, 1994; Martelletti et al, 1994) and carcinoid syndrome (Zambrzycki and Elliott, 1993).

5-HT IN THE CENTRAL NERVOUS SYSTEM (CNS)

The first time it was suspected that 5-HT might act as a central neurotransmitter was in 1953 when Gaddum realized that lysergic acid diethylamide (LSD), a hallucinogenic drug, acted as an antagonist of 5-HT on peripheral tissues and might also owe its central effects to antagonism of 5-HT.

The central pathway in which 5-HT is involved, includes several large groups of 5-HT-containing cells in the pons and upper medulla. These are found close to the midline (raphe) and are sometimes referred to as raphe nuclei. The main two clusters of 5-HT cells are rostral and caudal serotonin nuclei in the rat brain (Rang and Dale, 1991). The rostral serotonin nuclei which also contains many noradrenergic nerves has projections to different parts of the brain via the midial forebrain bundle. These areas of the brain include cortex, hippocampus, thalamus, limbic system and hypothalamus. However, the caudal serotonin nuclei has its projections to the medulla and spinal cord. Various experimental works have suggested that 5-HT interacts with several receptors (Peroutka, 1988) and the outcome of these different receptor activation are postsynaptic excitation and inhibition as well as presynaptic inhibition. 5-HT cells show a highly regular slow neuronal discharge pattern which can be antagonised by 5-HT_1 agonists, indicating the involvement of an inhibitory feedback mechanism.

Some Important CNS Functions of 5-HT

The brain has only about 1% of the total body 5-HT content (Green, 1985) and the main CNS related functions of 5-HT pathways include:

a) Different kinds of behavioural responses (Di Chiara et al, 1971).

b) Feeding behaviour (Marazziti et al, 1988; Curzon, 1990).

c) Regulation of sleep (Koella, 1988; Jouvet, 1967), wakefulness and general mood.

d) Regulation of neuronal sensory pathways.

e) Regulation of body temperature (Myers, 1981; Jacob and Girault, 1979) mediated by hypothalamus.

5-HT may also be involved in the central control of blood pressure, in learning and memory (McEntee and Crook, 1991; Altman and Normile, 1988), sex (Gorzalka et al, 1990; Fernandez-Guasti et al, 1987), neuroendocrine regulations (Van De Kar, 1991; Cowen et al, 1990), motor activity (Sternbach, 1991) and biological rhythms (Wesemann and Weiner, 1990).

THE AIMS OF THIS STUDY

Pithed and Anaesthetised Rats

One of the main objectives of this study was to investigate the cardiovascular responses to spinal nerve stimulation and drugs and to examine the various contributing factors which can modify these responses. The pithed rat was used by Bulloch & McGrath (1988) who suggested that co-transmission involving NA and ATP occurs in the sympathetically innervated blood vessels. This study re-examined this possibility and tried to elucidate whether co-transmission involving NA and ATP occurs and if so, to what extent this co-transmission contributes to the pressor responses to spinal nerve stimulation. This study also examined the role of the adrenal medullae and the catecholamines released from these glands into the blood stream (Bevan et al, 1980; Clutter et al, 1980) and investigated how ß-adrenoceptors influence the pressor responses to spinal nerve stimulation. The possibility that other locally released vasoactive agents, including AII (Rand et al, 1990; Majewski et al, 1984) and bradykinin (Rang & Dale, 1991) may also contribute to and complicate the pressor responses to spinal nerve stimulation was considered. This study also examined the effect of 5-HT which can be released from many sites including platelets into the blood circulation and affect the cardiovascular system (Saxena and Villalon, 1990; Vanhoutte, 1985). In addition, the role of NO (Moncada et al, 1991; Furchgott & Zawadzki, 1980) in the cardiovascular system and its effect on the responses to 5-HT were examined.

Lungs

This study examined the effect of some vasoactive agents including vasopressor agents (5-HT, PE, KCl, NA and adrenaline) and vasodepressor agents (isoprenaline and SNP) in the pulmonary circulation. An important objective of these experiments was to establish whether NO modulates pulmonary vascular tone and the responses to vasoactive agents, particularly 5-HT, in the pulmonary vasculature (Newby and Henderson, 1990).

Some prostaglandins also have vasoactive effects and may affect the pulmonary vascular resistance (Aiken, 1984; Wood et al, 1981) and these endogenously released prostaglandins may also modify the responses to vasoactive agents. An objective of this study was to determine whether endogenous prostanoids affect pressor responses to vasoconstrictors in this vascular bed.

Cyclic nucleotides (cAMP and cGMP) are the main second messengers involved in vasodilatation. Agonists that act on ß-adrenoceptors are known to exert thier vasorelaxant effect by increasing the synthesis of cAMP in the vascular smooth muscle cells (Furchgott & Vanhoutte, 1989 and Furchgott & Martin, 1985). One aim of this study was to determine the effects of the ß-adrenoceptor agonist on the vasoconstrictor effects of 5-HT, PE and KCl.

Hindquarters

Another aspect of this study was to investigate the effect of vasoactive agents in the hindquarters vasculature (Folkow et al, 1970; Cooper and

Wylie, 1979) which, unlike the pulmonary circulation, is a high resistance vascular bed. Fluid flow-induced shear stress may cause the release of endothelial vasodilator agents (Davies, 1989 and Griffith et al, 1988) which may be of great importance in keeping the vascular beds open to perfusion. One objective of this study was to determine whether NO (Hutcheson & Griffith, 1991) and perhaps PGI_2 (Pohl et al, 1986b and Koller & Kaley, 1990b) have any role in modifying tone and the responses of this vascular bed to vasoactive agents, especially 5-HT. Cumulative dose-response curves to vasopressor agents such as 5-HT, PE and KCl and vasodepressor agents such as carbachol and SNP, which were added to the Krebs-buffer that was perfused through the hindquarters vasculature, were also investigated.

It is known that in hypertension, the resistance of blood vessels is increased, perhaps in response to some abnormalities in the Ca^{2+} ion handling of arterial smooth muscle cells and this has been shown in spontaneously-hypertensive rats (SHR) (Bohr & Webb, 1988). In hypertension there is an increase in the incidence of myogenic tone, an increase in the responsiveness of the vascular bed to vasoconstrictor agents and a decrease to vasodilator agents. It is also reported that arteries from SHR have a spontaneous active tone (Asano et al, 1986 and Winquist & Bohr, 1983) that is known as myogenic tone which is independent of local innervation, circulating hormones and metabolic influences. This study investigated the responsiveness of vasculature of the rat hindquarters to both vasoconstrictors and vasodilators. Comparisons were made between the responses obtained in hindquarters preparations from SHR and WKY control rats.

METHOD

Experimental rats

The rats used for research purposes were kept in a controlled area. Their food was obtained from (Labsure CRM diet) and ordinary tap water was used for their drinking. The temperature and light of the animal house were controlled. The temperature was maintained between 18-22^{o}C and the lighting of the area was regulated in a cycle of twelve hours light (ie. 6:30-18:30 h) and twelve hours dark. Adult male rats were used in all the experiments throughout the project. Most experiments were carried out using Wistar rats but in some experiments Sprague Dawley, Spontaneously Hypertensive (SHR) and Wistar Kyoto (WKY) rats were used.

Krebs solution

Krebs physiological buffer which was originally described by Krebs and Henseleit in 1932 was used for perfusions and drug preparations.

Its composition (mM):

NaCl, 118.5; KCl, 4.75; $CaCl_2$, 2.5; $MgSO_4.7H_2O$, 1.0; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; Glucose, 22.2.

It was also gassed with a mixture of 95%O_2 / 5%CO_2.

Pithed rat preparation

Male Wistar rats (250-350 g) were anaesthetised withtrichloroethylene and respired artificially (stroke volume = 2.5 ml, rate = 55 min^{-1}) with air through a polythene cannula that was tied into the trachea. Each rat was pithed by inserting through the orbit a steel guide tube, through which a flexible teflon tube containing the electrode was passed (Gillespie and Muir, 1967; Gillespie et al, 1970). This teflon sheathed electrode was inserted into the foramen magnum and down the spinal canal to the point of furthest penetration in the sacral region (Fig-4). An indifferent electrode was inserted under the skin behind the skull and along the length of the spine. The left carotid artery was cannulated to record blood pressure and the femoral vein was cannulated to permit the administration of drugs. Blood pressure and pressor responses were recorded with a Grass pressure transducer and were displayed on a Linseis pen recorder. The temperature ($30^{o}C$) of the rat was maintained during the experiment with a table lamp. In most studies a 1 cm electrode was used (Fig-4). This was easily achieved since the relative lengths of the teflon shielding tube and the electrode itself were known. The position of the electrode in the spinal column was determined radiographically. In preliminary experiments the effect of stimulating at different positions was investigated and the responses obtained were then related to the position of the electrode within the spine. From such experiments, information was also obtained about the positions of the spinal outflows in rats of different sizes (100-400 g). These studies provided data that was used to chart the spinal outflows and the consequences of stimulating at different positions (Fig-5). In most studies stimulation with a 1 cm electrode between the eight and the ninth thoracic vertebrae (T_8 and T_9) was used. This position was chosen since it corresponded to that used in previous studies where purinergic nerves and co-transmission had been

studied (Bulloch & McGrath, 1988). In most experiments, electrical stimulation was with supramaximal voltage, 0.4 ms pulse-duration for 5 and 50 s at 2 Hz and 20 Hz. Gallamine (10 mg kg^{-1}, iv.) was administered as a muscle relaxant to stop muscle twitching during electrical stimulation. During the experiment further injections were usually necessary to maintain neuromuscular blockade.

Adrenalectomy

Rats were pithed according to the method described above and acute bilateral adrenalectomy was performed via lateral incisions above each kidney. Adrenalectomy made the blood pressure unstable and caused a fall in the basal blood pressure. Therefore, after adrenalectomy, pithed rats were left for about 15 to 20 min until the blood pressure became stable before carrying out the rest of the experiment. Blood pressure was measured via the left carotid artery and drugs were administered via the right femoral vein.

Anaesthetised rat preparation

Adult male Wistar rats (250-350 g) were injected with sodium pentobarbitone (60 mg kg^{-1}, ip.), which was enough to keep the rat unconscious for approximately 2 h. Further doses of sodium pentobarbitone were administered throughout the experiments to maintain anaesthesia. The trachea was then cannulated and each rat was respired artificially with an air pump (stroke volume = 2.5 ml, rate = 55 min^{-1}).

The left carotid artery and one femoral vein were also cannulated to allow blood pressure measurement and drug administration, respectively. The carotid artery was then connected through its cannula to a Grass pressure transducer and a Linseis pen recorder to record the basal blood pressure as well as any changes induced by drug administrations. Saline (0.2 ml) was injected after each intravenous drug injection to wash the drug into the circulation. The areas exposed by dissection were also covered with a saline moistened tissue paper to prevent the underlying living tissues getting dried.

Blood pressure measurement (tail cuff method)

Rats were kept in a warming chamber (temperature at $37^{o}C$) for about 15 min to cause vasodilatation before measuring blood pressure using the rat tail artery. Prior warming of the rat made recording of the blood pressure easier. Each rat was then brought out of the warming chamber and was placed in a restrainer. The tail of the rat was passed through an inflatable cuff and a pressure transducer which were connected to a pre-calibrated storage oscilloscope. The position of the inflatable cuff was in the base of the tail, close to the body and the pressure transducer was distal, midway along the tail.

In order to measure the systolic blood pressure, a motor driven piston was used to inflate the basal cuff which eventually stop the arterial blood flow in the tail. The pressure could be rapidly released by deflating the cuff. The point on the calibrated screen of the oscilloscope where the pulsing of the blood pressure first disappeared and later reappeared again, due respectively to inflation and deflation of the cuff, were recorded and indicated the systolic blood pressure.

Lungs preparation

Male Wistar rats (250-350 g) were anaesthetised with sodium pentobarbitone (60 mg kg^{-1}, ip.) and heparinised with heparin sulphate (2000 i.u. kg^{-1}, ip.) to prevent blood clotting. Once the rat was under deep anaesthesia and unresponsive to mechanical stimuli such as pressing its foot, the trachea was dissected, exposed and cannulated with a polythene cannula. After tracheotomy the rat was killed by exanguination. A stainless steel cannula which was connected to a 20 cm syringe, filled with Krebs-buffer was then quickly inserted into the pulmonary artery through an incision in the right ventricle of the heart and then tied in place. Another incision was made in the left atrium to allow the Krebs-solution contaminated, with blood to escape. Usually, 20-30 ml Krebs-buffer under approximately a 20 cm H_2O pressure would be sufficient to wash the blood out of the pulmonary circulation.

When all the blood was washed out, the lungs changed colour from pink to white. During this process the lungs were mechanically ventilated with an air pump which was connected to the tracheal cannula. This facilitated the washing of the blood out of the pulmonary circulation with Krebs-buffer.

The lungs were then left in situ and placed on a heating water blanket at a temperature of 37^{o}C and were usually perfused at a flow rate of 5 ml min^{-1} with Krebs-buffer at 35^{o}C (Fig-32). The perfusion pump used in these experiments was a peristaltic, constant output pump, which produced a pulsatile flow through the pulmonary circulation. The lungs were perfused in situ and were covered with a plastic blanket to keep them warm and moist. Pulmonary artery perfusion pressure was monitered using a Statham pressure transducer (model no. P21B) which was connected to the

perfusion circuit. The perfusion pressure and any drug-induced changes in the perfusion pressure were then recorded on a Linseis pen recorder. All the measurements either for changes in the basal perfusion pressure or for the effects of drugs were recorded from the upper parts of the traces (equivalent to the systolic pressure). The mean basal pulmonary arterial pressure was usually at 12-15 mm Hg. Drugs were administered by simply perfusing the pulmonary circulation with Krebs-buffer, containing the drugs and obtaining a stable equilibrium response.

Hypoxic (14% O_2) gas preparation

Douglas bags were prepared and used as a reservior to hold a mixture, containing 14% O_2, 5% CO_2 and 81% N_2 gasses. This low O_2 gas mixture which was much lower than the usual 95% O_2 mixture used to gas Krebs-buffer, was used to study the effect of perfusing a hypoxic solution through the pulmonary vasculature.

Hindquarters preparation (Brown, 1982)

Experimental rats were heparinized (2000 i.u. kg^{-1}, ip.) about 10 min before killing by exanguination. The abdominal cavity was cut open, the intestine and rest of the organs in the cavity were removed. The abdominal artery was then dissected free from the surrounding connective tissues to insert a polythene cannula for the Krebs-buffer perfusion. The cannula which was connected to a 20 ml syringe containing the Krebs-buffer solution was tied in place with the tip of it being located just above the abdominal arterial bifurcation that gives rise to the common iliac arteries which supply the

legs. Then the rat was cut in half below the kidneys and the hindquarters circulation was washed with Krebs-buffer to remove any blood left in the blood vessels. Usually 20-30 ml of Krebs-buffer solution under a 20 cm H_2O pressure was sufficient to remove the residual blood in the hindquarters vasculature.

The hindquarters were place on a heating water jacket (37^{o}C) and connected to an adjustable peristaltic perfusion pump, which perfused Krebs-buffer (35^{o}C) through the vasculature of the hindquarters. The perfusion rate was usually at 5 ml min^{-1} and the apparatus assembly for the hindquarters preparation was the same as it was for the lungs preparation. All the measurements either for the basal perfusion pressure or the effects of drugs were recorded from the upper parts of the traces.

Rectal temperature was measured by a thermometer to monitor the temperature of the hindquarters.

Drugs

The following drugs were used in this study:

Adrenaline (B.D.H.), α,ß-mATP (Sigma), ascorbic acid (May & Baker), captopril (Sigma), carbamylcholine chloride (carbachol) (Sigma), flurbiprofen (Sigma), gallamine triethiodide (Sigma), guanethidine sulphate (Ciba), heparin sodium (Sigma), 5-hydroxytryptamine hydrochloride (Sigma), indomethacin (Sigma), isoprenaline (Sigma), KCl (Fisons), ketanserin (Sigma), L-arginine hydrochloride (Sigma), L-NAME (Sigma), L-phenylephrine hydrochloride (Sigma), noradrenaline bitartrate (Sigma), prazosin

hydrochloride (pfizer), propranolol hydrochloride (Sigma), sodium nitroprusside (Sigma), sodium pentobarbitone (May & Baker), yohimbine (Sigma).

Drugs were prepared in 0.9% saline for use in the experiments with pithed and anaesthetised rats. In experiments in lungs and hindquarters, drugs were dissolved in the Krebs-buffer solution.

Statistical analysis

The results were expressed as (Means ± SEM). Comparisons of data were carried out using Student's t-test, where appropriate, the non parametric Mann-Whitney test. A probability value of < 0.05 was considered to be significant.

Regression line analysis

In some experiments, comparisons of concentration-response relationships to an agonist in the absence and presence of an antagonist were performed through the comparison of the regression lines of the curves. This can be done by the method described by Bowman and Rand (1980) and using the following formulae, the two regression lines were tested for coincidence be a t-test.

$$t = \frac{\left| \frac{\bar{Y}_A - \bar{Y}_B}{\bar{X}_A - \bar{X}_B} - \bar{b}_{YX} \right|}{\sqrt{\left[\bar{S}_{Y(X)}^2 \left\{ \left(\frac{1}{(\bar{X}_A - \bar{X}_B)^2} \right) \left(\frac{1}{N_A} + \frac{1}{N_B} \right) + \left(\frac{1}{SX_A + SX_B} \right) \right\} \right]}}$$

where: $\bar{Y}_A$= mean of Y values in A

$\bar{X}_A$= mean of X values in A

$\bar{Y}_B$= mean of Y values in B

$\bar{X}_B$= mean of X values in B

N_A= number of observation in A

N_B= number of observation in B

$\bar{b}_{YX}$= combined regression coefficient

$$= \frac{(SXY)_A + (SXY)_B}{SX_A + SX_B}$$

$\bar{S}_{Y(X)}^2$= combined residual variance

$$= \frac{SY(X)_A + SY(X)_B + \frac{(b_A - b_B)^2}{\frac{1}{SX_A} + \frac{1}{SX_B}}}{N_A + N_B - 3}$$

where: $SX = \Sigma X^2 - \frac{(\Sigma X)^2}{N}$

$SY = \Sigma Y^2 - \frac{(\Sigma Y)^2}{N}$

$SXY = \Sigma XY - \frac{\Sigma X \Sigma Y}{N}$

$SY(X) = SY - \frac{(SXY)^2}{SX}$

RESULTS

Effects of Electrical Stimulation of the Sympathetic Outflow in the Pithed Rat Using the Spinal Electrode and the Effects of Drugs on the Responses Produced

Effects of electrical stimulation of the spinal sympathetic outflow

Electrical stimulation of the sympathetic outflow (NS) in the pithed rat at different levels within the spinal canal produced characteristic increases in the heart rate (HR) and blood pressure (BP). The magnitude of these responses differed according to the region of the thoraco-lumber outflow stimulated. The results obtained are shown in Fig-5. The largest pressor responses were obtained at the eighth to the ninth thoracic vertebrae (T_{8-9}) (Figs-5,6). Stimulation at this position also produced a large increase in HR, which suggested that the adrenals were also being stimulated at this point, since the cardio-accelerator nerves arise at the sixth cervical to the second thoracic vertebrae (C_6-T_2) (Gillespie et al., 1970) and could not account for such a response. Subsequent experiments in adrenalectomised rats confirmed that a large component of the pressor response produced by stimulation at T_{8-9} was caused by the release of catecholamines from the adrenal medullae (Figs-7,10). In this study, the largest increases in HR occurred at the second to the fourth thoracic vertebrae (T_{2-4}), which is close to the point where the cardio-accelerator nerves leave the column. At this point, the pressor response was small. The most likely explanation of the cardio-acceleration caused by stimulation at other segments is that

circulating catecholamines from the adrenal glands are responsible for increasing HR.

Role of adrenal medulla and effect of propranolol

Pressor responses to nerve stimulation at T_{8-9} were usually biphasic and this was most marked at higher frequencies of stimulation (Fig-6). The first phase occurred during stimulation and the second phase persisted after stimulation had stopped (Fig-6). The occurence of such a biphasic response also suggests that there may be a substantial adrenal component in the response to spinal nerve stimulation at T_{8-9}. This possibility was examined in a number of ways. First, the effects of propranolol (2 mg kg^{-1}) on responses to nerve stimulation were examined (Fig-8). Propranolol inhibited the cardio-accelaration (not shown) and usually potentiated the second phase of the pressor response, which persisted after nerve stimulation had stopped. This is consistent with the possibility that electrical stimulation at T_{8-9} released catecholamines from the adrenal medullae. In some experiments, where the resting BP was very low (BP < 18-22 mmHg), propranolol did not potentiate the second phase of the pressor response, but instead inhibited the pressor response, particularly to stimulation at 20 Hz (Fig-9). It may be that in these animals, blockade of cardiac $ß_1$-adrenoceptors by propranolol, impaired their ability to maintain a pressor response, since the heart could not cope with pumping blood into the constricted peripheral vasculature. Another possibility is that propranolol may have impaired the renin-angiotensin mechanism.

Effect of adrenalectomy

The effects of adrenalectomy on the responses to spinal nerve stimulation was examined in the pithed rats. Adrenalectomy inhibited both components of the pressor response to nerve stimulation and usually converted the biphasic response into a small monophasic response that occurred only during nerve stimulation (Figs-7,10). Adrenalectomy did not inhibit responses to injected NA (Fig-11).

Effect of alpha, beta-methylene ATP (α,ß-mATP)

α,ß-mATP (2x0.05 mg kg^{-1} and then 0.1 mg kg^{-1}) produced large pressor responses, which showed tachyphylaxis that developed slowly and was readily reversible with time (Fig-12). In experiments in which desensitization to the pressor effect of α,ß-mATP developed, responses to nerve stimulation also declined but not to a greater extent than did responses to equi-active doses of exogenous NA (9 μg kg^{-1}). Cessation of administration of α,ß-mATP restored the sensitivity to α,ß-mATP as measured by the return of the pressor response to α,ß-mATP but did not restore the sensitivity to NA or to spinal nerve stimulation (Fig-12). Further administration of α,ß-mATP produced a pressor response that again showed tachyphylaxis but the standard responses to NA and spinal nerve stimulation were unaffected (Fig-12).

Effects of prazosin and yohimbine

Prazosin (2 mg kg^{-1}) inhibited both components of the pressor response to stimulation of the spinal autonomic outflows at T_{8-9} and inhibited pressor responses to injected NA (0.3 μg kg^{-1}) (Fig-13). The prazosin-induced decline in pressor responses to spinal nerve stimulation matched the prazosin-induced decline in pressor responses to injected NA (Fig-13).

Yohimbine (1 mg kg^{-1}) inhibited pressor responses to injected NA (0.1, 1.3, 22.2 μg kg^{-1}) and converted the biphasic pressor responses to spinal nerve stimulation into monophasic responses of brief duration. In these residual responses, the secondary response that persisted after nerve stimulation was abolished but the pressor response that occurred during stimulation was slightly potentiated, probably reflecting inhibition of presynaptically-mediated autoinhibition and increased release of NA (Fig-14). Any residual nerve stimulation-induced pressor response that remained after treatment with prazosin and yohimbine was abolished by guanethidine (2 mg kg^{-1}) (Fig-15). In some pithed rats, yohimbine (2 mg kg^{-1}) caused the basal BP to fall and become unstable and this was followed quickly by death.

Effect of captopril

In the presence of propranolol (2 mg kg^{-1}), captopril (1 mg kg^{-1}) did not affect the basal BP (36 mg kg^{-1}) in the pithed rat (Fig-16). However, pressor responses to spinal nerve stimulation at T_{8-9} and to injected NA (0.3 μg kg^{-1}) were inhibited by captopril (1 mg kg^{-1}). In the presence of captopril (1 mg kg^{-1}), prazosin (2 mg kg^{-1}) further inhibited the pressor responses to nerve stimulation and NA (Figs-16,17).

Effect of L-NAME

In the absence of propranolol, L-NAME (45 mg kg^{-1}) increased the basal blood pressure to a similar extent in anaesthetized (Mean = 59.0 mm Hg, SEM = 13.6 mm Hg, n = 3) and pithed (Mean = 68.5 mm Hg, SEM = 14.8 mm Hg, n = 4) rats but in the presence of propranolol (2 mg kg^{-1}) which lowered the basal blood pressure, L-NAME (45 mg kg^{-1}) raised the basal blood pressure to a lesser extent in pithed rats (Mean = 12.3 mm Hg, SEM = 1.8 mm Hg, n = 19) than in anaesthetized rats (Mean = 55.6 mm Hg, SEM = 8.8 mm Hg, n = 12) (Fig-18). In the pithed rat, the extent to which L-NAME raised the basal BP was proportional to the initial level of the basal BP in the absence (correlation coefficient $R = 0.96$, $n = 5$, $0.01 < P < 0.001$) and presence (correlation coefficient $R = 0.64$, $n = 19$, $0.01 < P < 0.001$) of propranolol (2 mg kg^{-1}) (Fig-19) but in anaesthetised rats, there was no linear correlation between the basal blood pressure and L-NAME-induced increase in the blood pressure either in the absence or presence of propranolol (2 mg kg^{-1}) (Fig-20).

L-NAME (45 mg kg^{-1}) occasionally enhanced pressor responses to spinal nerve stimulation (NS) and NA but overall it did not potentiate pressor responses to NS (Figs-21,22), or injected NA (0.1-15 μg kg^{-1}) (Fig-23) but did potentiate pressor responses to injected 5-HT (25 μg kg^{-1}) in the presence of propranolol (2 mg kg^{-1}) (Fig-24). After administration of L-NAME (45 mg kg^{-1}), recovery from the enhanced pressor responses to drugs or spinal nerve stimulation was usually slower than the recovery prior to administration of L-NAME (Figs-21 and 24). The effect of α,β-mATP was lethal after L-NAME (45 mgkg^{-1}) and the rat did not survive (Fig-25).

Effects of L-arginine

L-arginine (45 mg kg^{-1}) lowered the basal BP, reversed the effects of L-NAME (45 mg kg^{-1}) and inhibited pressor responses to injected NA and to nerve stimulation (Figs-26,27). L-arginine also shortened the duration of action of responses to injected NA and to spinal nerve stimulation (Fig-26).

Effects of 5-hydroxytrytamine (5-HT)

In pithed rats 5-HT (6-50 μg kg^{-1}) produced dose-related pressor responses in the absence and presence of L-NAME (45 mg kg^{-1}) while propranolol (2 mg kg^{-1}) was present throughout the experiments (Fig-28). Pressor responses to 5-HT were potentiated and prolonged by L-NAME (45 mg kg^{-1}) (Figs-28,29). 5-HT (6 μg kg^{-1}) inhibited the pressor components to the spinal nerve stimulation (2 Hz, 20 Hz, 100 pulses) of the sympathetic outflow at T_{8-9}. This inhibitory effect of 5-HT occurred in the presence of both propranolol (2 mg kg^{-1}) and L-NAME (45 mg kg^{-1}) (Fig-22). In anaesthetised rats, 5-HT produced a triphasic response in the blood pressure which consisted of an initial brief depressor component, a secondary pressor component followed by a final prolonged depressor component. This triphasic response to 5-HT was most marked at higher doses of 5-HT (12-55 μg kg^{-1}). L-NAME (45 mg kg^{-1}) produced a large increase in the baseline blood pressure and enhanced and prolonged the secondary pressor component and reduced but did not abolish the depressor components to 5-HT (Figs-30,31).

The Effects of Drugs in the Vasculature of the Perfused Pulmonary Circulation of the Rat

Preliminary experiments, performed using different perfusion rates (3, 5 and 12 ml min^{-1}), indicated that a perfusion rate of 5 ml min^{-1} was satisfactory. Higher perfusion rates produced preparations that were unstable, gave variable results and developed oedema early in the experiment. In this study, drugs were perfused for a sufficient period to produce an equilibrium response.

Effects of vasoconstrictors in the perfused pulmonary vasculature

Effects of Phenylephrine (PE)

PE was administered in a range of concentrations (0.1-100 μM), each of which was perfused until a stable equilibrium response was obtained (Fig-33). PE produced small pressor responses, the size of which was dependent on the manner of perfusion. PE produced very small pressor responses when perfused cumulatively with no interval between each drug concentration perfused (Fig-33 Panel A). These small pressor responses to PE were better maintained than were pressor responses to 5-HT.

Effects of 5-hydroxytryptamine (5-HT)

5-HT (0.1-10000 μM) was administered by perfusion to obtain an equilibrium response. 5-HT produced very small pressor responses when it

was perfused cumulatively in increasing concentrations (Fig-34 Panel A). The pressor responses to 5-HT were not well-maintained.

Effects of Potassium Chloride (KCl)

KCl produced well-maintained pressor responses in the perfused pulmonary vasculature. In order to obtain a dose-response curve to KCl, increasing concentrations of KCl (3-80 mM) were perfused until at each concentration, a stable, equilibrium response was obtained (Fig-35 Panel A).

Effects of Noradrenaline (NA)

NA (1-100 μM) produced small pressor responses even when perfused continuously to produce equilibrium responses. Recovery was quick after washing the drug out of the pulmonary circulation with Krebs-buffer (Fig-36, right hand panel). In some experiments, NA (1-100 μM) produced poorly-maintained pressor responses, particularly at submaximal (10 μM) and maximal (100 μM) concentrations. In these experiments, the addition of propranolol to the perfusate during the declining phase of the pressor response to NA (100 μM) raised the tone and produced a well-maintained pressor response to NA (Fig-36, left hand panel).

Effects of Adrenaline

Adrenaline (10 and 100 μM) produced small, poorly-maintained pressor responses when perfused continuously to produce equilibrium responses

(Fig-37 Panel A). Adrenaline-induced responses were small even when there were recovery intervals between each concentration perfused.

Effects of KCl on 5-HT responses

Some pressor responses to 5-HT (1-100 μM) administered in the presence of L-NAME (400 μM), were potentiated in preparations, which had previously been perfused with and constricted by a submaximal concentration of KCl (40 mM). In these preparations the pressor responses to submaximal and maximal concentrations of 5-HT were larger than those obtained in preparations that had not previously been exposed to KCl (Fig-38).

Responses to PE in Wistar and Sprague Dawley rats

PE (0.01-100 μM) produced pressor responses in the perfused pulmonary vasculature of Wistar and Sprague Dawley rats. When the cumulative concentration response curves to PE were compared, it was clear that the submaximal pressor responses (0.1-1 μM) obtained in lungs from Sprague Dawley rats were larger than those obtained in Wistar rats (Fig-39).

Effects of antagonists in the perfused pulmonary vasculature

Effects of Ketanserin

Ketanserin (1 nM) had little effect on the basal perfusion pressure but inhibited the pressor responses to equilibrium perfused concentrations of 5-HT (1, 10 and 100 μM) in the presence of L-NAME (400 μM) (Fig-40). It is likely that the 5-HT_2 receptor is involved in the pulmonary vasoconstriction caused by 5-HT.

Effects of a combination of L-NAME, Propranolol and Yohimbine on pressor responses to PE

The pressor responses to PE (0.1-100 μM) in the perfused pulmonary vasculature of the rat were enhanced by perfusing the vasculature with a combination of L-NAME (400 μM), propranolol (10 μM) and yohimbine (1 μM). This was reflected in the cumulative concentration-response curve to PE (Fig-41).

Effects of L-NAME

L-NAME (400 μM) caused a small but prolonged rise in the basal perfusion pressure in the perfused lungs and enhanced the pressor responses to 5-HT, PE, KCl and adrenaline (Figs-34,42,43;44;35;37).

Effects of Flurbiprofen

Flurbiprofen (2 μM) had little effect on the basal perfusion pressure in the Krebs-buffer perfused pulmonary circulation. Flurbiprofen only enhanced responses to the middle range of concentrations of KCl (30 mM) but did not affect the responses to other concentrations of KCl (Fig-45). Flurbiprofen (2 μM) had no effect on the responses to PE (0.01-100 μM) (Fig-46). In addition, Flurbiprofen (2 μM) enhanced the pressor responses to a middle range concentration of 5-HT (20 μM) perfused to equilibrium in the presence of L-NAME (400 μM) (Fig-47). Flurbiprofen (2 μM) increased the rate of rise of the pressor responses to NA (0.1-10 μM) (Fig-48) but had little effect on the pressor responses of adrenaline (0.1-100 μM) (Fig-49).

Effects of hypoxia and propranolol

When the percentage of oxygen in the gas mixture used to oxygenate the Krebs-buffer was reduced and the composition of the gas mixture was changed from 95%O_2 and 5%CO_2 to 14%O_2, 5%CO_2 and 81%N_2, the pressor responses to NA were reduced (Figs-50,51). The addition of propranolol (10 μM) to the perfusate, gassed with 14%O_2, increased the pressor responses to NA (Figs-52,53).

Effects of vasodilators in the perfused pulmonary vasculature

Effects of Isoprenaline

Isoprenaline (10^{-7}-10^{-4} M) caused vasorelaxation in the pulmonary vasculature, in which the tone had been increased by perfusing the vessels with Krebs-buffer, containing vasopressor agents which included 5-HT (50 μM), PE (100 μM) and KCl (50 mM). In these experiments each concentration of isoprenaline was perfused until a stable, equillibrium response was obtained. The concentrations of the vasoconstrictors were submaximal. Isoprenaline almost completely inhibited the vasoconstrictor effects of 5-HT (Fig-54) and PE (Fig-55) but only partially inhibited the vasoconstrictor effect of KCl (Fig-56). The vasorelaxant effect of isoprenaline (10^{-8}-10^{-5} M) was unaffected by L-NAME (400 μM) (Fig-54).

Effects of SNP

SNP (10^{-10}-10^{-4} M) caused vasorelaxation in the pulmonary vasculature, in which the tone had been raised by perfusing the vessels with Krebs-buffer, containing vasopressor agents, which included 5-HT (20 μM), PE (50 μM) and KCl (40 mM). In these experiments, each concentration of SNP was perfused until a stable, equilibrium response was obtained. The concentrations of the vasoconstrictors were submaximal and increasing concentrations of SNP produced correspondingly increased inhibitory responses. In the presence of L-NAME (400 μM), SNP (10^{-10}-10^{-4} M) readily inhibited the pressor responses to 5-HT (20 μM) and to PE (50 μM) (Figs-57,58) but in the absence of L-NAME (400 μM), SNP only partially

inhibited the pressor response to PE (50 μM) (Fig-58). SNP (10^{-10}-10^{-4} M) produced small concentration-dependent relaxations of KCl-induced tone and these relaxations were enhanced in the presence of L-NAME (400 μM) (Fig-59). Apparently, flurbiprofen (2 μM) also increased the inhibitory effect of increasing concentration of SNP on the ongoing pressor response of PE (50 μM) (Fig-60).

The Effects of Drugs in the Vasculature of the Perfused Hindquarters Circulation of the Rat

Effects of vasoconstrictors in the vasculature of the perfused hindquarters

Effects of 5-HT

5-HT (0.1-100 μM) had a concentration-dependent vasoconstrictor effect which was well-maintained in the vasculature of the rat hindquarters and this was reflected in the increased resistance of the hindquarters to perfusion (Fig-61). The pressor responses to 5-HT, each concentration of which was perfused until a stable, equilibrium response was obtained, was large enough and readily allowed a cumulative concentration-response curve to be produced (Fig-62 panels A & B). The pressor effect of 5-HT (0.001-10 μM) was additive with the pressor response to PE (10 μM) (Fig-63).

Effects of PE

PE (0.1-100 μM) had a vasoconstrictor effect in the vasculature of the rat hindquarters, and this was reflected in the cumulative concentration-response curves (Fig-64 panels A & B). Responses to PE were well-maintained (Fig-65).

Effects of KCl

KCl (10-300 mM) also increased the resistance of the vasculature of the rat hindquarters to perfusion. Pressor responses to KCl (10-300 mM) were large and well-maintained (Figs-66,67 panels A & B).

Comparisons of the maximal pressor responses to KCl, 5-HT and PE in SHR and WKY hindquarters vasculatures

SHR rats (Mean = 184.29 mmHg, SEM = 6.85 mmHg, n = 7, $P < 0.001$) have higher basal blood pressure than WKY rats (Mean = 120.00 mmHg, SEM = 3.78 mmHg, n = 7) and this is illustrated in Fig-68. There was no significant difference between the pressor responses to KCl, 5-HT and PE in hindquarters from SHR compared with responses to these vasoconstrictors in WKY control rats (Fig-69). Fig-70 shows sample traces of pressor responses to increasing concentrations of cumulatively perfused PE (0.1-100 μM) and 5-HT (0.1-10 μM) in a SHR hindquarters vasculature. A comparison of the peak pressor responses to KCl (100 mM), 5-HT (10 μM) and PE (100 μM) in SHR and WKY control hindquarters vasculature showed a significant difference ($P < 0.01$) between the peak pressor responses of

KCl and PE in SHR rats (Fig-71 lower panel). The peak pressor response to KCl was larger than those to both 5-HT and PE in WKY control rats (Fig-71 upper panel).

Effects of antagonists in the vasculature of the perfused hindquarters

Effects of L-NAME

L-NAME (400 μM) increased the basal perfusion pressure in the rat hindquarters and apparently enhanced the pressor responses to 5-HT (0.01-100 μM) when comparison was made between responses to 5-HT, before and after administration of L-NAME. However, when comparison was made between responses to 5-HT after L-NAME and responses to 5-HT administered for the second time to hindquarters preparations, which had not received L-NAME and therefore which acted as time controls, L-NAME did not significantly potentiate the responses to 5-HT (Fig-72).

Effects of Indomethacin

Indomethacin (10 μM) produced a small, transient pressor response, followed by a slight depressor effect on the basal perfusion pressure in the hindquarters of the rat. Indomethacin (10 μM) did not affect the pressor responses to 5-HT (0.01-100 μM) (Fig-73).

Effects of vasodilators in the vasculature of the perfused hindquarters

Effects of SNP

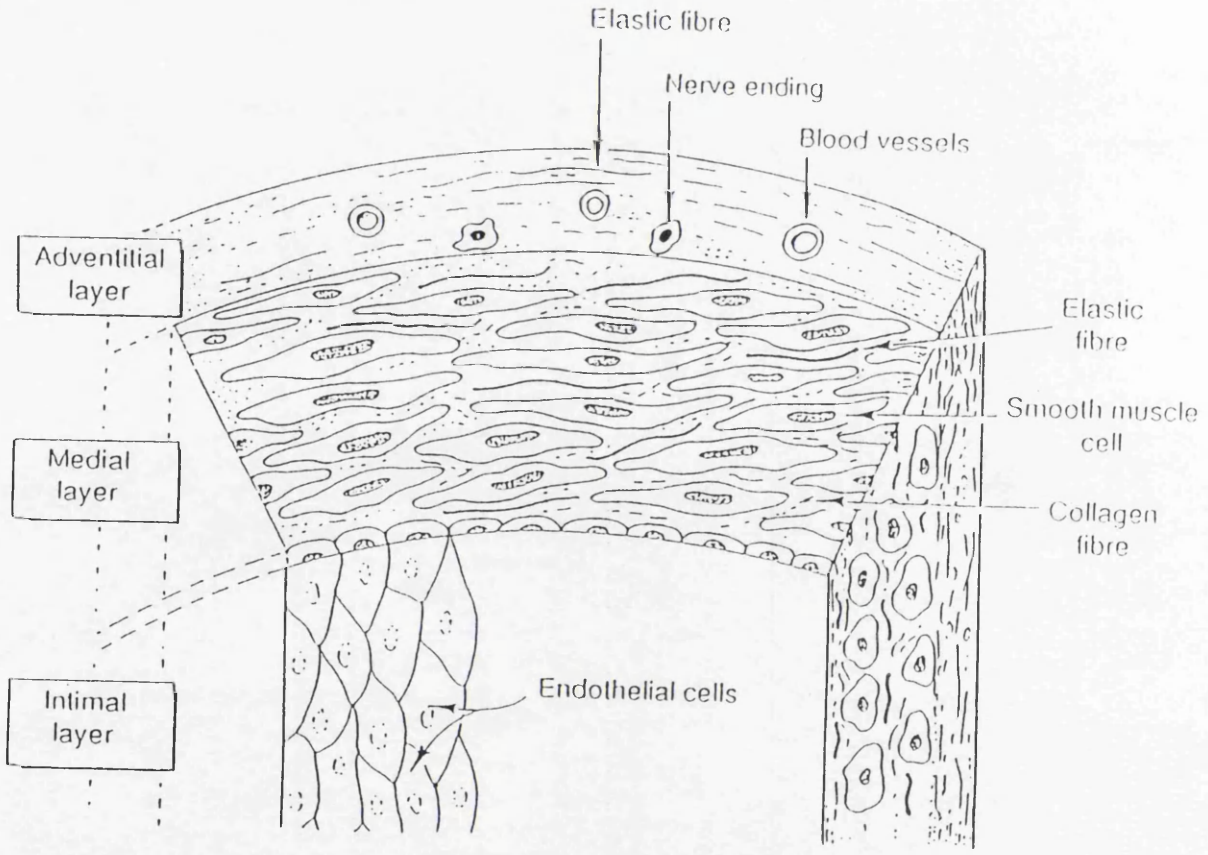
SNP (10^{-9}-10^{-4} M), perfused cumulatively to equilibrium, produced concentration-dependent inhibitions of pressor responses to submaximal concentrations of 5-HT (1 μM), PE (10 μM) and KCl (60 mM) in the Krebs-buffer perfused hindquarters vasculature of Wistar rats. SNP completely inhibited pressor responses to 5-HT (Fig-62 panels C & D) and PE (Fig-64 panels C and D) and almost completely inhibited KCl (Fig-67 panels C & D)-induced pressor responses. SNP as a source of endogenous NO, was therefore, very effective in opposing the pressor responses to agonists that activated the PI-mechanism, but was less effective in inhibiting responses to KCl.

In hindquarters vasculature from SHR and WKY rats, SNP (10^{-9}-10^{-5} M) did not completely inhibit the pressor response to a submaximal concentration of PE (10 μM)(Fig-74). However, there was no significant difference between the inhibitory effect of SNP on PE-induced pressor responses in hindquarters vasculature from SHR and WKY rats. This observation indicates that, SNP was less effective in inhibiting pressor responses to PE in SHR and WKY rats' hindquarters vasculature than it was in Wistar rats.

Effects of carbachol

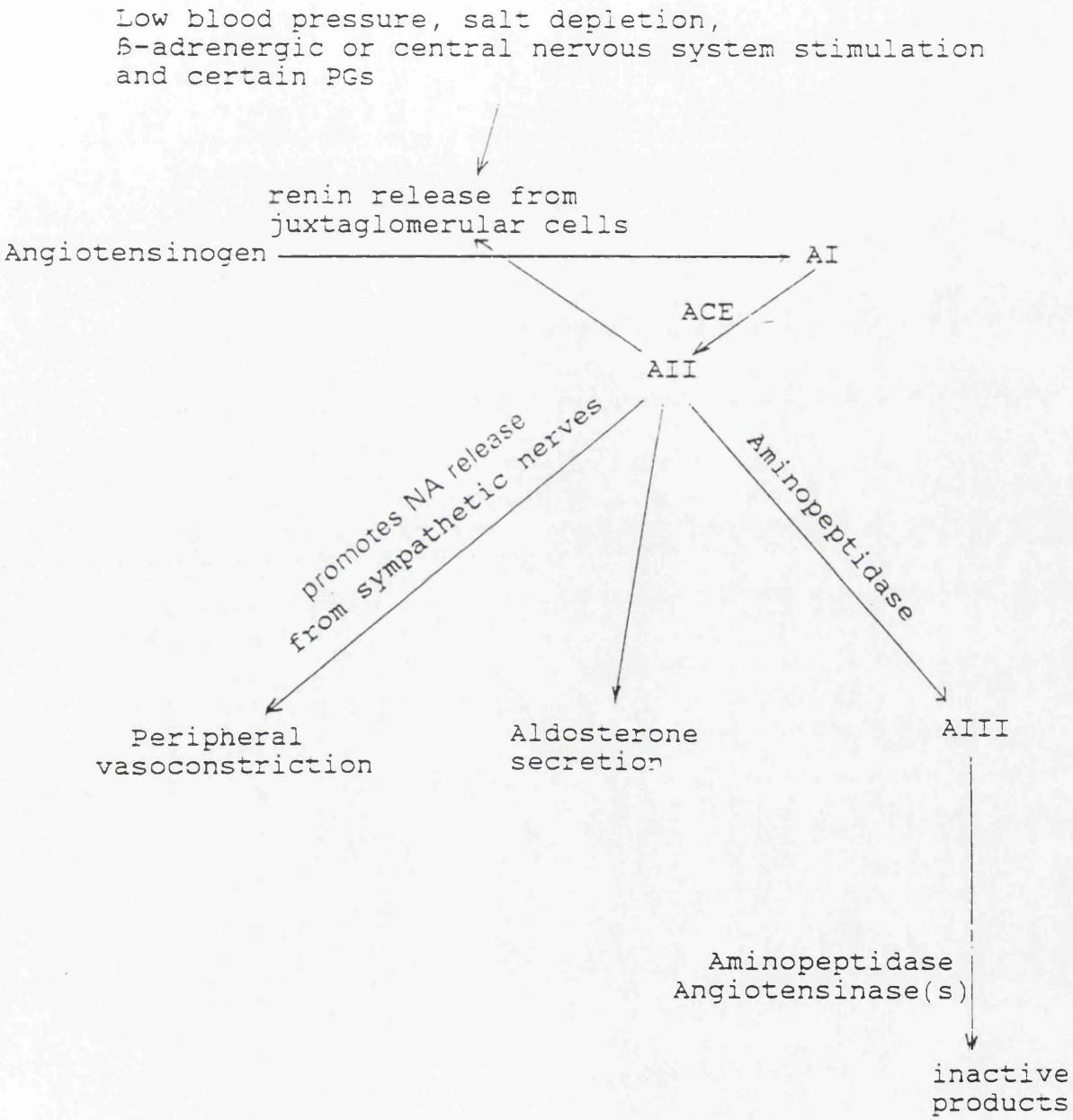
The effects of carbachol (10^{-9}-10^{-4} M) were examined in the perfused hindquarters of SHR and WKY rats. Carbachol was perfused cumulatively in increasing concentrations in Krebs-buffer to obtain equilibrium responses in preparations in which the tone had already been raised by a submaximal concentration of PE (10 μM). Carbachol produced a complex concentration-response curve, which consisted of an early pressor response at low concentrations, followed by inhibitory responses at high concentrations. There was no difference between the complex effects of carbachol in SHR and in WKY rats (Fig-75).

Fig-1 The structure of a blood vessel wall.



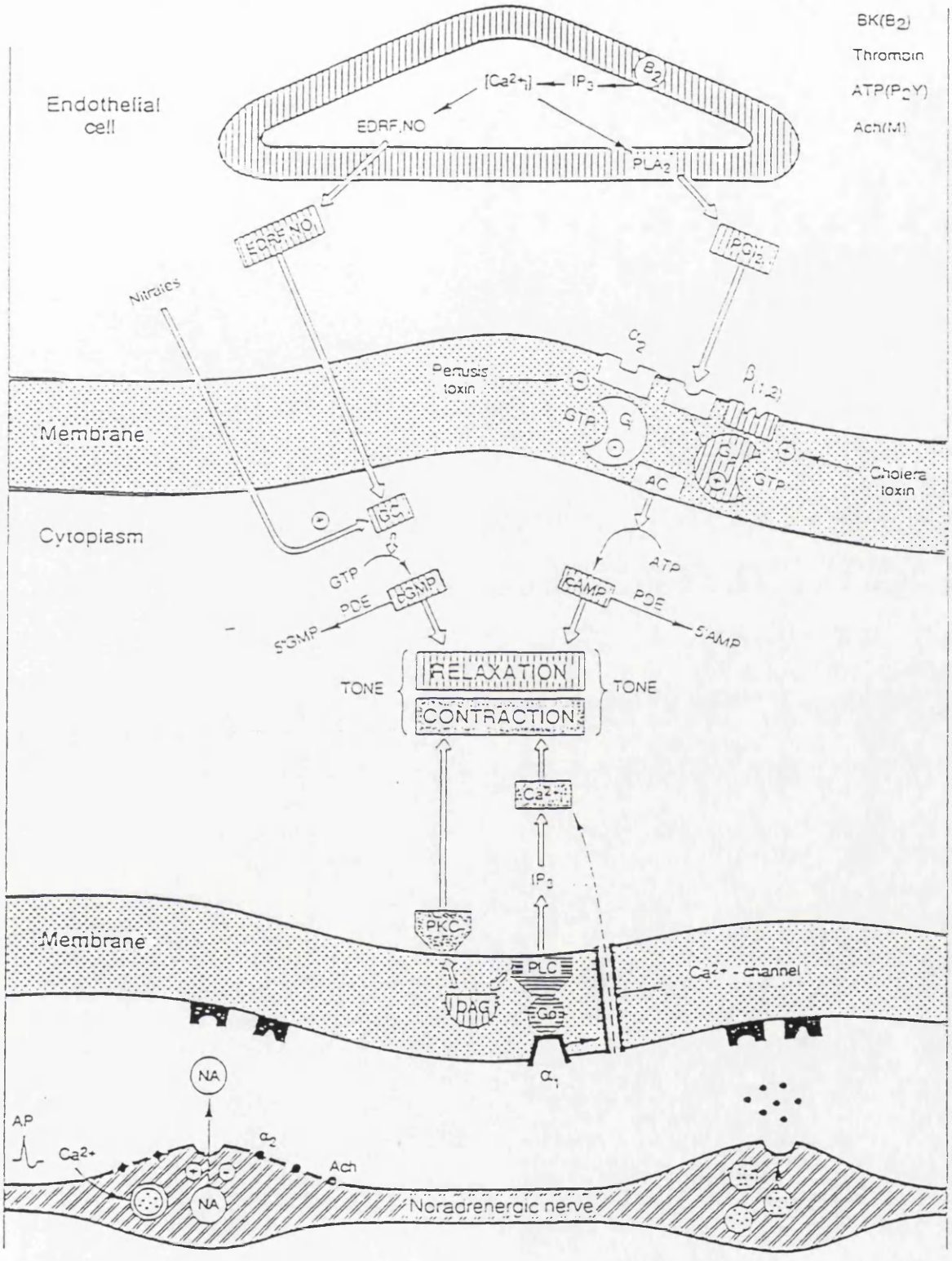
General structure diagram of a typical blood vessel wall. This diagram represents the layers of a blood vessel wall including intima, media and adventitia (Sharifi, 1992).

Fig-2
The renin-angiotensin system.

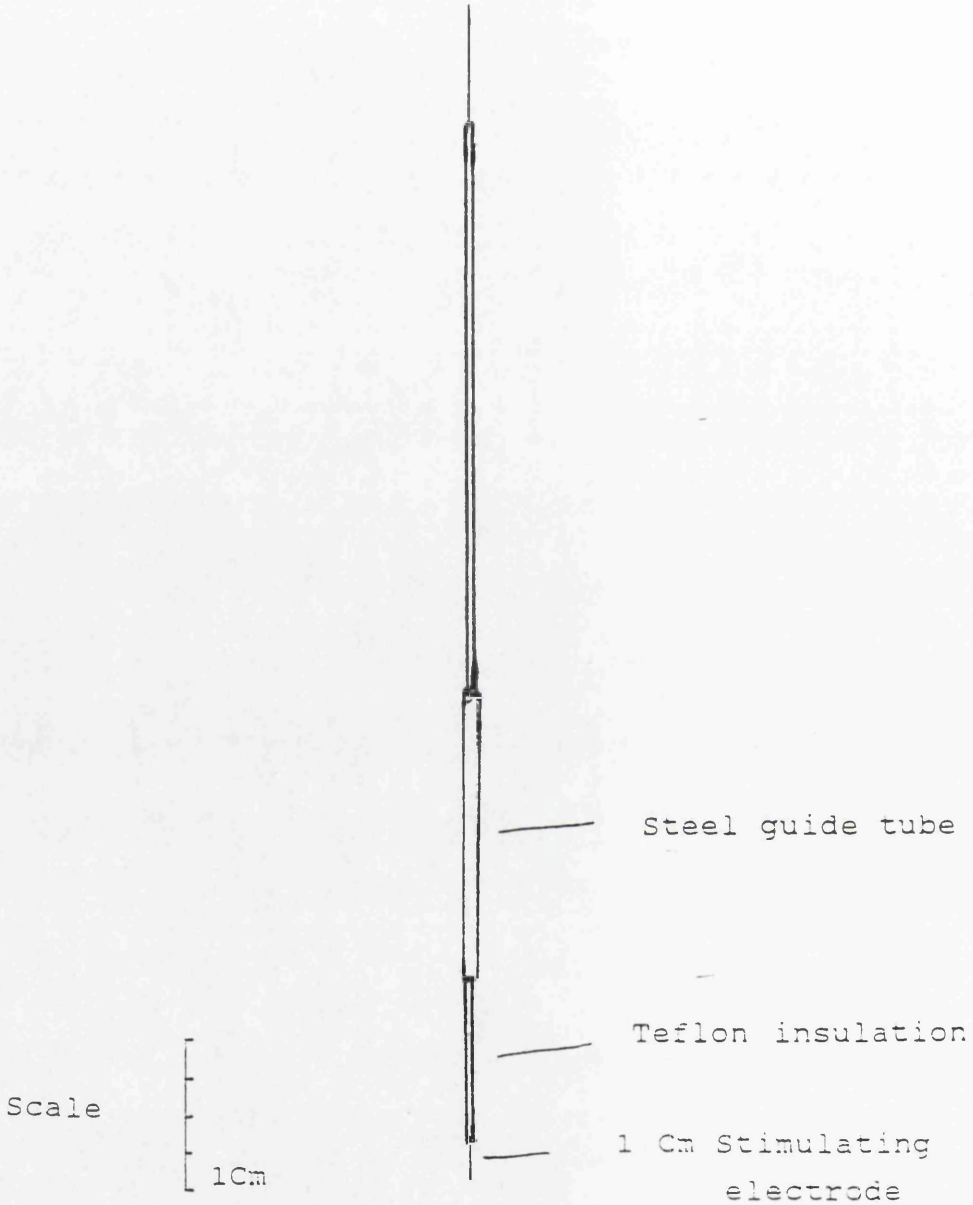


Renin-Angiotensin System
Formation of angiotensin I (AI), angiotensin II (AII), angiotensin III (AIII) from angiotensinogen and the major effect of AII. ACE (angiotensin converting enzyme).

Fig-3
The underlying mechanisms that modulate vascular smooth muscle tone.

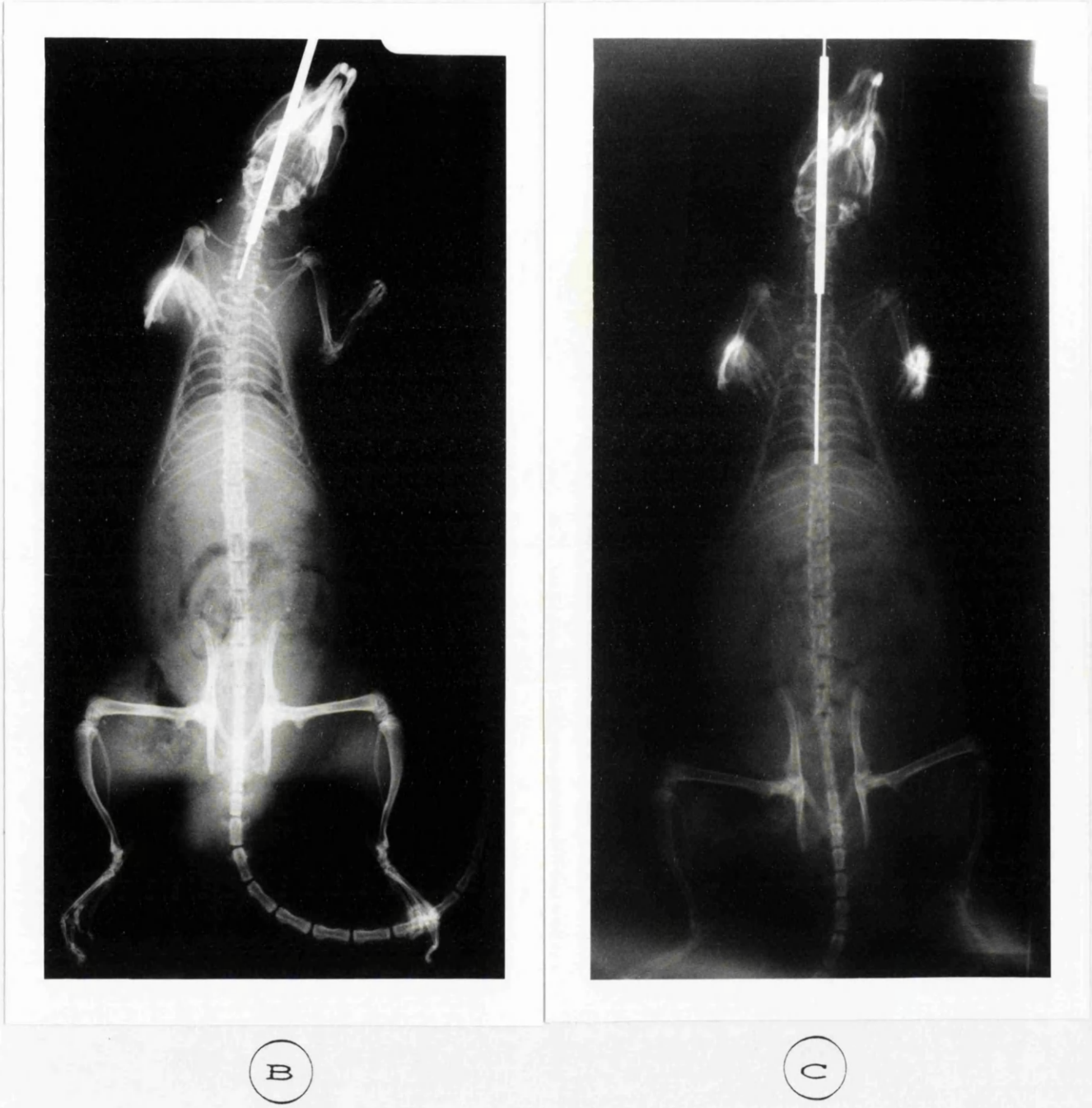


A schematic diagram showing the principal mechanisms that modulate tone in vascular smooth muscle. The endothelial cell release EDRF (NO) and PGI_2, which respectively activate cytosolic guanylate cyclase (GC) to produce cGMP, and membrane adenylate cyclase (AC) to produce cAMP. AC, unlike GC, is regulated via both excitatory and inhibitory receptors, which control the enzyme by activating G-proteins (G_i and G_s). NA released from noradrenergic nerves acts on α_1-adrenoceptors to cause PI-hydrolysis, which results in the production of IP_3 and DAG which cause contraction. NA release is regulated via various presynaptic receptors. The tone of vascular smooth muscle is the resultant of the opposing mechanisms that cause contraction and relaxation (Sharifi, 1992).


Steel guide tube
Teflon insulation
1 Cm Stimulating electrode
Scale
1Cm

A

-Fig-4
Radiographs of pithed rats with pithing rod electrodes in the vertebral canals.

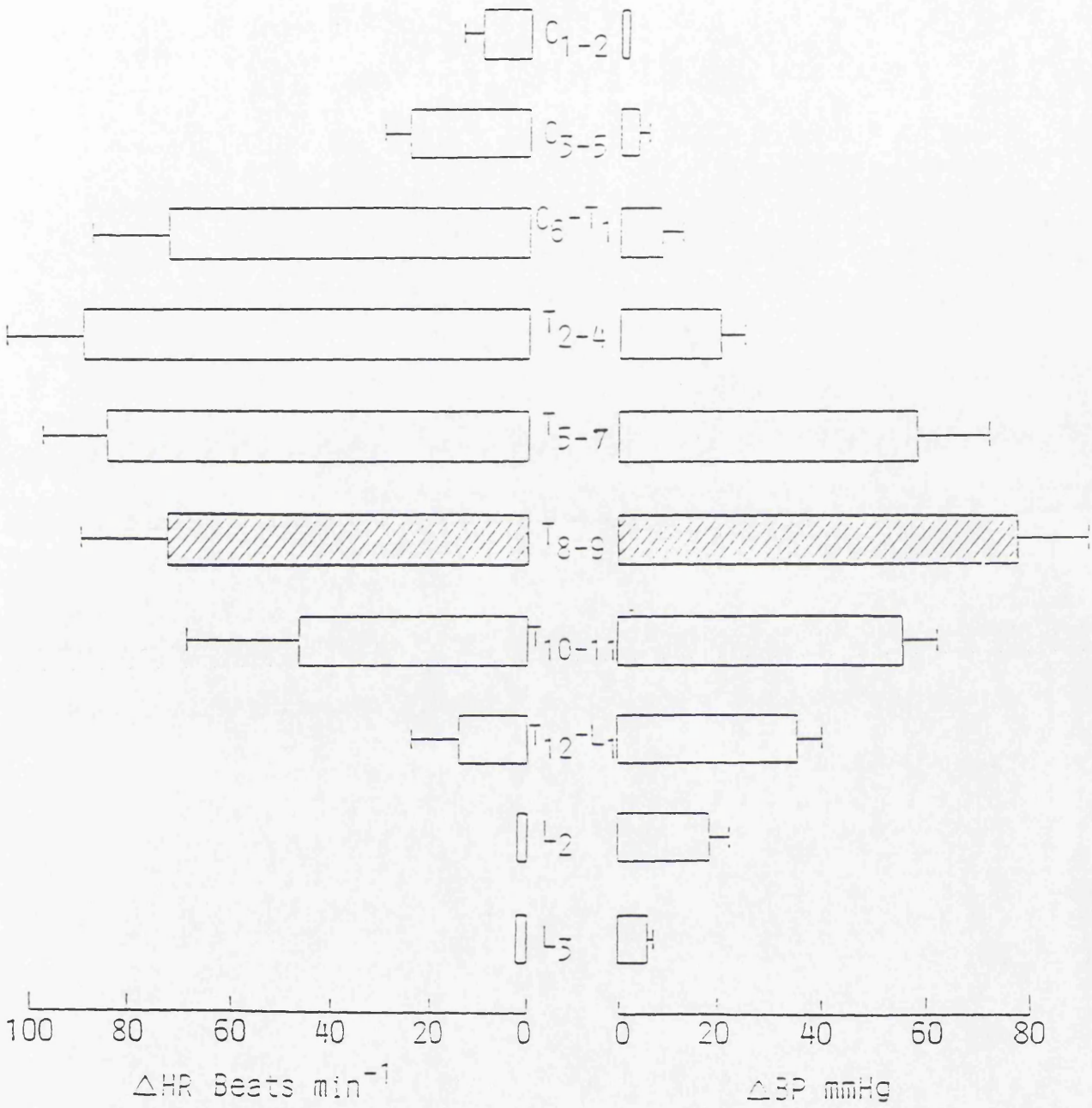


A- Diagram of the pithing rod electrode assembly illustrating the stimulating electrode, the teflon insulation tube and the steel guide tube.

B&C- Two radiographs of pithed rats illustrating the pithing rod electrodes inserted through the eye orbit, the foramen magnum and into the vertebral canal. Radiograph-B showing the position of the electrode at T_{2-4} which gave the maximal increase in heart rate (beats min^{-1}). Radiograph-C showing the position of the electrode at T_{8-9} which gave the maximal increase in blood pressure (mmHg). The teflon insulation is not radio-opaque and therefore can not be observed.

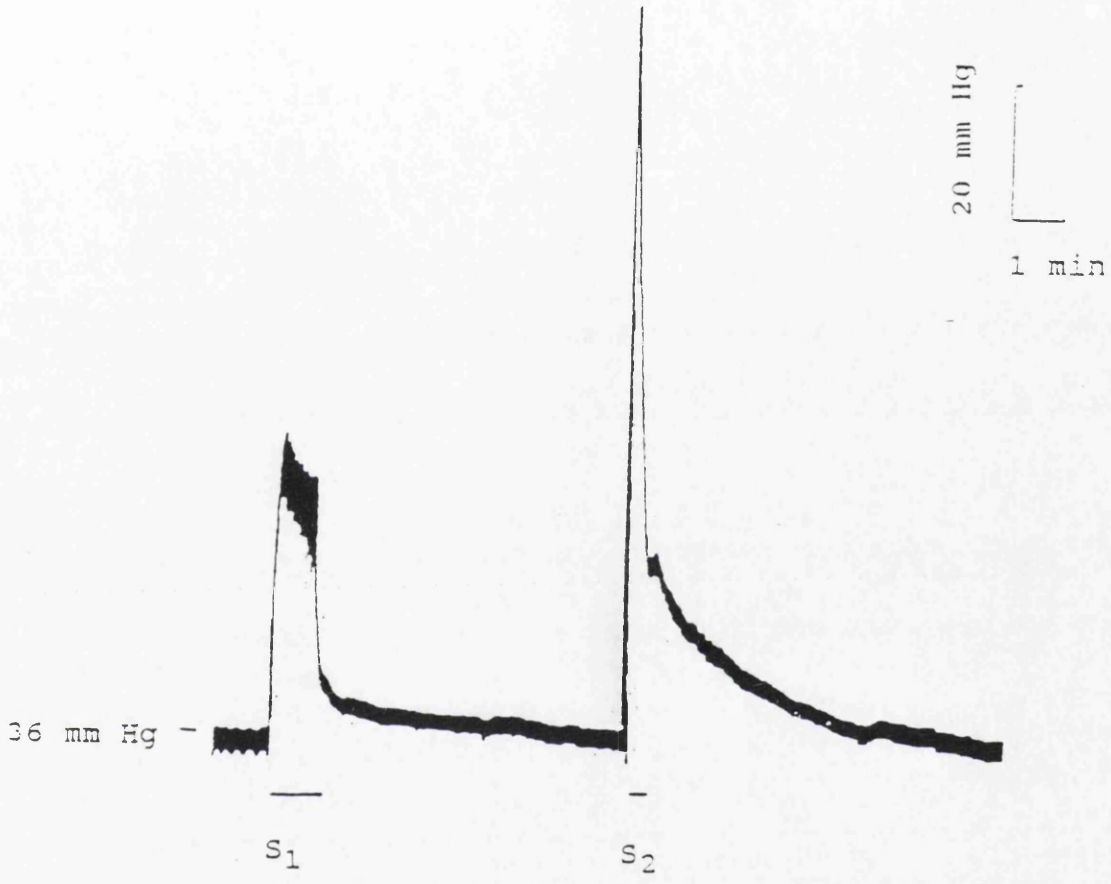
-Fig-5

Responses illustrated as increases in heart rate and in blood pressure to the spinal nerve stimulation in the pithed rats.



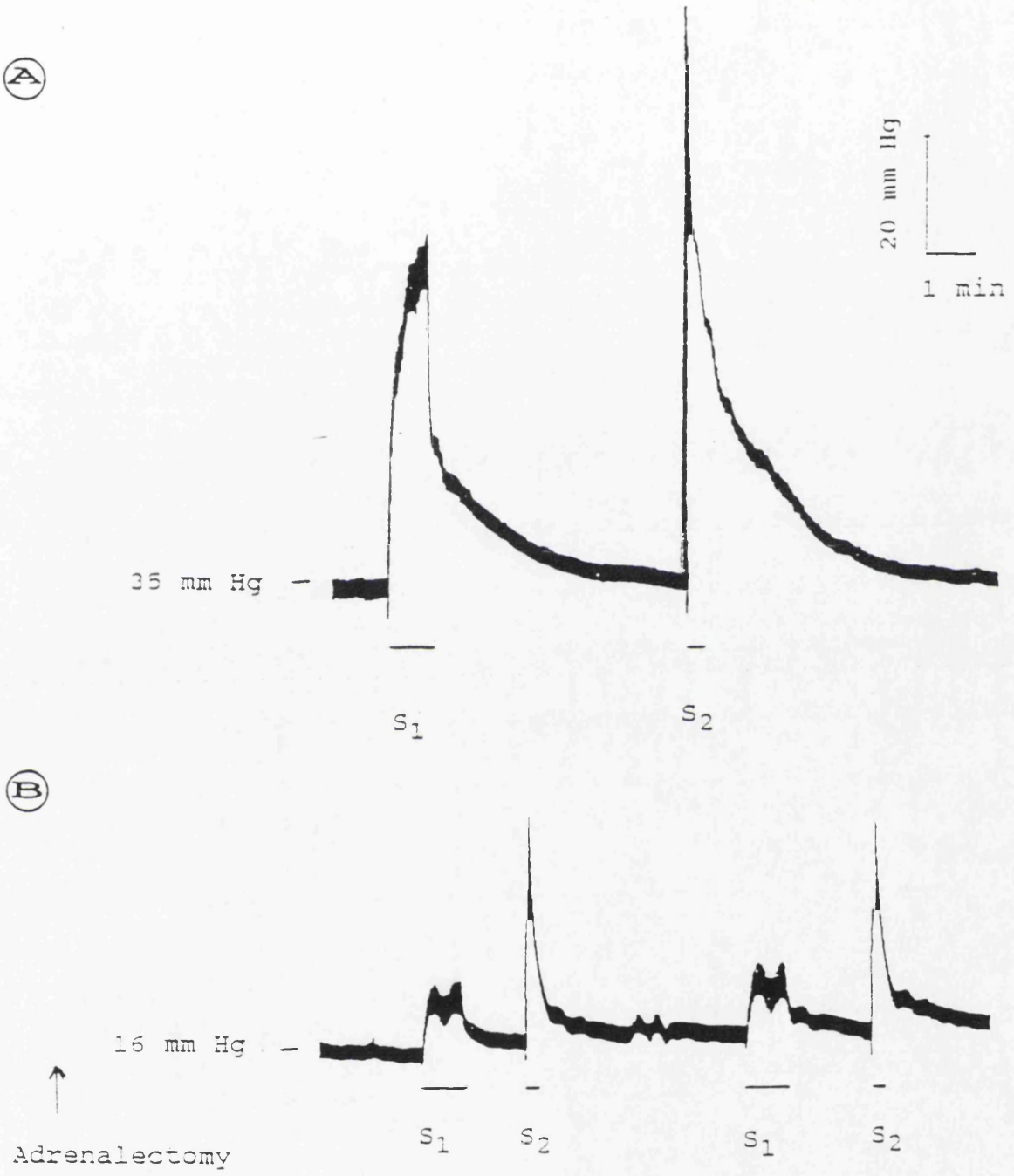
Responses in the pithed rat to preganglionic stimulation of the sympathetic outflow at 20 Hz using a teflon shielded electrode with a 1 cm electrode and supramaximal voltage and 0.4 ms pulse-duration. The histograms show the responses (Mean ± SEM, n=4) expressed as increases in heart rate (HR, basal HR = 216.7 beats min^{-1} ± 23.3 beats min^{-1}, n=3) and increases in blood pressure (BP, Basal BP = 23.1 mm Hg ± 42 mm Hg, n=4). The positions of the electrode are indicated by the corresponding vertebrae, from the first cervical (C_1) through the thoracic vertebrae (T_1-T_{12}) down to the third lumbar (L_3). The position of the electrode was checked radiographically. In most experiments the electrode was located at T_{8-9}, where the maximal increase in BP was obtained.

Fig-6
Pressor responses to nerve stimulation in the pithed rat.



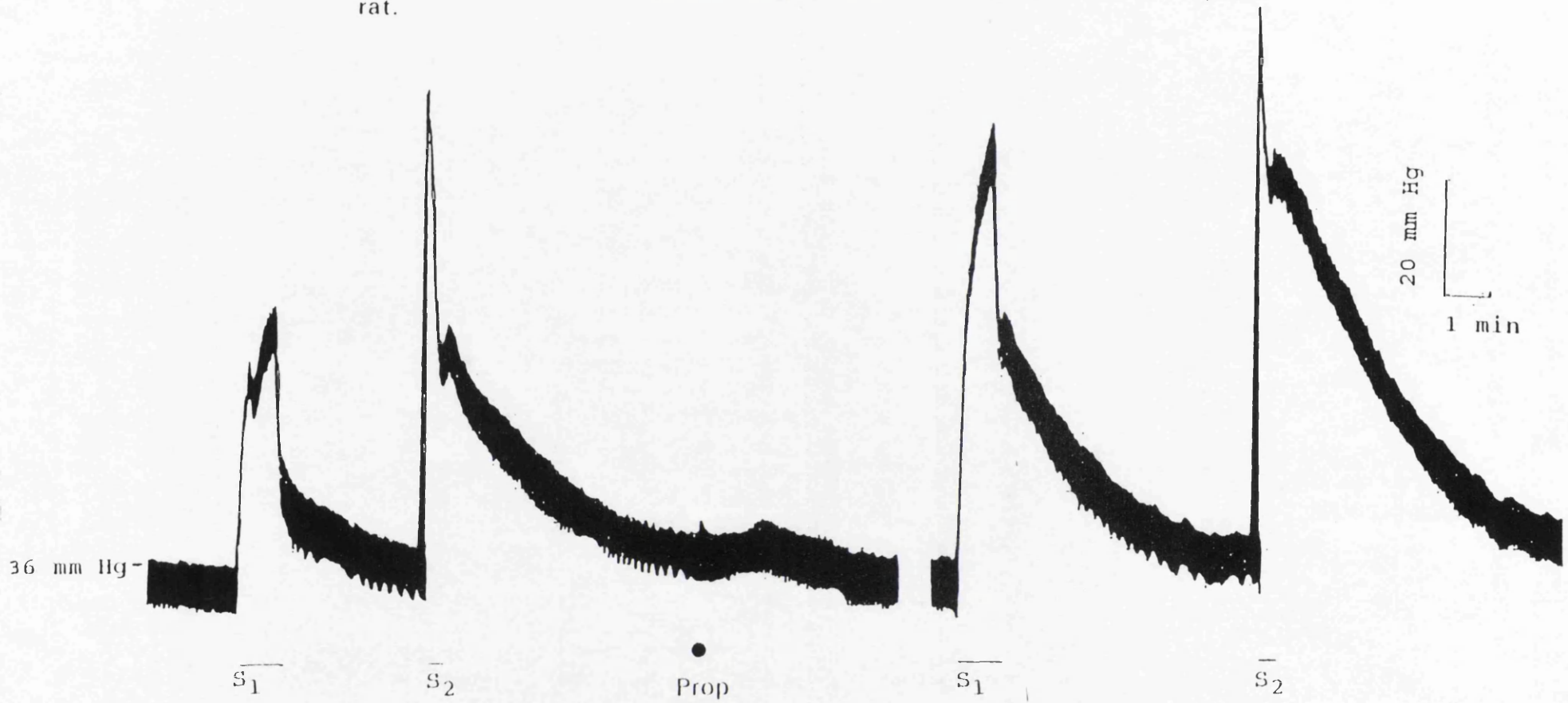
The characteristic pressor responses to electrical stimulation of the spinal sympathetic outflow at T_{8-9} with supramaximal voltage, 0.4 ms pulse-duration at 2 Hz (S_1) and 20 Hz (S_2) for 100 pulses in the pithed rat. The periods of nerve stimulations are shown as (—— = 50s and — = 5s). The original basal blood pressure is also shown to the left of the trace.

Fig-7
Effect of adrenalectomy on pressor responses to nerve stimulation in the pithed rat.



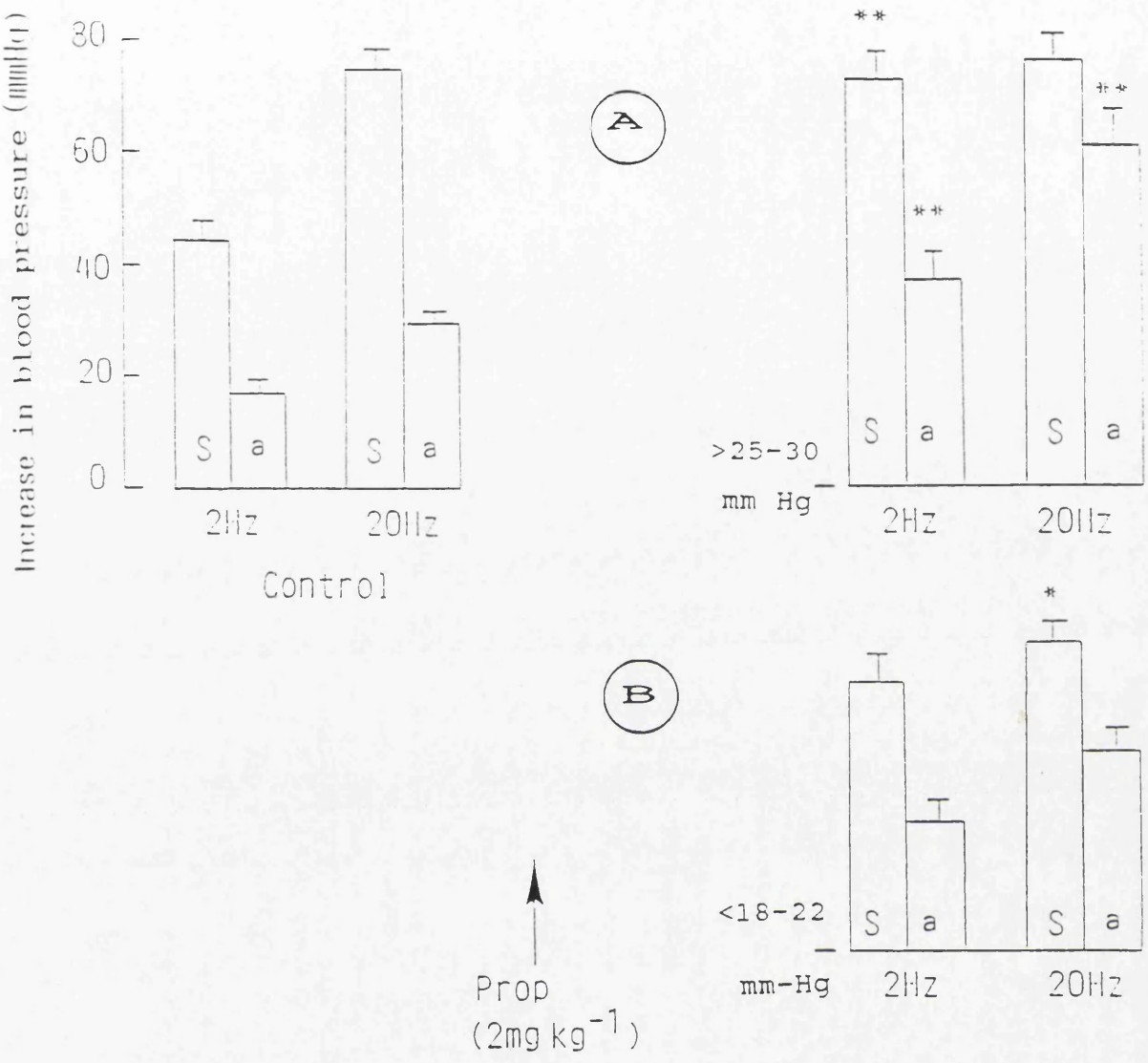
The characteristic pressor responses to electrical stimulation of the spinal sympathetic outflow at T_{8-9} with supramaximal voltage, 0.4 ms pulse-duration at 2 Hz (S_1) and 20 Hz (S_2) for 100 pulses in the pithed rat. The trace in panel B is a continuation of that in panel A. Adrenalectomy lowered the basal blood pressure and substantially reduced the pressor responses to nerve stimulation. Propranolol (2 mg kg^{-1}, iv.) was present throughout the experiment. The periods of nerve stimulations are shown as (—— = 50s and — = 5s). The original basal blood pressures are also shown to the left of the traces in each panel.

Fig-8 Effect of propranolol on pressor responses to nerve stimulation in the pithed rat.



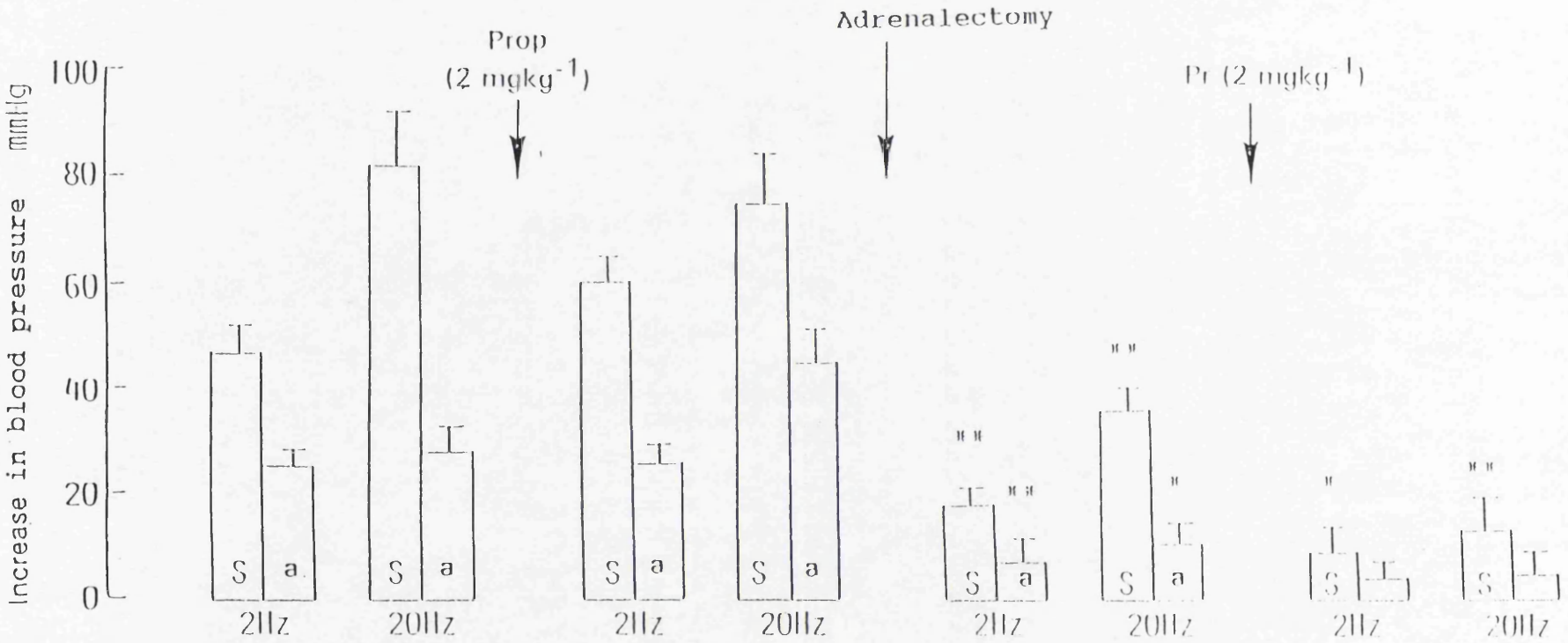
The pressor responses to electrical stimulation of the spinal sympathetic outflow at T_{8-9} with supramaximal voltage, 0.4 ms pulse-duration at 2 Hz (S_1) and 20 Hz (S_2) for 100 pulses in the pithed rat. Propranolol (Prop, 2 mg kg^{-1}, iv.) enhanced both components of the pressor responses to nerve stimulation. The periods of nerve stimulations are shown as (——=50s and — —=5s). The original basal blood pressure is also shown to the left of the trace.

Fig-9
Effect of propranolol on pressor responses to nerve stimulation in pithed rats with low and high basal blood pressures.



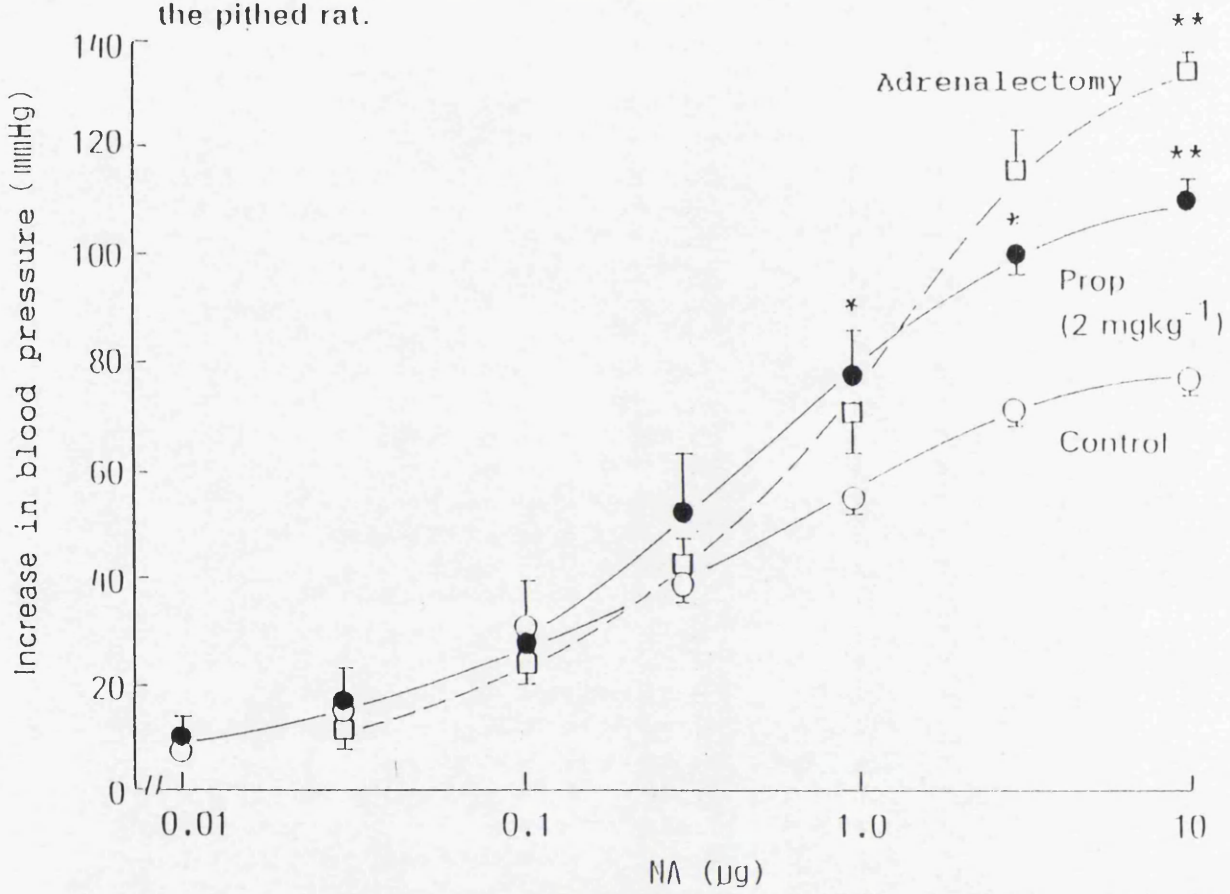
Histograms showing pressor responses (mm Hg, Mean ± SEM, n≥10) to electrical stimulation at T_{8-9} at 2 Hz and 20 Hz in control (untreated) pithed rats and in pithed rats treated with propranolol (Prop, 2 mg kg^{-1}, iv., A and B). In the histograms, "S" indicates the pressor response obtained during electrical stimulation and the "a" indicates the peak post-stimulation response, obtained after stimulation had stopped. Stimulation at 20 Hz produced larger responses than those obtained at 2 Hz and all of these responses with the exception of the response obtained during stimulation at 20 Hz, were usually increased by propranolol (2 mg kg^{-1}). In some rats with low resting blood pressures (Panel B), propranolol did not increase responses to stimulation but reduced the response obtained during stimulation at 20 Hz. It may be that in these rats, the balance of the opposing effects of propranolol reduced the capacity to maintain a pressor response to electrical stimulation. The ability of propranolol to block vascular $ß_2$-adrenoceptors would normally tend to enhance the pressor effects of circulating catecholamines but this would only be possible as long as the ability of propranolol to block cardiac β_1-adrenoceptors did not impair cardiac output and the capacity of the heart to maintain a pressor response. The basal blood pressures are also shown for panel A (>25-30 mm Hg) and panel B (<18-22 mm Hg). These were the resting blood pressures after propranolol administration and before nerve stimulation. (* $0.05>P>0.01$, ** $0.01>P>0.001$).

Fig-10 Effects of propranolol, adrenalectomy and prazosin, on pressor responses to nerve stimulation in the pithed rat.



Histograms showing pressor responses (mm Hg, Mean ± SEM, n = 5) in the pithed rat to electrical stimulation at T_{8-9} at 2 Hz and 20 Hz ("S" indicates the response obtained during stimulation and "a" indicates the post-stimulation response). Also shown are the effects of propranolol (Prop, 2 mg kg^{-1}, iv.) and of adrenalectomy, which significantly reduced the responses to stimulation at 2 Hz and 20 Hz and also inhibited the post-stimulation responses. The residual responses, which survived adrenalectomy were further inhibited by prazosin (Pr, 2 mg kg^{-1}, iv.). The starting basal blood pressure was (Mean = 38.6 mm Hg, SEM = 6.62 mm Hg, n = 5). (* $0.05 > P > 0.01$, ** $0.01 > P > 0.001$).

Fig-11 Effects of propranolol and adrenalectomy on dose-response curves to NA in the pithed rat.



Dose-response curves to NA administered intravenously in pithed rats. Responses are expressed as increases in BP (mm Hg, Mean ± SEM, n = 3). ○–○ indicates control responses to NA. ●–● indicates resppnses to NA obtained after administration of propranolol (Prop, 2 mg kg^{-1}, iv.) and □–□, indicates responses to NA in the presence of propranolol (2 mg kg^{-1}, iv.) and after adrenalectomy. Responses to high doses of NA were enhanced by propranolol and in adrenalectomised rats. (* $0.05 > P > 0.01$, ** $0.01 > P > 0.001$).

-Fig-12
Tachyphylaxis to pressor responses to α,ß-mATP, NA and nerve stimulation in the pithed rat.

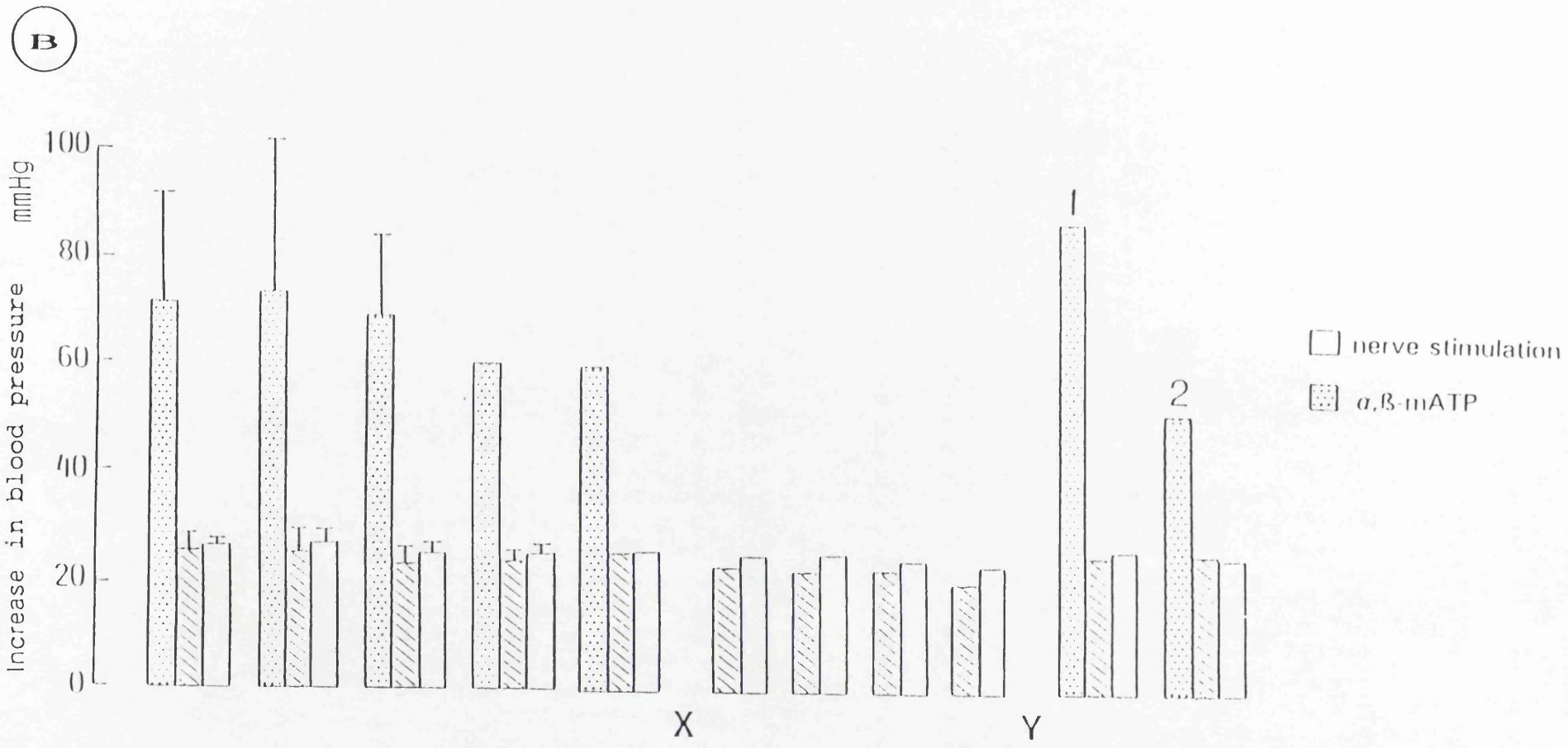
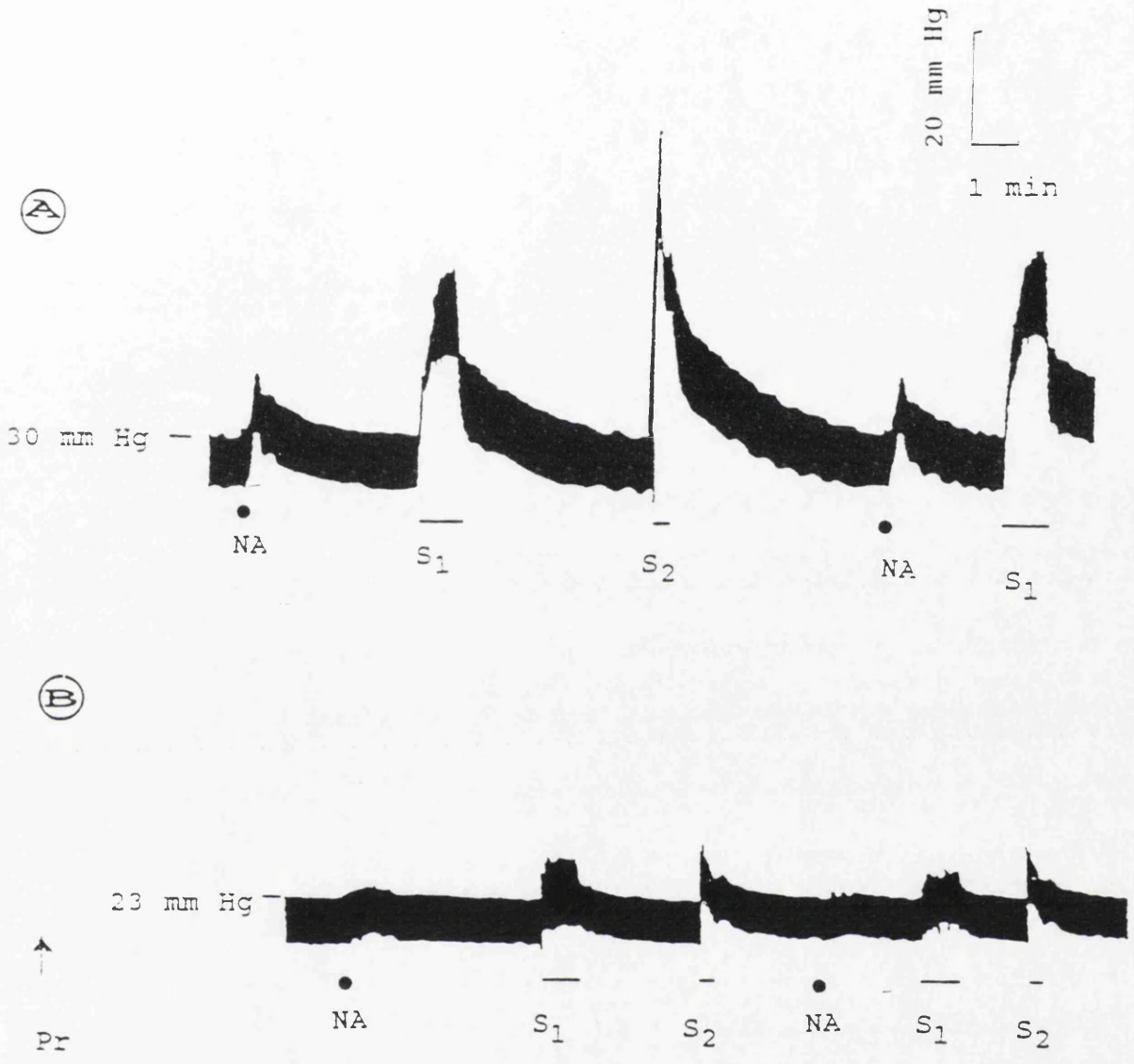

B
Increase in blood pressure mmHg
100
80
60
40
20
0
X
Y
1
2
nerve stimulation
α,ß-mATP

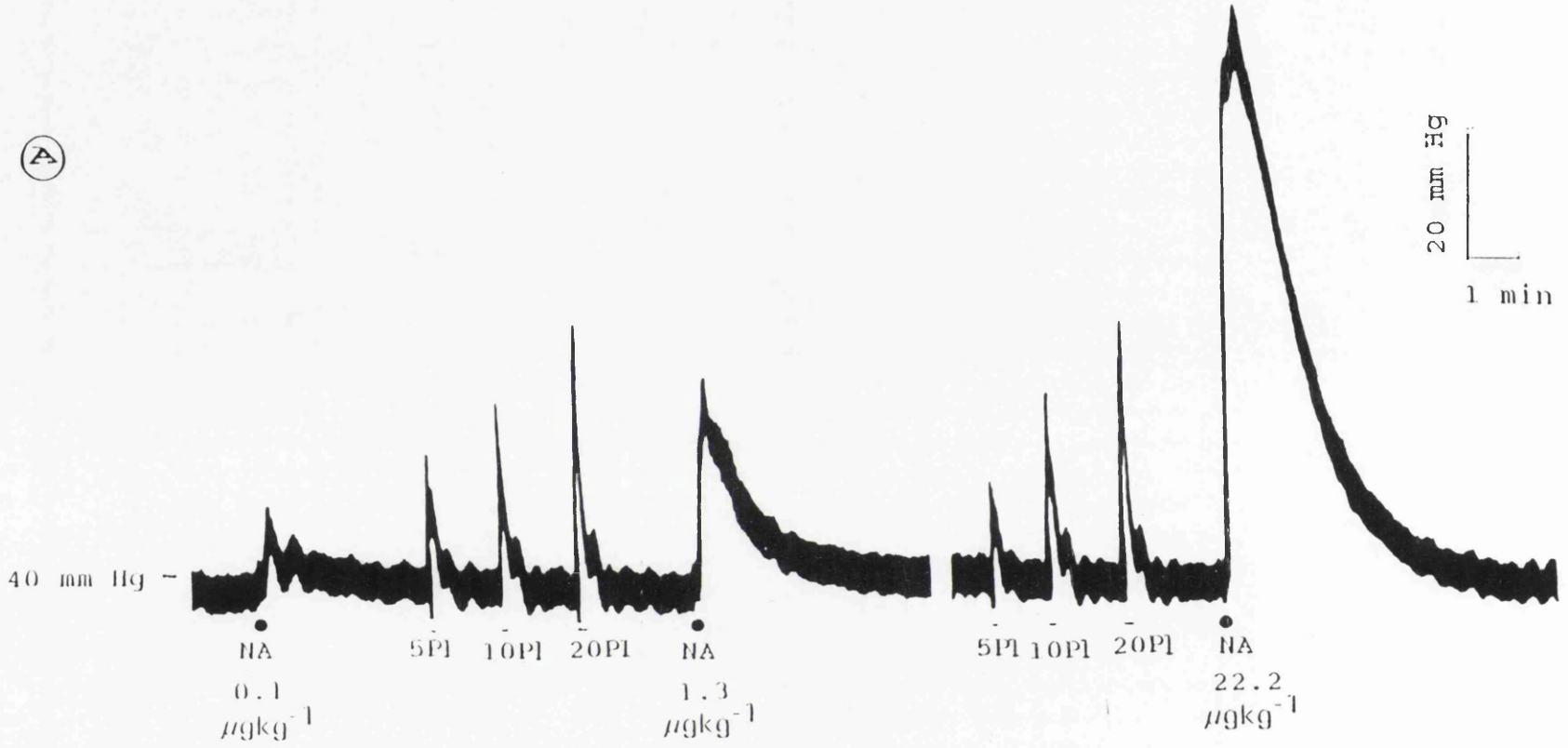
Fig-12 Histograms showing pressor responses (mm Hg, Mean ± SEM) in the propranolol-pretreated (2 mg kg^{-1}, iv.) pithed rat to intravenously injected NA (9 μg kg^{-1}, iv., hatched columns) and to spinal nerve stimulation at 20 Hz. The lower panel (B) is a continuation of the upper panel (A). Also shown are the responses to intravenously injected α,β-mATP (shaded columns, starting with two 0.05 mg kg^{-1} intravenous injections of α,β-mATP and thereafter injections of 0.1 mg kg^{-1}). Repeated administration of NA and repeated electrical stimulation at 15 minute intervals produced pressor responses that declined with time. Responses to α,β-mATP also declined. There was no evidence that during or after tachyphylaxis to α,β-mATP had occurred the pressor response to electrical stimulation declined to a greater extent than did the standard response to NA. When the administration of α,β-mATP was interrupted (between X and Y), the pressor response to α,β-mATP recovered (1) and then quickly desensitised again (2) but throughout this period of recovery and desensitisation, the responses to electrical stimulation and to NA were unchanged. The starting basal blood pressure was (Mean = 44.33 mm Hg, SEM = 5.84 mm Hg, n = 3).

Fig-13
Effect of prazosin on pressor responses to a single dose of NA and nerve stimulation in the pithed rat.



The pressor responses to NA (0.3 μg kg^{-1}, iv.) and to electrical stimulation of the spinal sympathetic outflow at T_{8-9} with supramaximal voltage, 0.4 ms pulse-duration at 2 Hz (S_1) and 20 Hz (S_2) for 100 pulses in the pithed rat. The trace in panel B is a continuation of that in panel A. Prazosin (Pr, 2 mg kg^{-1}, iv.) substantially reduced the pressor responses to NA and nerve stimulation but did not abolish them completely. Propranolol (2 mg kg^{-1}, iv.) and Captopril (1 mg kg^{-1}, iv.) were present throughout this experiment. The periods of nerve stimulations are shown as (——= 50s and – – = 5s).

Fig-14
Effect of yohimbine on pressor responses to increasing doses of NA and to nerve stimulation in the pithed rat.



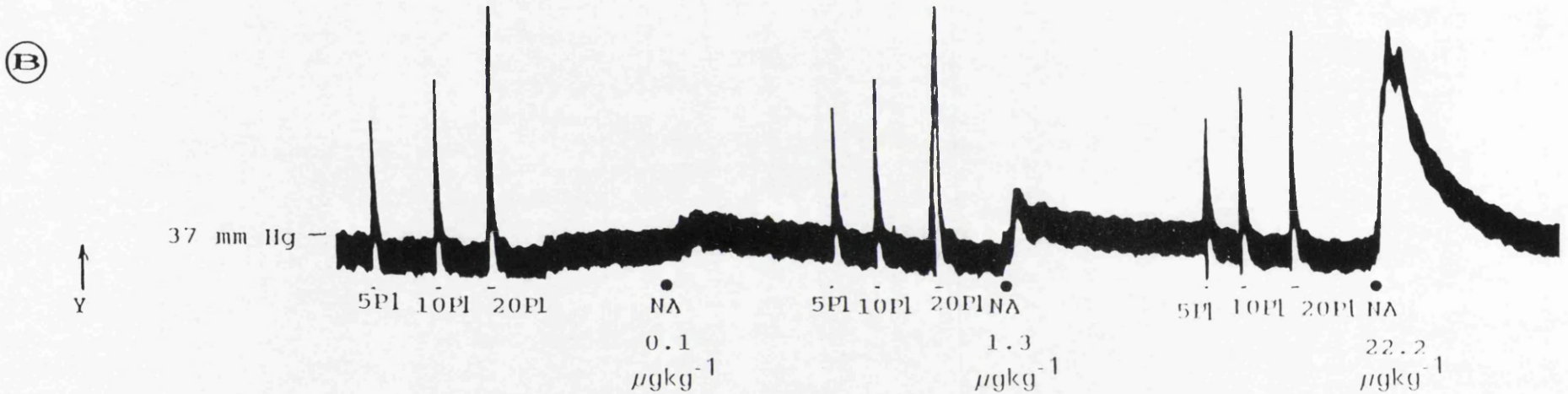
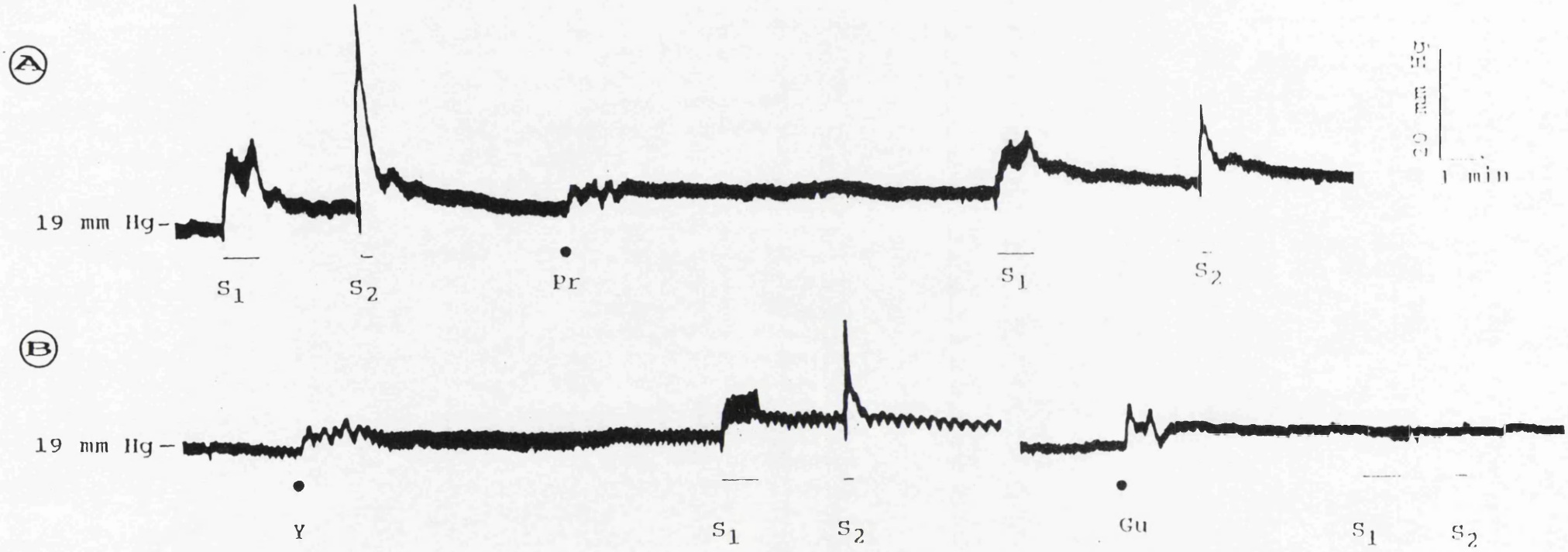


Fig-14 Pressor responses to increasing concentrations of NA (0.1, 1.3 and 22.2 μg kg^{-1}, iv.) and to electrical stimulation of the spinal sympathetic outflow at T_{8-9} with supramaximal voltage, 0.4 ms pulse-duration at 5 Hz for 5, 10 and 20 pulses (Pl) in the pithed rat. The trace in panel B is a continuation of that in panel A. The pressor responses to nerve stimulations were increased with the length of the train. NA produced dose-dependent pressor responses. Yohimbine (Y, 1 mg kg^{-1}, iv.) (which was present throughout the second part of the experiment, Panel B), reduced NA-induced pressor responses but slightly enhanced the pressor responses to nerve stimulations. Propranolol (2 mg kg^{-1}, iv.) was present during the experiment. The periods of nerve stimulations are shown as (-=1s, -=2s and – =4s).

Fig-15 Effects of prazosin, yohimbine and guanethidine administered consecutively on pressor responses to nerve stimulation in the pithed rat.



Pressor responses to electrical stimulation of the spinal sympathetic outflow at T_{8-9} with supramaximal voltage, 0.4 ms pulse-duration at 2 Hz (S_1) and 20 Hz (S_2) for 100 pulses in the pithed rat. The trace in panel B is a continuation of that in panel A. Prazosin (Pr, 2 mg kg^{-1}, iv.) inhibited the pressor responses to nerve stimulation. Yohimbine (Y, 2 mg kg^{-1}, iv.) inhibited the pressor response to 2 Hz stimulation but enhanced the pressor response at 20 Hz nerve stimulation. Finally, Guanethidine (Gu, 2 mg kg^{-1} ,iv.) abolished the pressor components to nerve stimulation. Propranolol (2 mg kg^{-1}, iv.) was also present during the experiment. The periods of nerve stimulations are shown as (——= 50s and – – = 5s).

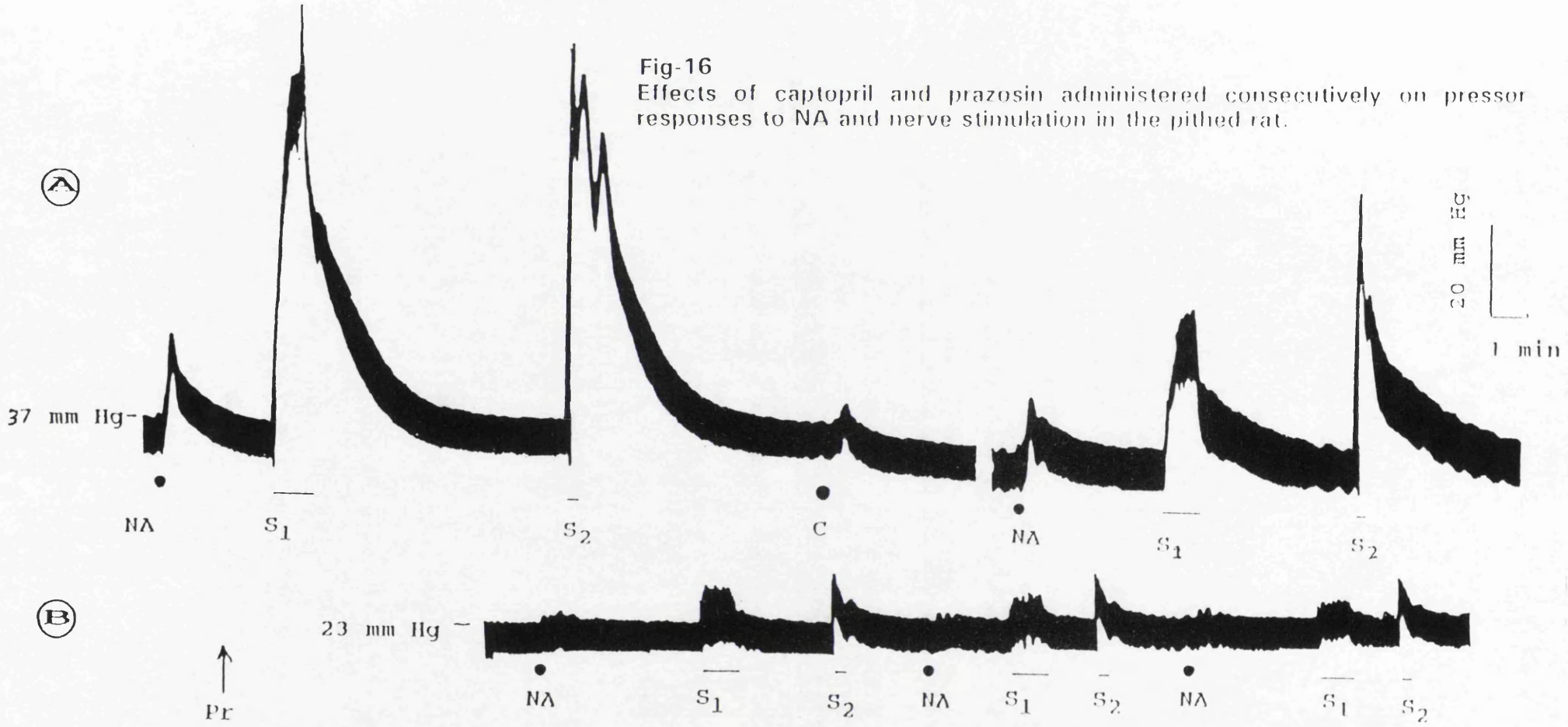
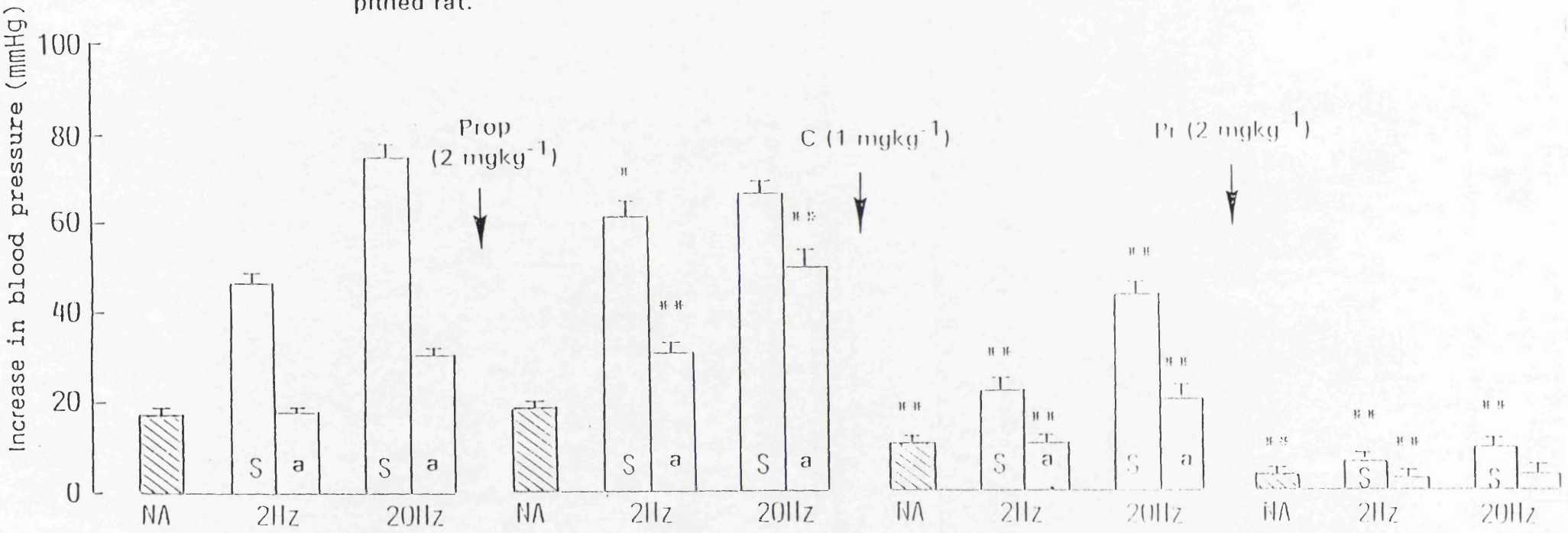


Fig-16
Effects of captopril and prazosin administered consecutively on pressor responses to NA and nerve stimulation in the pithed rat.

Pressor responses to NA (0.3 μg kg^{-1}, iv.) and to electrical stimulation of the spinal sympathetic outflow at T_{8-9} with supramaximal voltage, 0.4 ms pulse-duration at 2 Hz (S_1) and 20 Hz (S_2) for 100 pulses in the pithed rat. The trace in panel B is a continuation of that in panel A. Captopril (C, 1 mg kg^{-1}, iv.) lowered the basal blood pressure slightly and reduced the pressor responses to both NA and nerve stimulations. Prazosin (Pr, 2 mg kg^{-1}, iv.) further reduced the pressor responses to NA and nerve stimulation but did not abolish them completely. Propranolol (2 mg kg^{-1}, iv.) was also present during the experiment. The periods of nerve stimulations are shown as (—— = 50s and – – = 5s).

Fig-17 Effects of propranolol, captopril and prazosin, administered consecutively, on pressor responses to a single dose of NA and nerve stimulation in the pithed rat.



Histograms showing pressor responses (mm Hg, Mean ± SEM, $n \geq 12$) in the pithed rat to electrical stimulation at $T_{8\text{-}9}$ at 2 Hz and 20 Hz ("S" indicates the response obtained during stimulation and "a" indicates the post-stimulation response). In addition, this figure shows the pressor responses (mm Hg) to NA (9 $\mu g\ kg^{-1}$, iv., hatched histogram). Also shown are the effects of propranolol (Prop, 2 mg kg^{-1}, iv.), which potentiated pressor responses to electrical stimulation but did not affect the pressor response to NA. Next, captopril (C, 1 mg kg^{-1}, iv.) significantly inhibited the responses to electrical stimulation and to NA and these responses were inhibited by prazosin (Pr, 2 mg kg^{-1}, iv.). The starting basal blood pressure was (Mean = 33.0 mm Hg, SEM = 2.54 mm Hg, n = 4). (* $0.05 > P > 0.01$, ** $0.01 > P > 0.001$).

Fig-18
Effect of L-NAME on blood pressure in the absence and presence of propranolol in pithed and anaesthetised rats.

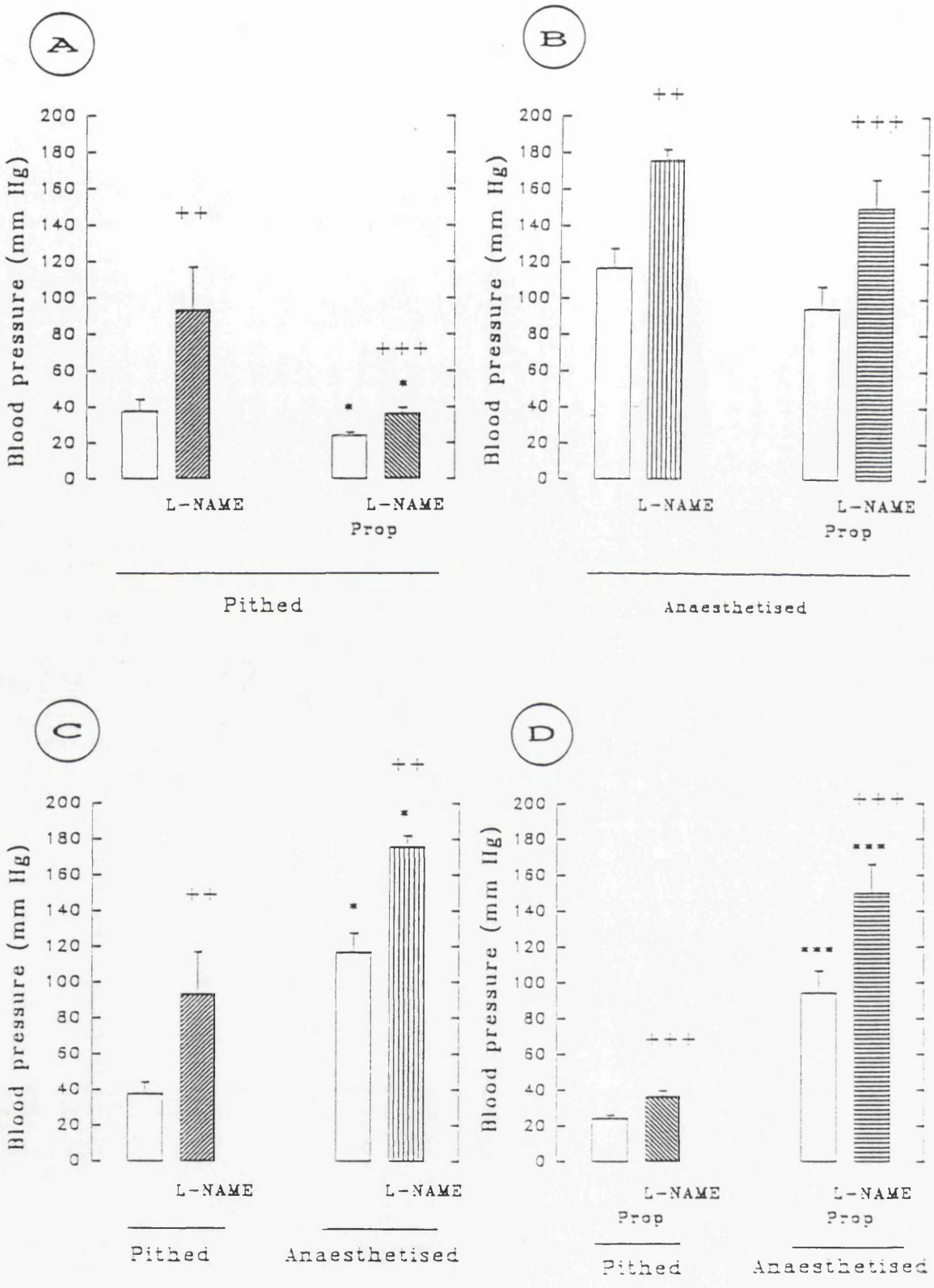
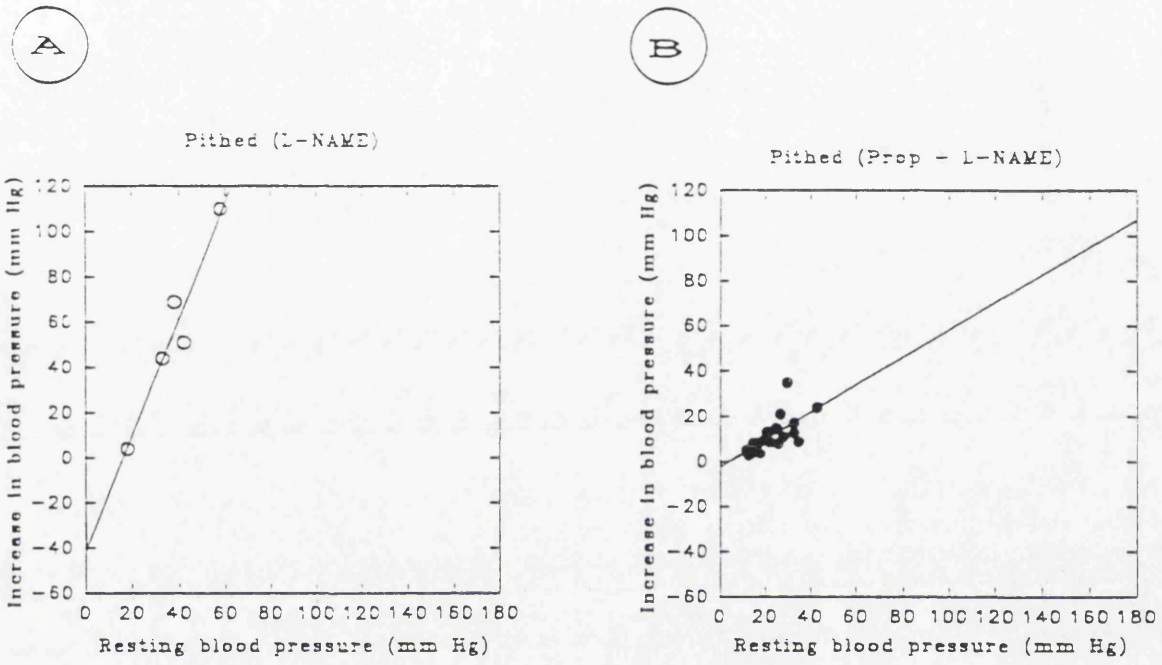


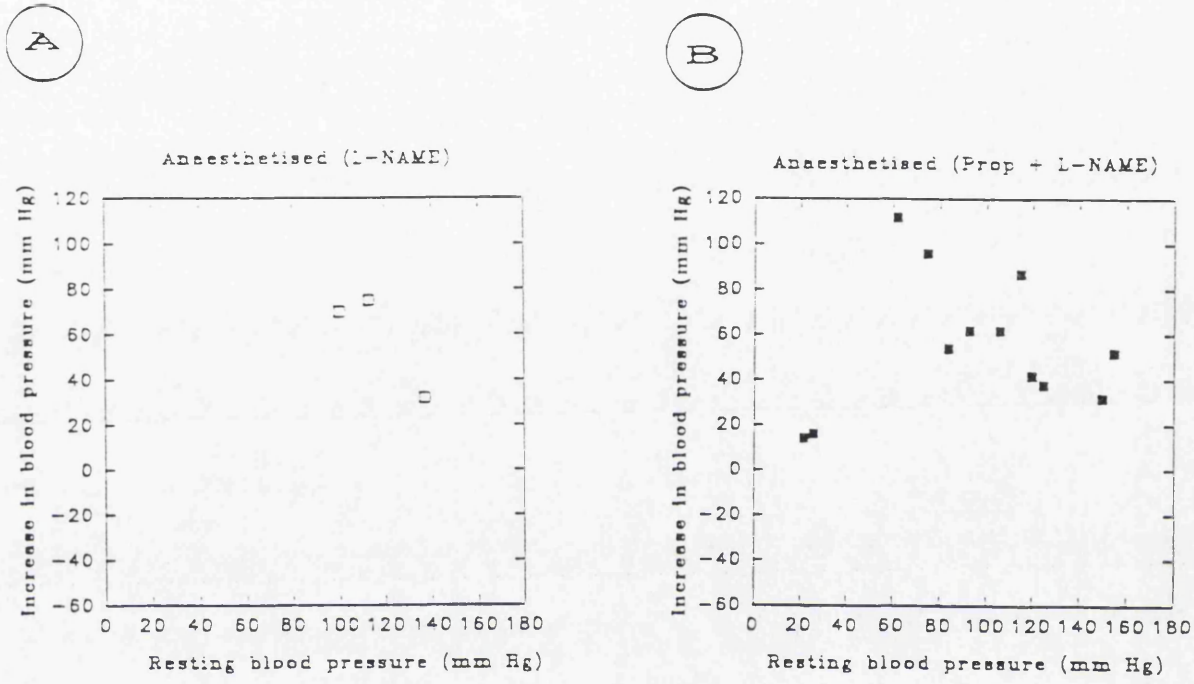
Fig-18
In pithed and anaesthetised rats, L-NAME (45 mg kg^{-1}, iv.) increased the basal blood pressure (mm Hg, Mean ± SEM, $n \geq 3$) in the absence and presence of propranolol (Prop, 2 mg kg^{-1}, iv.). In all four panels (A,B,C,D), the basal blood pressures are shown as open columns and the ones with L-NAME are shown as patterned columns. Panel A, illustrates the effect of propranolol in lowering the basal blood pressure and lowering the size of L-NAME-induced increase in blood pressure in the pithed rat. Panel B, illustrates no effect of propranolol on the basal blood pressure and the L-NAME-induced increase in blood pressure in the anaesthetised rat. Panel C, illustrates, higher basal blood pressure and higher maximal blood pressure, induced by L-NAME in the anaesthetised rat compared with the pithed rat. Panel D, illustrates, higher basal blood pressure and higher maximal blood pressure, induced by L-NAME in the presence of propranolol in the anaesthetised rat compared with the pithed rat. The asterisks above each test column show the significant differences with their correspondent column in the control group in each panel (* $0.05 > P > 0.01$, *** $P < 0.001$). All L-NAME-induced rises, in the absence and presence of propranolol, in the pithed and anaesthetised rats are significant (+ $0.05 > P > 0.01$, + + + $P < 0.001$).

Fig-19
Correlation between the resting blood pressure and the L-NAME-induced increase in blood pressure in the absence and presence of propranolol in the pithed rats.



The regression lines showing the linear relationship between the resting blood pressure (mm Hg) and the L-NAME (45 mg kg^{-1}, iv.)-induced increases in blood pressure (mm Hg) in the absence (panel A) and presence (panel B) of propranolol (Prop, 2 mg kg^{-1}, iv.) in the pithed rats. The extent to which L-NAME raised the basal blood pressure was proportional to the initial basal blood pressure in the absence (panel A, correlation coefficient $R=0.96$, $n=5$, $0.01>P>0.001$) and presence (panel B, correlation coefficient $R=0.64$, $n=19$, $0.01>P>0.001$) of propranolol.

Fig-20
Scattergrams between the resting blood pressure and the L-NAME-induced increase in blood pressure in the absence and presence of propranolol in the anaesthetised rats.



The scattered data, showing no linear relationship between the resting blood pressure (mm Hg) and the L-NAME (45 mg kg^{-1}, iv.)-induced increases in blood pressure (mm Hg) in the absence (Panel A) and presence (Panel B) of propranolol (Prop, 2 mg kg^{-1}, iv.) in the anaesthetised rats.

Fig-21 Effect of L-NAME on pressor responses to nerve stimulation in the pithed rat.

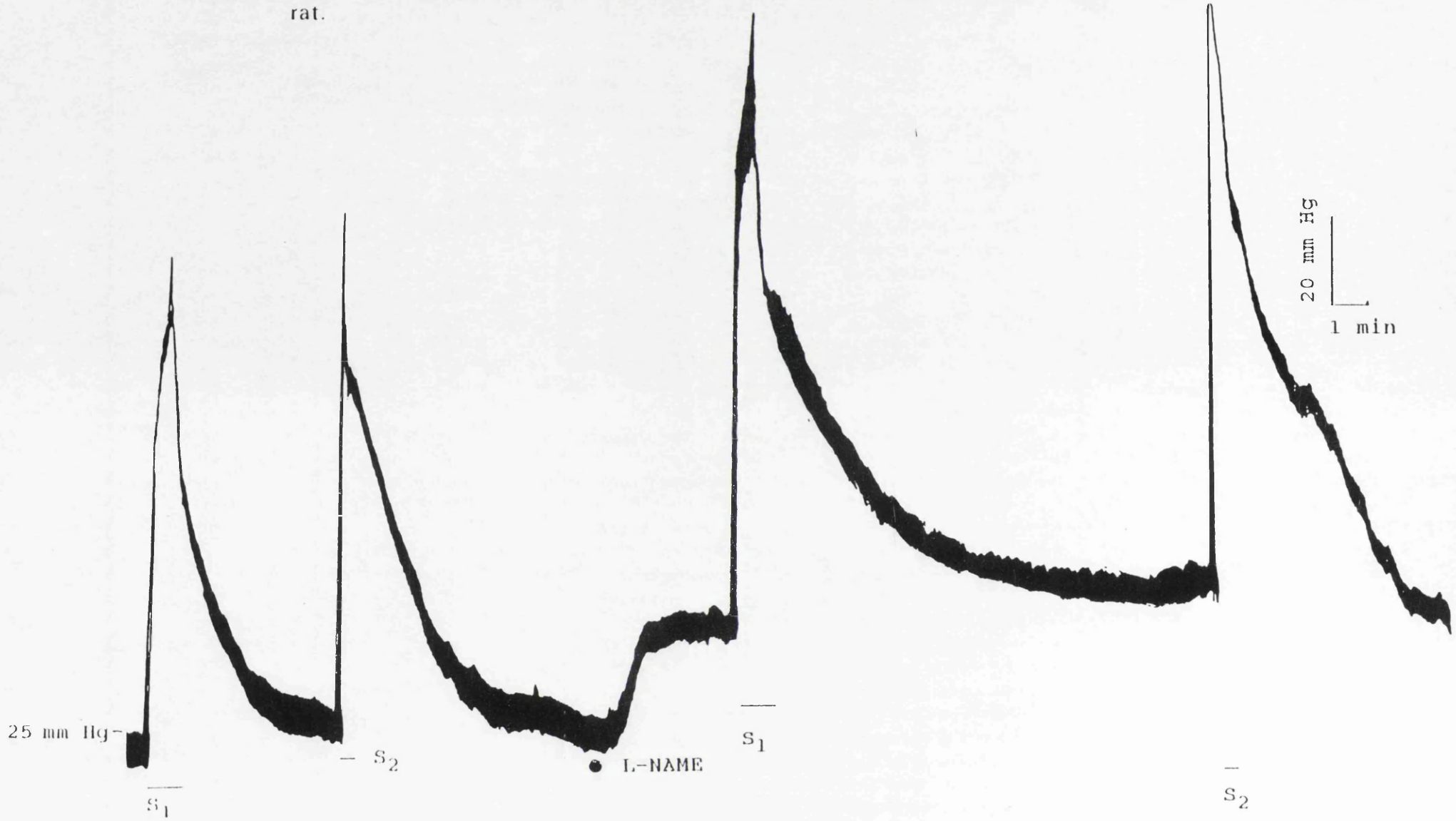
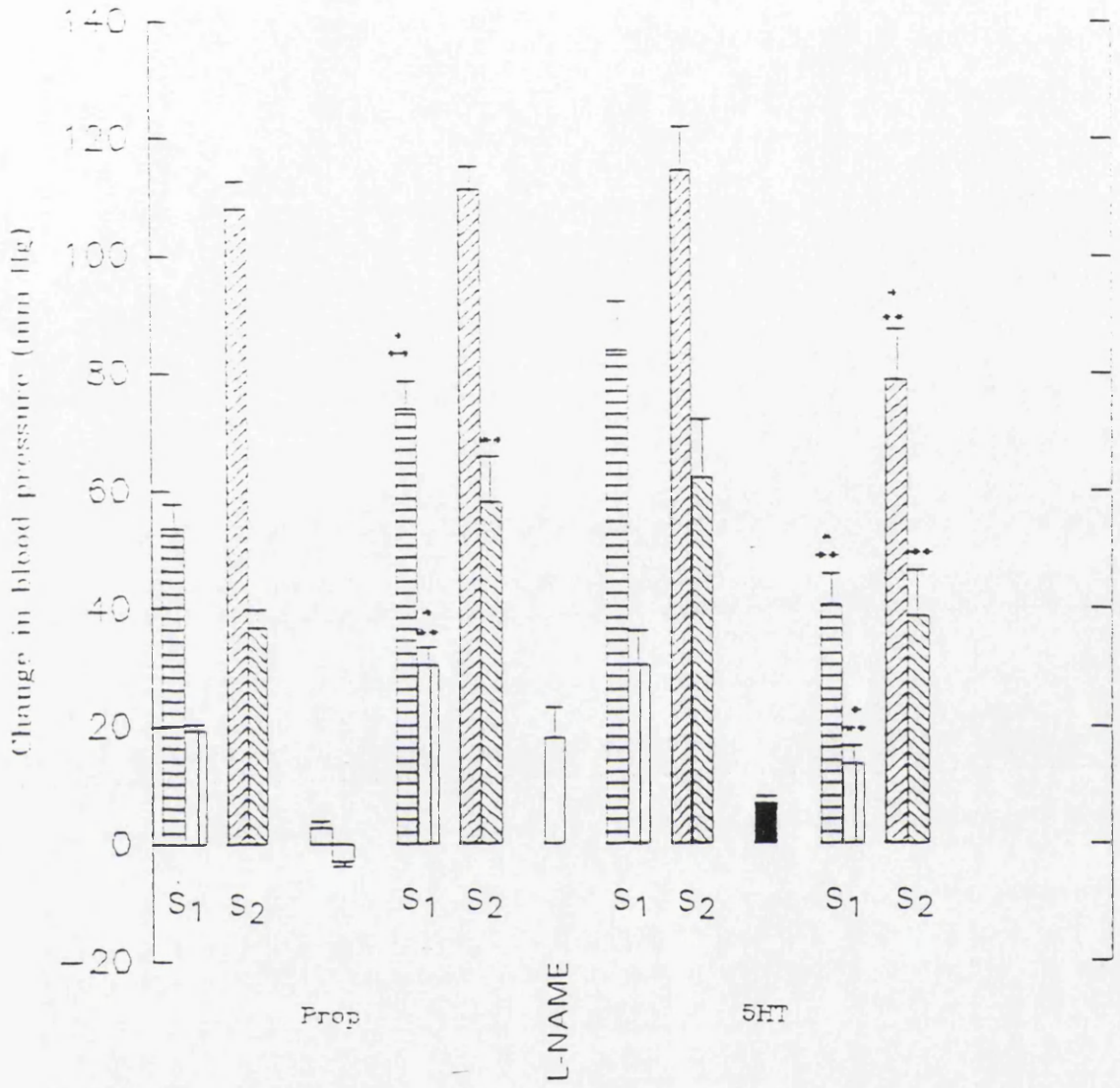


Fig-21
The pressor responses to electrical stimulation of the spinal sympathetic outflow at T_{8-9} with supramaximal voltage, 0.4 ms pulse-duration at 2 Hz (S_1) and 20 Hz (S_2) for 100 pulses in the propranolol-treated (2 mg kg^{-1}, iv.) pithed rat. L-NAME (45 mg kg^{-1}, iv.) increased the basal blood pressure and slightly increased the pressor responses to nerve stimulation in this experiment. The periods of nerve stimulations are shown as (—— = 50s and – – = 5s).

Fig-22
Effects of Propranolol, L-NAME and 5-HT; administered consecutively on pressor responses to nerve stimulation in the pithed rat.



Histograms of pressor responses (mm Hg, Mean ± SEM, n≥11) in the pithed rat to electrical stimulation of the spinal sympathetic outflow at T_{8-9} with supramaximal voltage, 0.4 ms pulse-duration at 2 Hz (S_1) and 20 Hz (S_2) for 100 pulses. Stimulation at 2 Hz and 20 Hz produced characteristic responses, which are shown here as peak responses obtained during stimulation with the secondary, smaller post-stimulus responses that persisted after stimulation ceased, indicated by the smaller adjoining histogram. Propranolol (Prop, 2 mg kg^{-1}, iv.) enhanced the pressor responses to electrical stimulation at 2 Hz and also enhanced the secondary, smaller post-stimulus response that persisted after stimulation at 2 Hz and 20 Hz. After propranolol, the administration of L-NAME (45 mg kg^{-1}, iv.) raised the basal blood pressure but had no other effect. 5-HT (6 μg kg^{-1}, iv.) inhibited the pressor responses obtained during and also persisting after electrical stimulation of the sympathetic nerves at 2 Hz and 20 Hz. The starting basal blood pressure was (Mean = 34.32 mm Hg, SEM = 2.63 mm Hg, n=11). (** $0.01>P>0.001$, *** $P<0.001$).

Fig-23
Effect of L-NAME on pressor responses to increasing doses of NA and to nerve stimulation in the pithed rat.

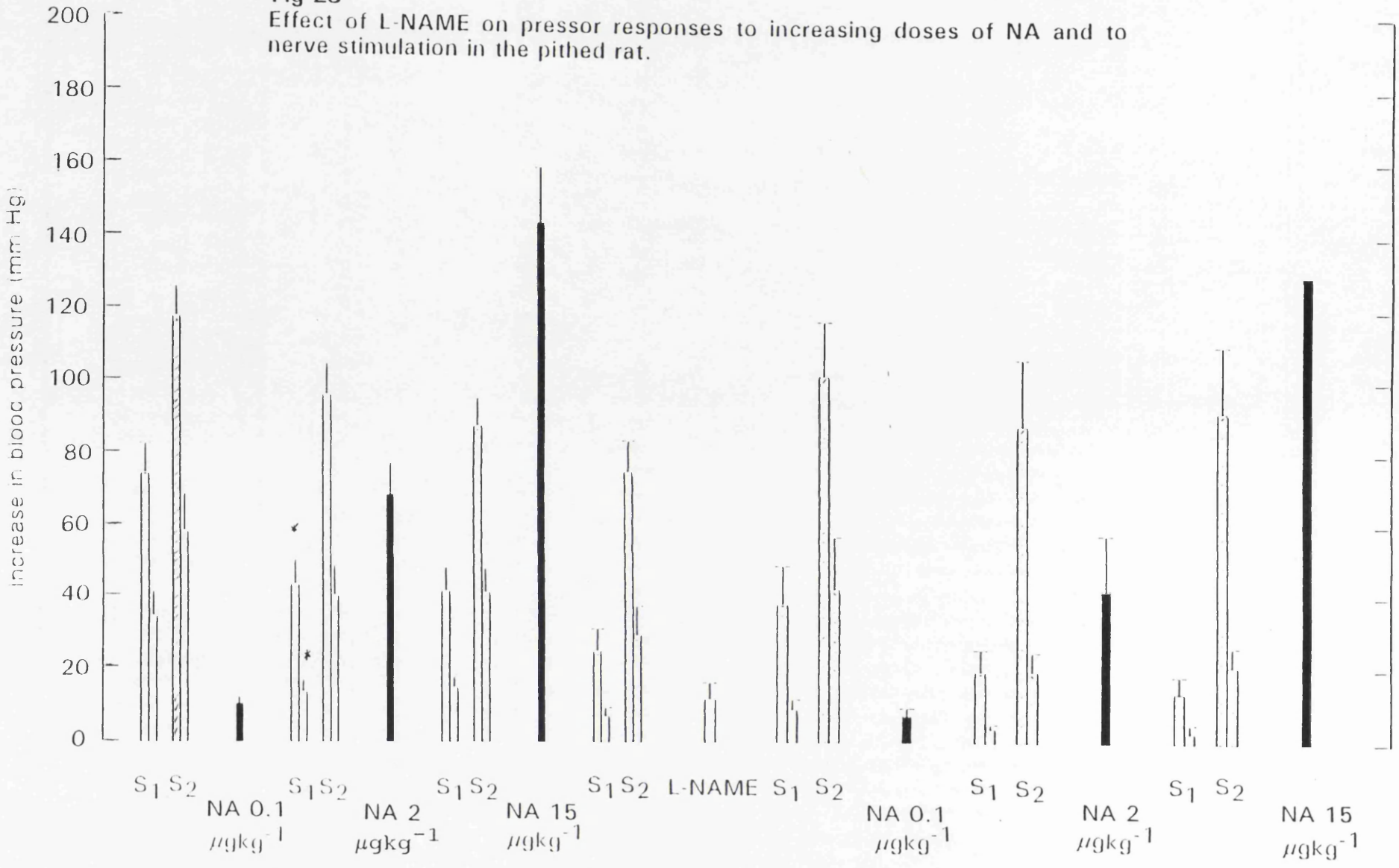
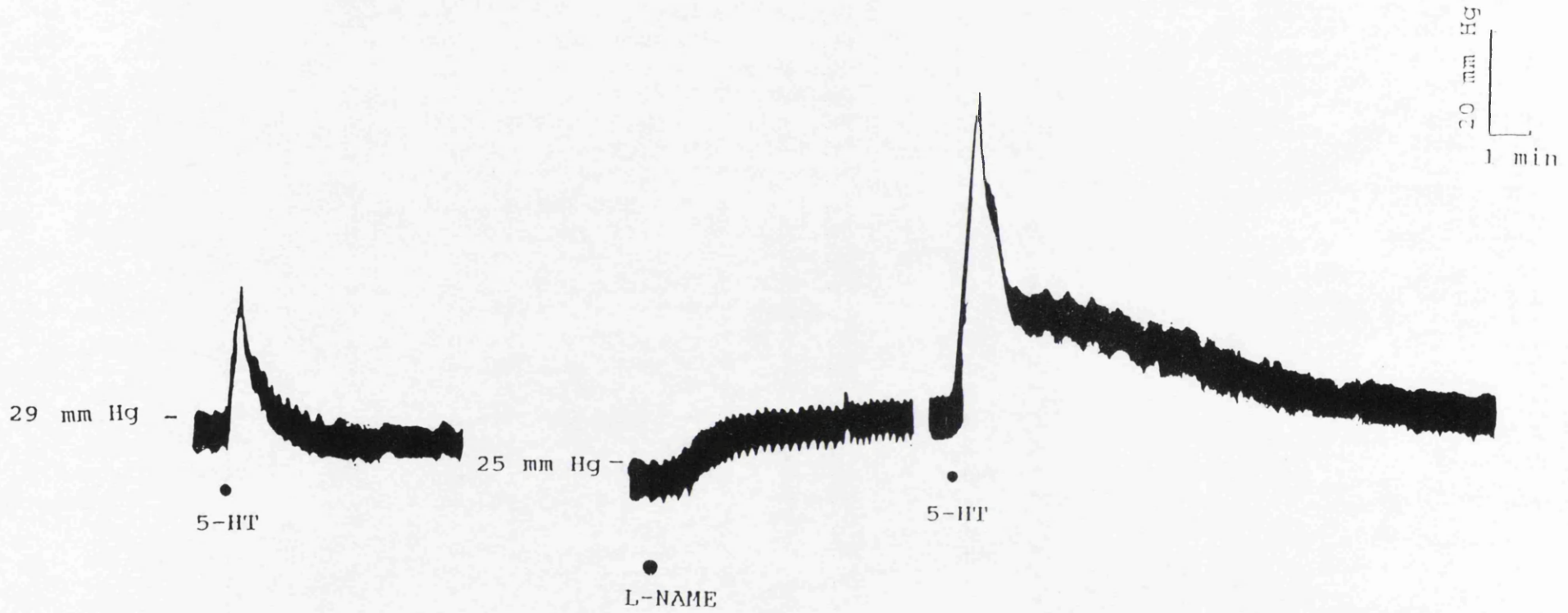


Fig-23

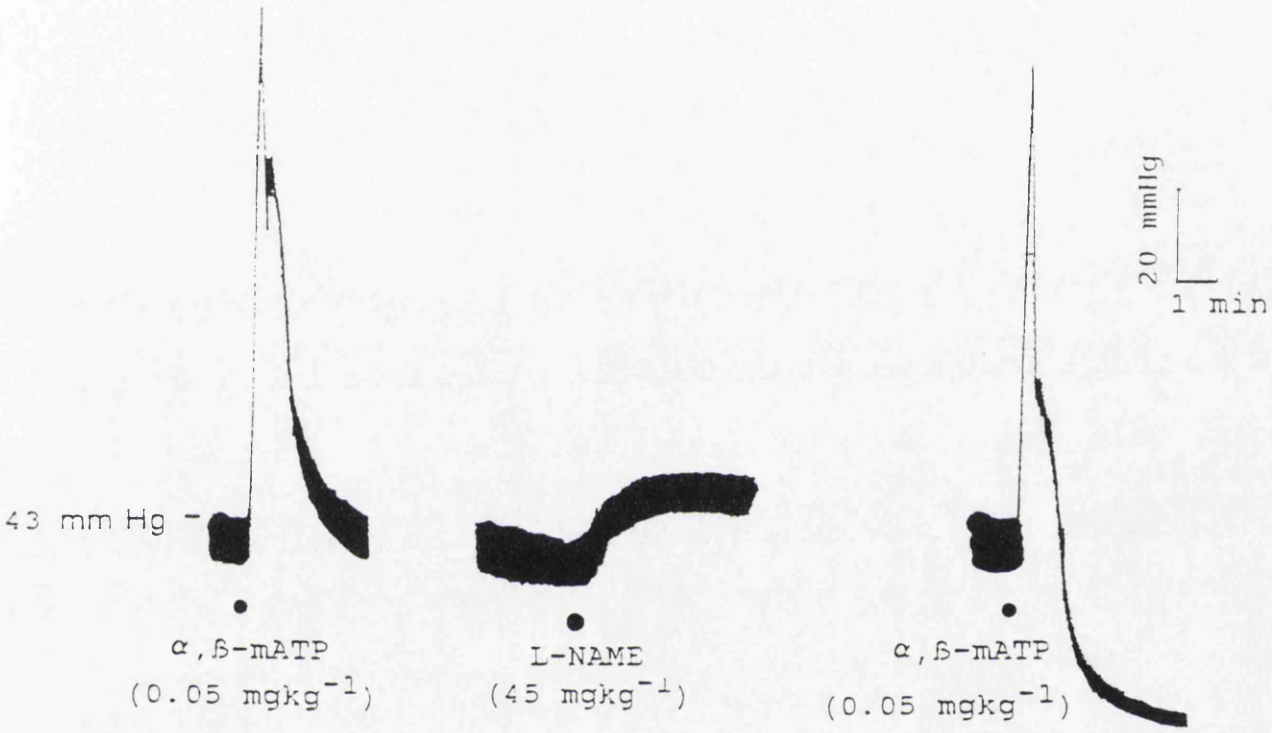
Pressor responses in the pithed rat (mm Hg, Mean ± SEM, $n \geq 3$) to NA and to stimulation of the spinal sympathetic outflow at T_{8-9} with supramaximal voltage, 0.4 ms pulse-duration at 2 Hz (S_1) and 20 Hz (S_2) for 100 pulses in the absence and presence of L-NAME (45 mg kg^{-1}, iv.). Propranolol (2 mg kg^{-1}, iv.) was present throughout the experiment. Stimulation at 2 Hz (S_1) and 20 Hz (S_2) produced characteristic responses, which consisted of a peak response during stimulation, followed by a smaller post-stimulus response. These responses are represented by the paired histograms with the adjoining, smaller histograms indicating the post-stimulation response in each case. NA (0.1, 2 and 15 μg kg^{-1}, iv.) was injected at intervals between the stimulations, before and after L-NAME (45 mg kg^{-1}, iv.). The lowest dose of NA (0.1 μg kg^{-1}, iv.) inhibited the standard pressor responses to nerve stimulation but although higher doses produced bigger pressor responses they had no such inhibitory effect on the responses to spinal nerve stimulation. L-NAME raised the resting blood pressure but did not affect the responses either to NA (0.1, 2 and 15 μg kg^{-1}, iv.) or to spinal nerve stimulation. The starting basal blood pressure was (Mean = 50.7 mm Hg, SEM = 4.55 mm Hg, $n = 5$). (* $0.05 > P > 0.01$).

Fig-24
Effect of L-NAME on pressor response to a submaximal dose of 5-HT in the pithed rat.



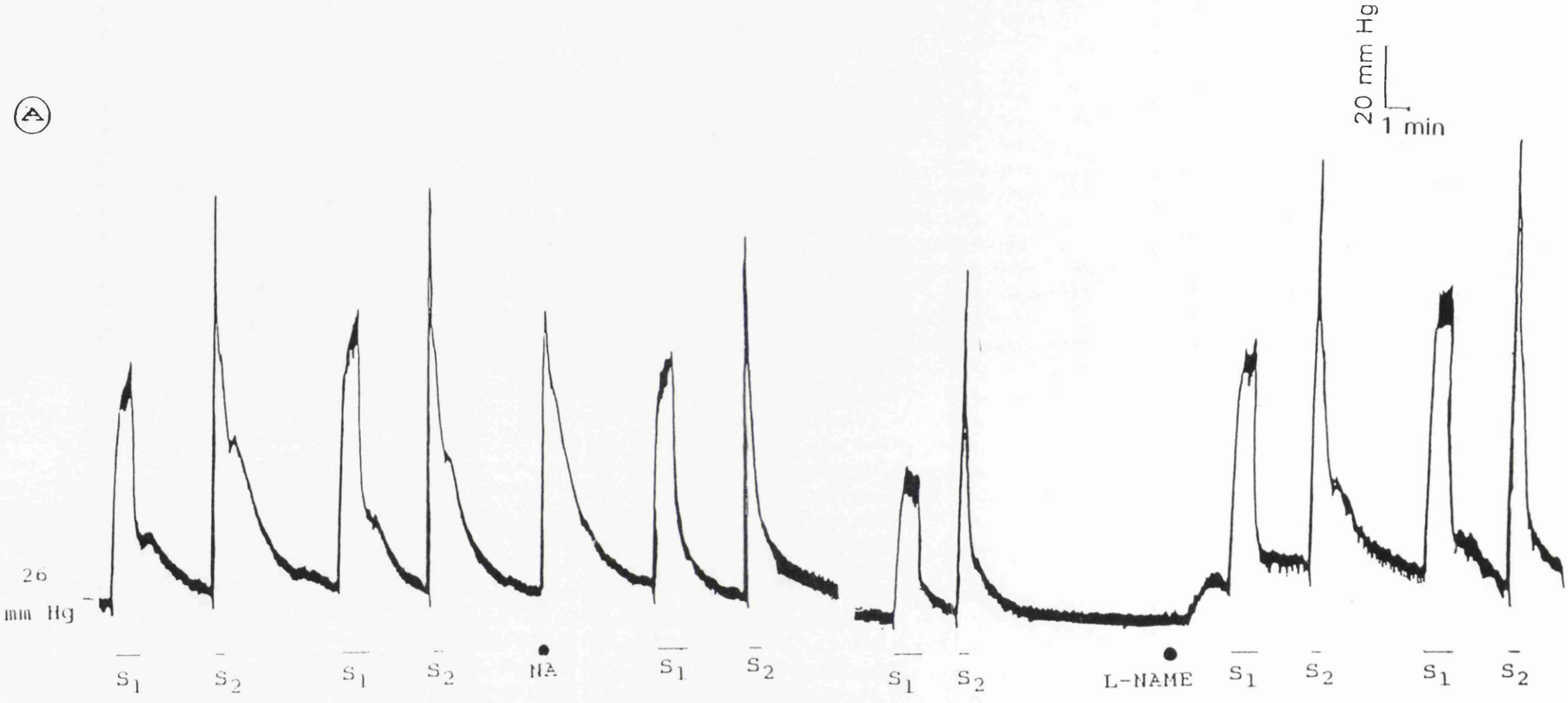
The pressor response to a submaximal concentration of 5-HT (25 μg kg^{-1}, iv.) in the absence and presence of L-NAME (45 mg kg^{-1}, iv.) in the pithed rat. L-NAME caused a small rise in the basal blood pressure and enhanced and prolonged the pressor response to 5-HT.

Fig-25
Effect of L-NAME on the pressor response to α,ß-mATP in the pithed rat.



The pressor responses to α,ß-mATP (0.05 mgkg^{-1}, iv.) in the absence and presence of L-NAME (45 mgkg^{-1}, iv.) in the pithed rat. L-NAME produced a small rise in the basal blood pressure and enhanced the toxicity of α,ß-mATP. Propranolol (2 mgkg^{-1}, iv.) was present throughout this experiment.

Fig-26 The effects of L-NAME and L-arginine administered consecutively on the pressor responses to nerve stimulation and to NA in the pithed rats.



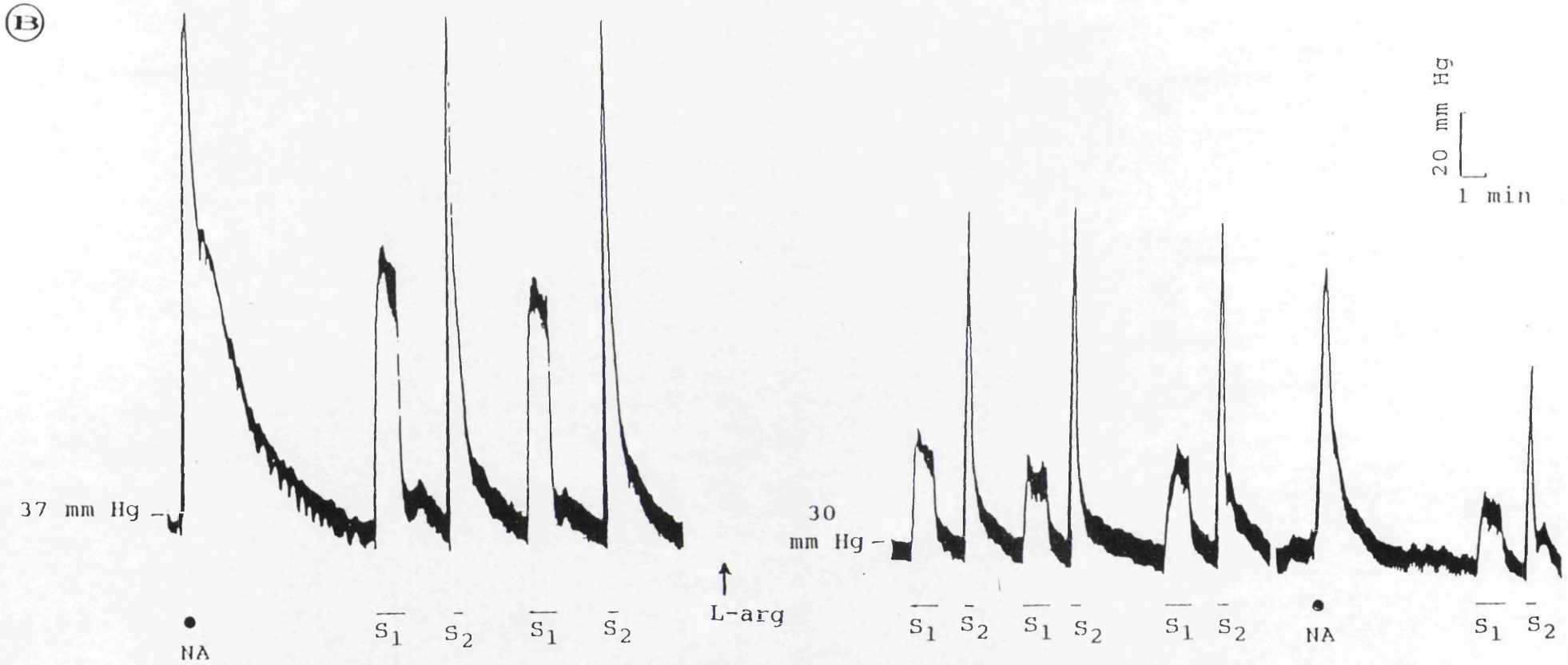

B
37 mm Hg
30
mm Hg
20 mm Hg
1 min
NA
S1
S2
L-arg
NA

Fig-26
Pressor responses to electrical stimulation of the spinal sympathetic outflow at T_{8-9} with supramaximal voltage, 0.4 ms pulse-duration at 2 Hz (S_1) and 20 Hz (S_2) for 100 pulses and to NA (2 μg kg^{-1}, iv.) in the pithed rat. Propranolol (2 mg kg^{-1}, iv.) was present throughout the experiment. The panels A and B are continuous. In panel A, stimulation at 2 Hz and 20 Hz produced characteristic, reproducible pressor responses. NA (2 μg kg^{-1}) also produced a pressor response, which was similar in magnitude to that produced by electrical stimulation at 2 Hz. After NA the pressor responses to electrical stimulation at 2 Hz declined slightly, after which L-NAME (45 mg kg^{-1}, iv.) increased the basal blood pressure and apparently enhanced the responses to electrical stimulation at 2 Hz and 20 Hz. In panel B, the standard response to NA (2 μg kg^{-1}) was enhanced after L-NAME (45 mg kg^{-1}). L-arginine (L-arg, 45 mg kg^{-1}, iv.) reversed the effect of L-NAME and reduced the pressor responses to NA and to nerve stimulation at 2 Hz and 20 Hz. After the pressor response to NA had declined and the blood pressure had returned to normal, pressor responses to electrical stimulation at 2 Hz and 20 Hz were further reduced. The periods of nerve stimulations are shown as (——= 50s and – = 5s).

Fig-27 Effects of L-NAME and L-arginine on the pressor responses to a submaximal dose of NA and to nerve stimulation in the pithed rat.

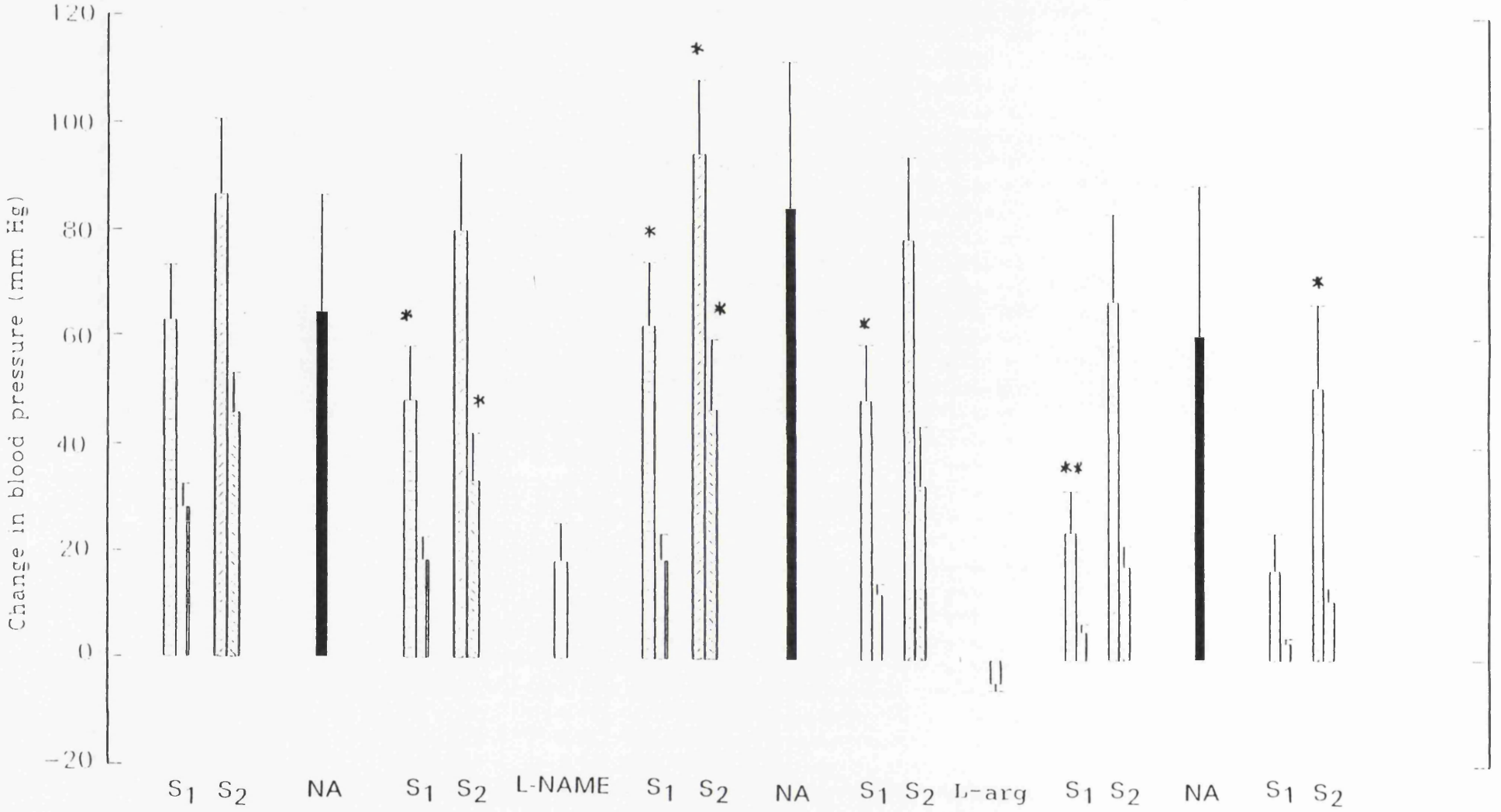


Fig-27

Histograms of pressor responses (mm Hg, Mean ± SEM, $n \geq 4$) in the pithed rat to electrical stimulation of the spinal sympathetic outflow at $T_{8,9}$ with supramaximal voltage, 0.4 ms pulse-duration at 2 Hz (S_1) and 20 Hz (S_2) for 100 pulses and also to NA (2 $\mu g\ kg^{-1}$, iv.). Propranolol (2 $mg\ kg^{-1}$, iv.) was present throughout the experiment. Stimulation at 2 Hz and 20 Hz produced characteristic responses, which are shown here as peak responses obtained during stimulation with the secondary, smaller post-stimulus responses that persisted after stimulation ceased, indicating by the smaller adjoining histogram. After NA (2 $\mu g\ kg^{-1}$, iv.) the pressor responses to electrical stimulation that occurred during stimulation at 2 Hz and the pressor response that persisted after stimulation at 20 Hz were inhibited. Thereafter, the injection of L-NAME (45 $mg\ kg^{-1}$, iv.) increased the basal blood pressure and enhanced the pressor responses to electrical stimulation at both 2 Hz and 20 Hz. After the subsequent administration of NA, the pressor response to electrical stimulation at 2 Hz was inhibited and was further inhibited after the administration of L-arginine (L-arg, 45 $mg\ kg^{-1}$, iv.). The starting basal blood pressure was (Mean = 33.0 mm Hg, SEM = 10.0 mm Hg, n = 4). (* $0.05 > P > 0.01$, ** $0.01 > P > 0.001$).

Fig-28
Effect of L-NAME on pressor responses to increasing doses of 5-HT in the pithed rat.

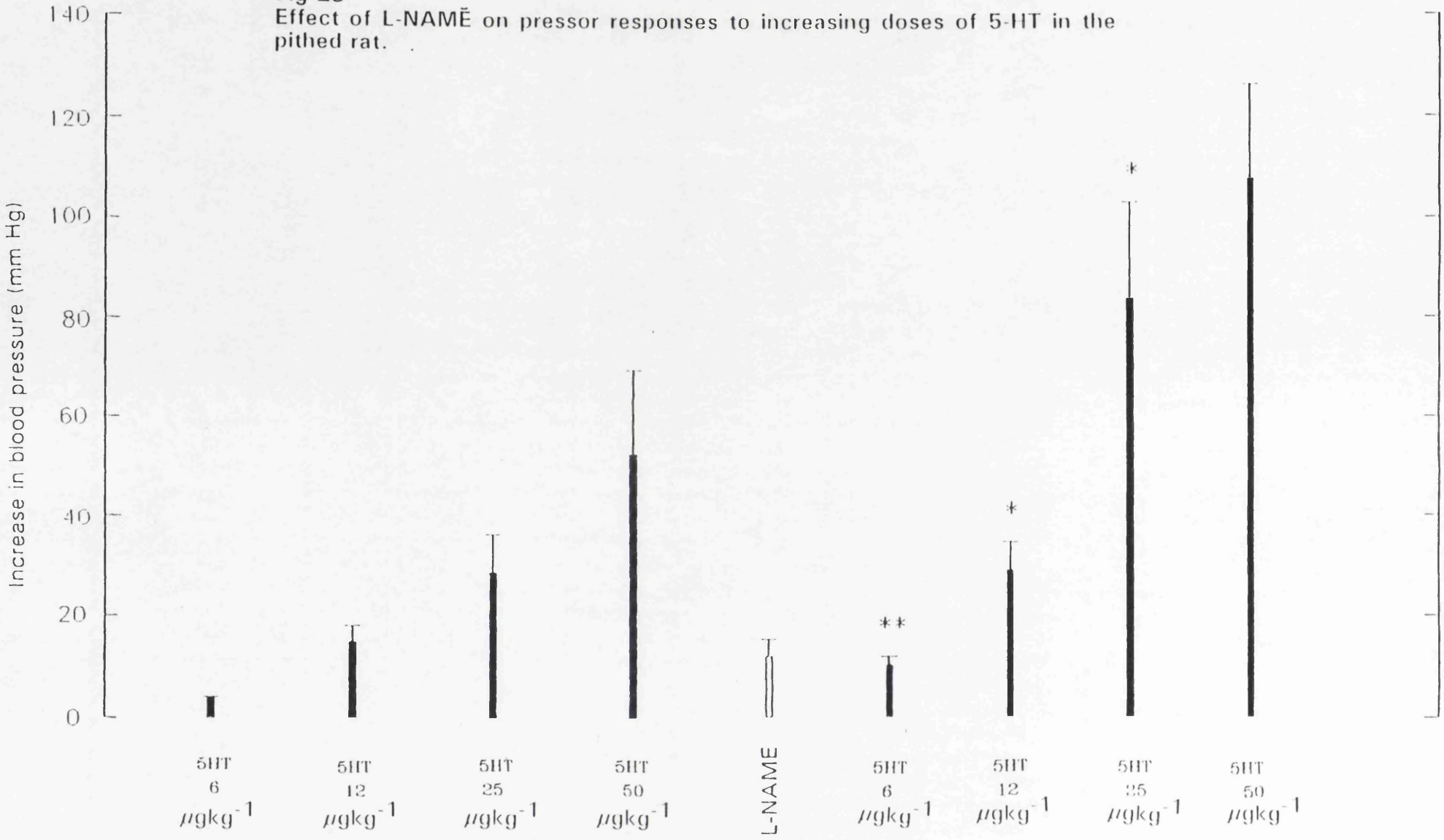
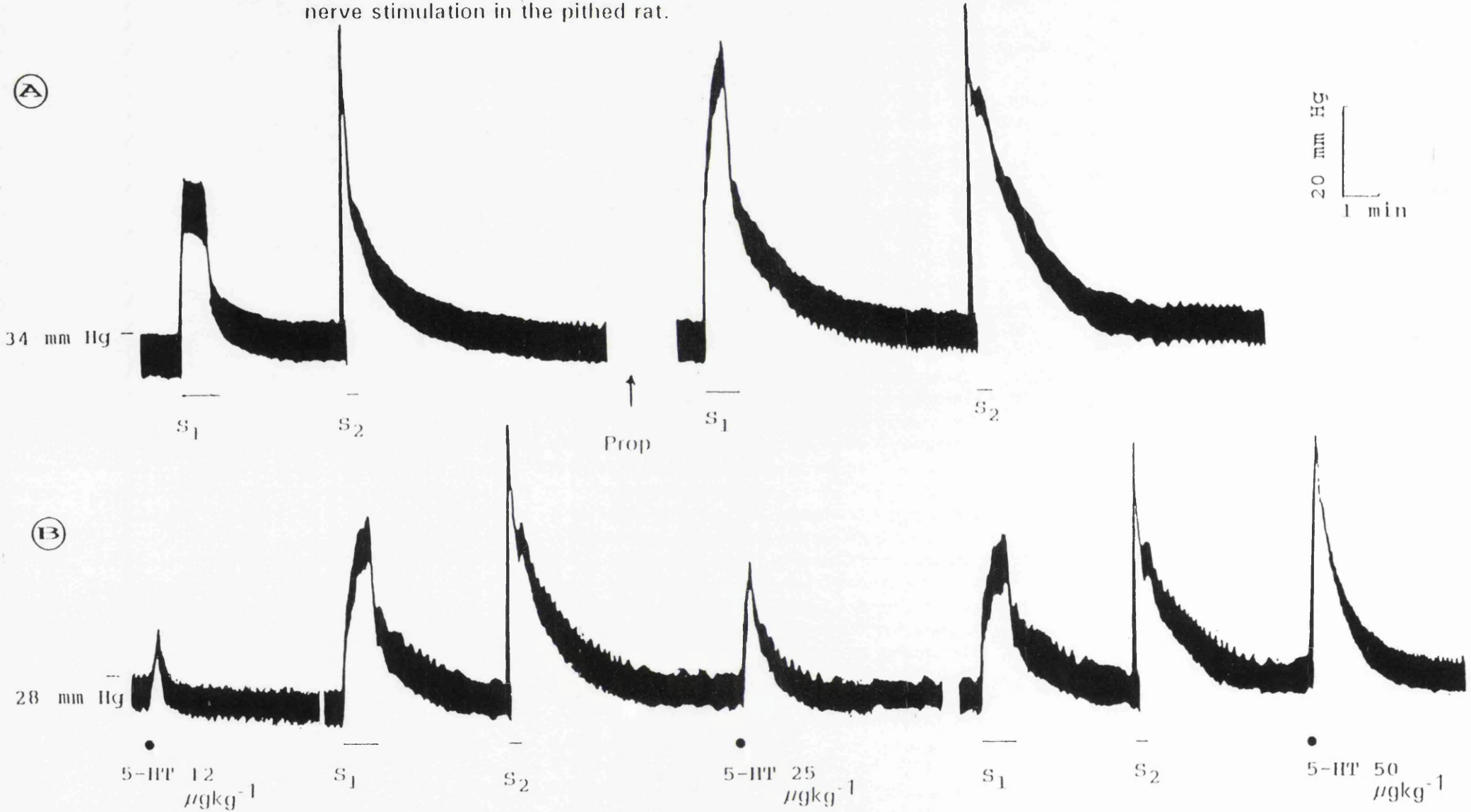
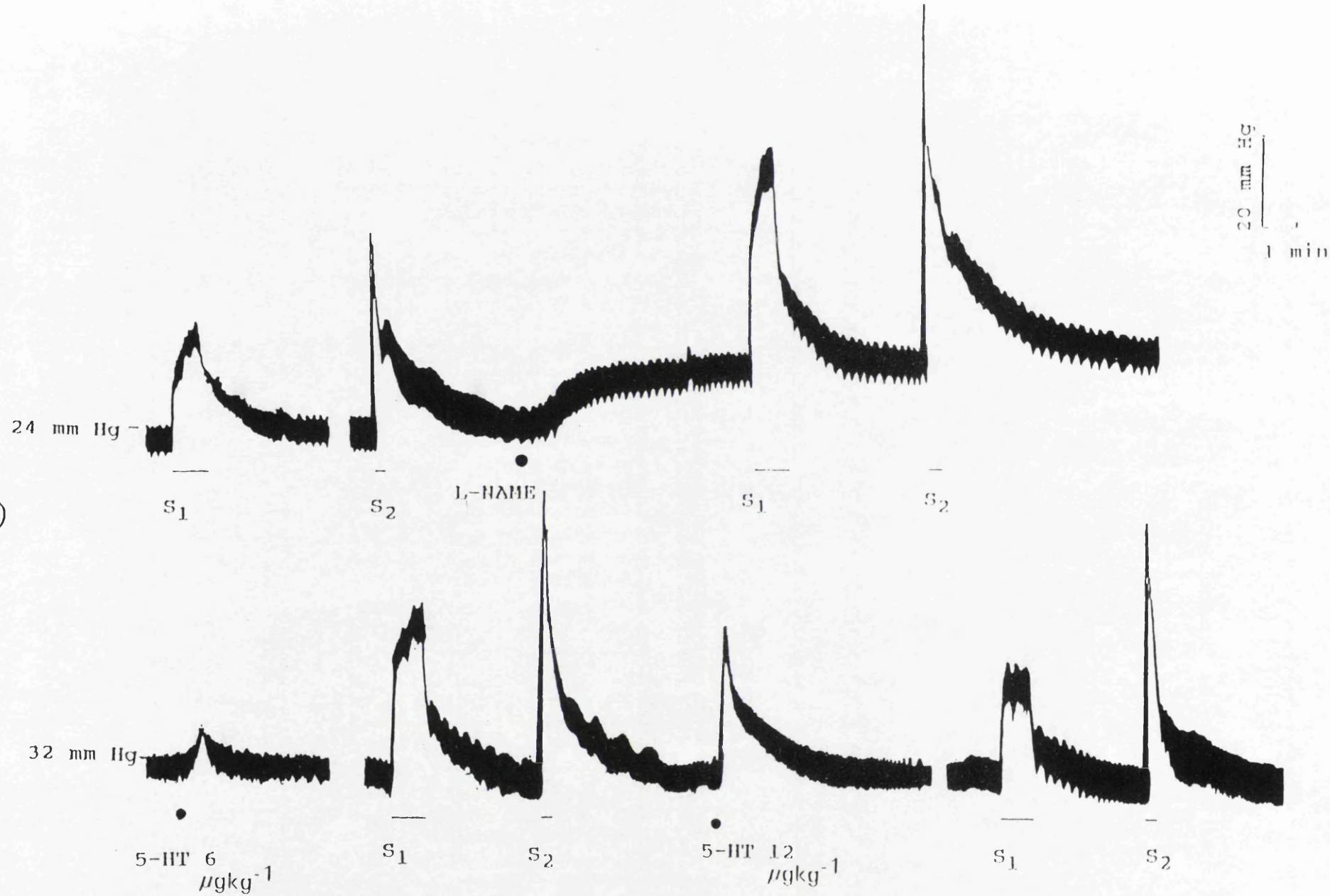


Fig-28
Histogram of pressor responses (mm Hg, Mean ± SEM, $n \geq 3$) in the pithed rat to 5-HT (6, 12, 25, 50 $\mu g\ kg^{-1}$, iv.) in the absence and presence of L-NAME (45 $mg\ kg^{-1}$, iv.). Propranolol (2 $mg\ kg^{-1}$, iv.) was present throughout the experiment. 5-HT produced dose-dependent pressor responses. L-NAME raised the basal blood pressure and enhanced the pressor responses to 5-HT (6, 12, 25 $\mu g\ kg^{-1}$, iv.). The starting basal blood pressure was (Mean = 33.05, mm Hg, SEM = 1.47 mm Hg, $n = 11$). (* $0.05 > P > 0.01$, ** $0.01 > P > 0.001$).

Fig-29 Effect of L-NAME on pressor responses to increasing doses of 5-HT and nerve stimulation in the pithed rat.




C
20 mm Hg
1 min
24 mm Hg
S1
S2
L-NAME
S1
S2
D
32 mm Hg
5-HT 6
μgkg-1
S1
S2
5-HT 12
μgkg-1
S1
S2

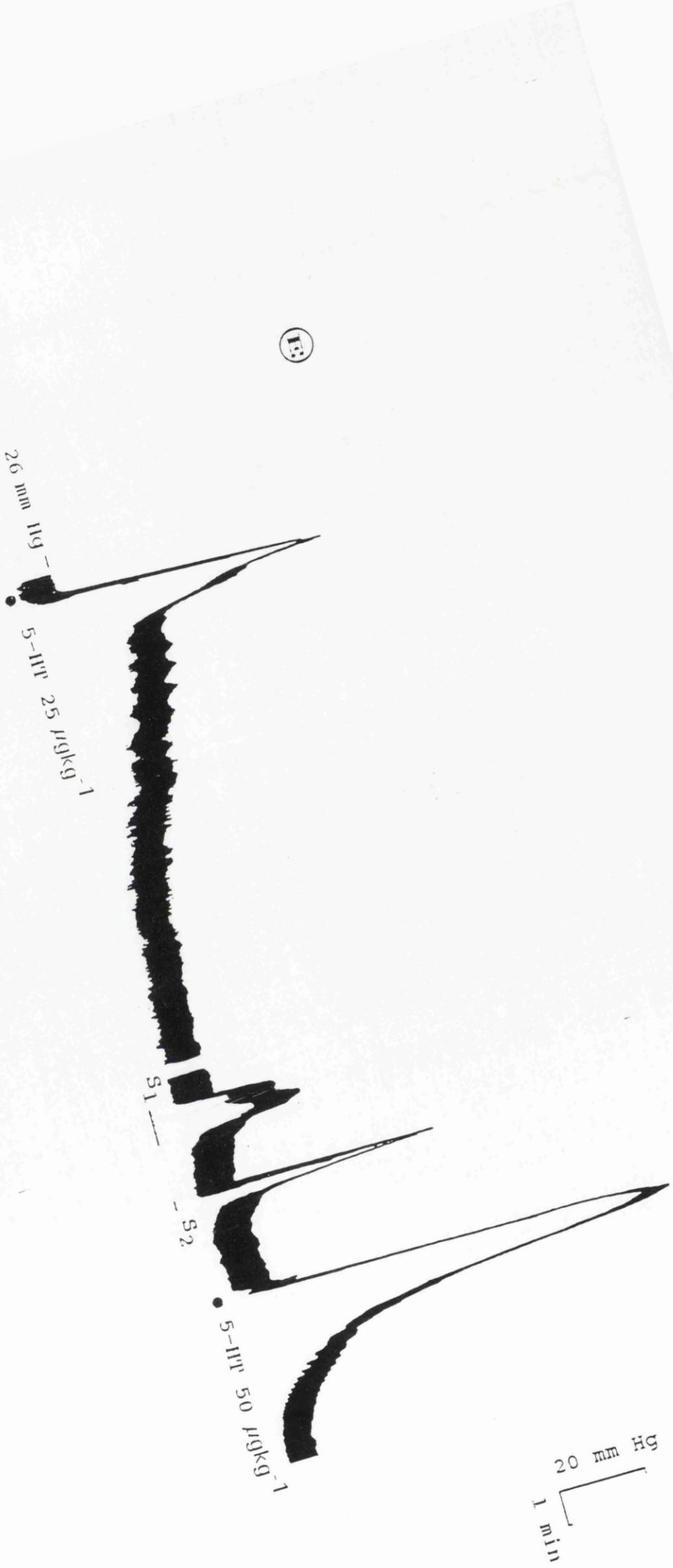
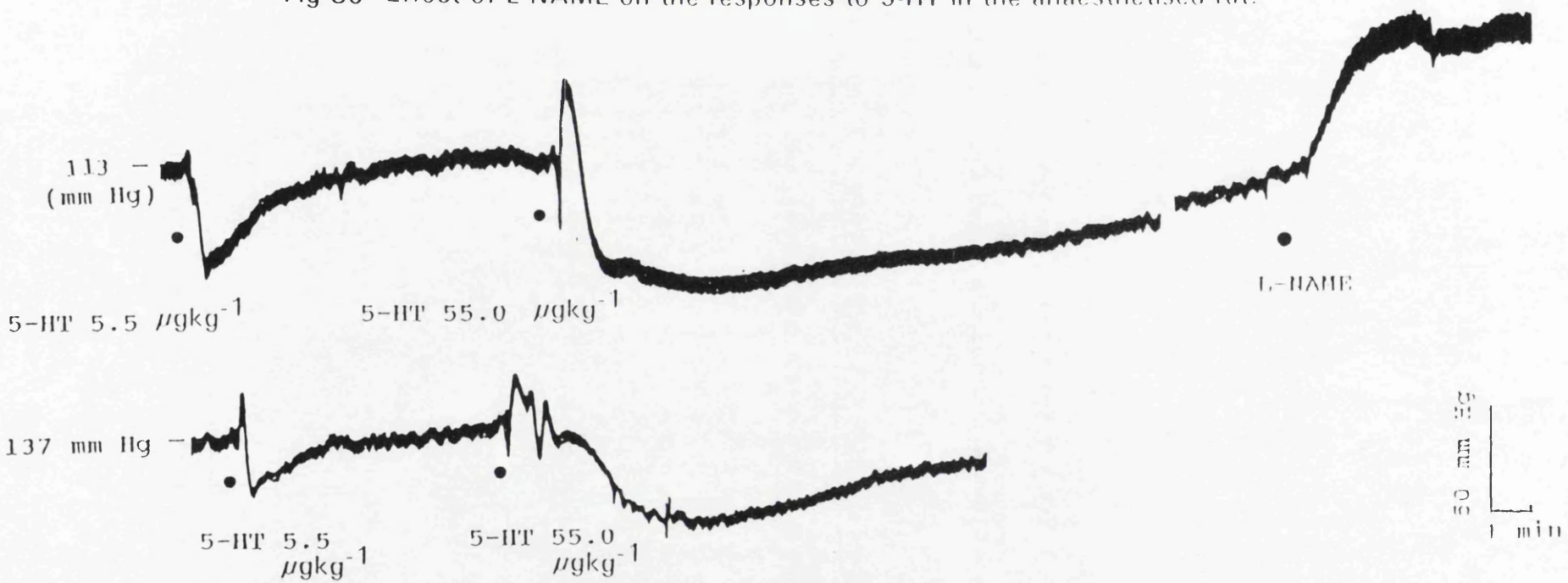

26 mm Hg
5-HT 25 μgkg⁻¹
S1
S2
5-HT 50 μgkg⁻¹
20 mm Hg
1 min

Fig-29

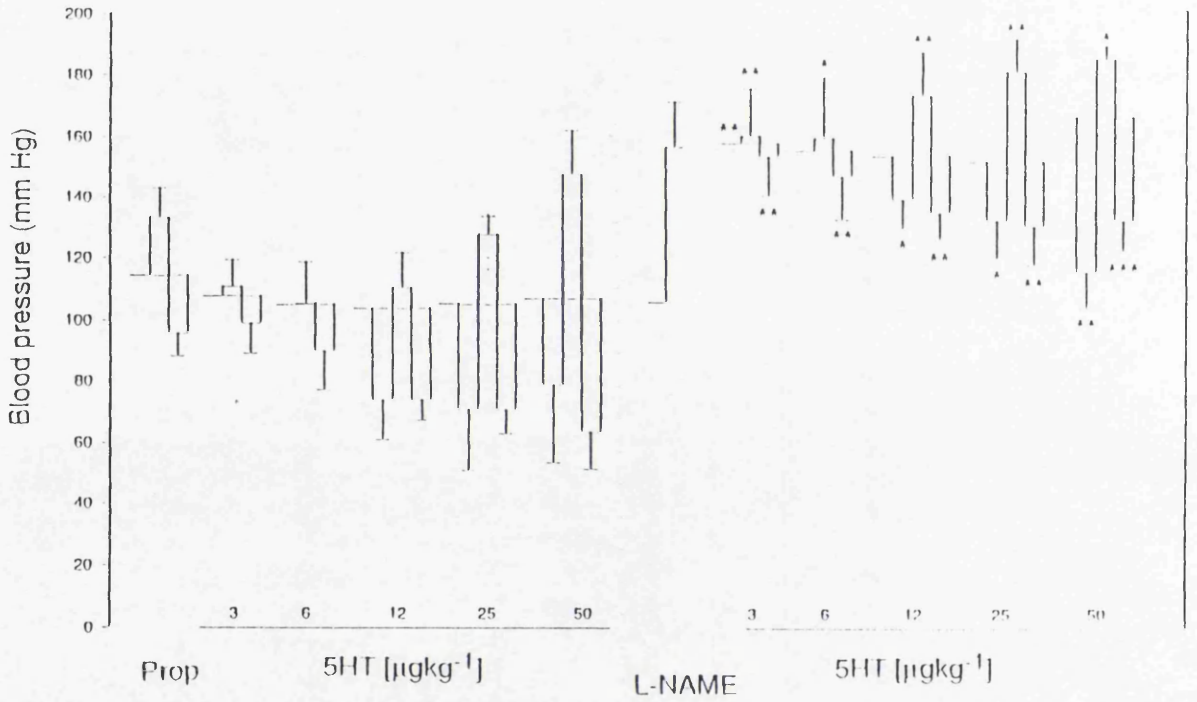
Traces (A ,B ,C ,D and E) obtained in a single experiment showing pressor responses (mm Hg) to electrical stimulation of the spinal sympathetic outflow at T_{8-9} with supramaximal voltage, 0.4 ms pulse-duration at 2 Hz (S_1) and 20 Hz (S_2) for 100 pulses in the pithed rat. Stimulation at 2 Hz and 20 Hz produced characteristic pressor responses. In trace A propranolol (Prop, 2 mg kg^{-1}, iv.) enhanced the pressor response obtained during stimulation at 2 Hz and also enhanced the secondary post-stimulus pressor response that persisted after stimulation at 2 Hz ceased. In addition, propranolol also enhanced the post-stimulus component of the pressor response to stimulation at 20 Hz. In trace B, 5-HT (12, 25 and 50 μg kg^{-1}, iv.) produced dose-related pressor responses to electrical stimulation. In trace C, L-NAME (45 mg kg^{-1}, iv.) raised the baseline tone and potentiated the pressor responses to electrical stimulation. In traces D and E the pressor responses to 5-HT (6, 12, 25 and 50 μg kg^{-1}, iv.) were enhanced after the administration of L-NAME (in trace C) in comparison with the responses to 5-HT in trace B. However, in traces D and E the standard pressor responses to electrical stimulation at 2 Hz and 20 Hz were reduced as the dose of 5-HT increased. The periods of nerve stimulations are shown as (——= 50s and – = 5s).

Fig-30 Effect of L-NAME on the responses to 5-HT in the anaesthetised rat.



Traces showing the effects of 5-HT (5.5 and 55.0 μg kg^{-1}, iv.) on the blood pressure in the anaesthetised (pentobarbitone, 60 mg kg^{-1}, ip.) rat, before and after administration of L-NAME (45 mg kg^{-1}, iv.). Propranolol (2 mg kg^{-1}, iv.) was present throughout the experiment. The lower trace is a continuation of the upper trace. 5-HT produced a complex effect, which consisted of an initial brief depressor component, a secondary pressor component followed by a final prolonged depressor component that was most marked at the higher dose of 5-HT (55.0 μg kg^{-1}, iv.). After L-NAME (45 mg kg^{-1}, iv.), which raised the basal blood pressure, the pressor response to the low dose of 5-HT (5.5 μg kg^{-1}, iv.) was slightly enhanced and the pressor response to the high dose of 5-HT (55.0 μg kg^{-1}, iv.) showed an irregular pattern. L-NAME also reduced the depressor components of 5-HT.

Fig-31 Histograms of the effect of L-NAME on the triphasic responses to 5-HT in the anaesthetised rats.



Histograms of the responses (mmHg, Mean ± SEM, $n \geq 4$) in the anaesthetised rats to 5-HT (3, 6, 12, 25 and 50 μgkg^{-1}, iv.) in the absence and presence of L-NAME (45 $mgkg^{-1}$, iv.). Propranolol (Prop, 2 $mgkg^{-1}$, iv.) was present throughout the experiment. 5-HT produced dose-dependent triphasic responses which was most marked at higher doses in the absence and presence of L-NAME. L-NAME raised the basal blood pressure, enhanced the pressor component but reduced the depressor components of the response to 5-HT (3, 6, 12, 25 and 50 μgkg^{-1}, iv.) (* $0.05 > P > 0.01$, ** $0.01 > P > 0.001$, *** $P < 0.001$).

Fig-32 The apparatus used to perfuse Krebs-buffer throughout the pulmonary circulation of the rat lungs.

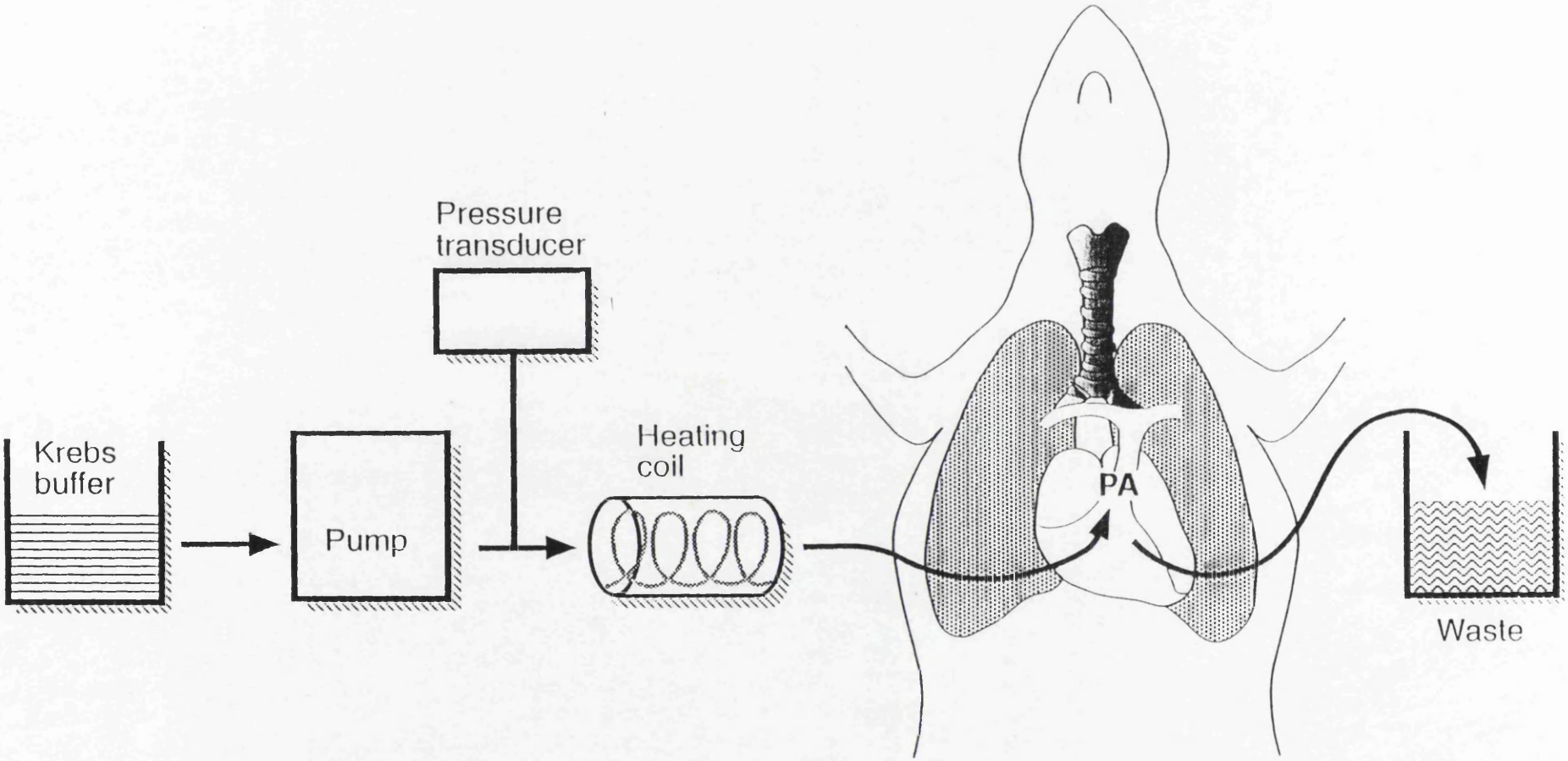
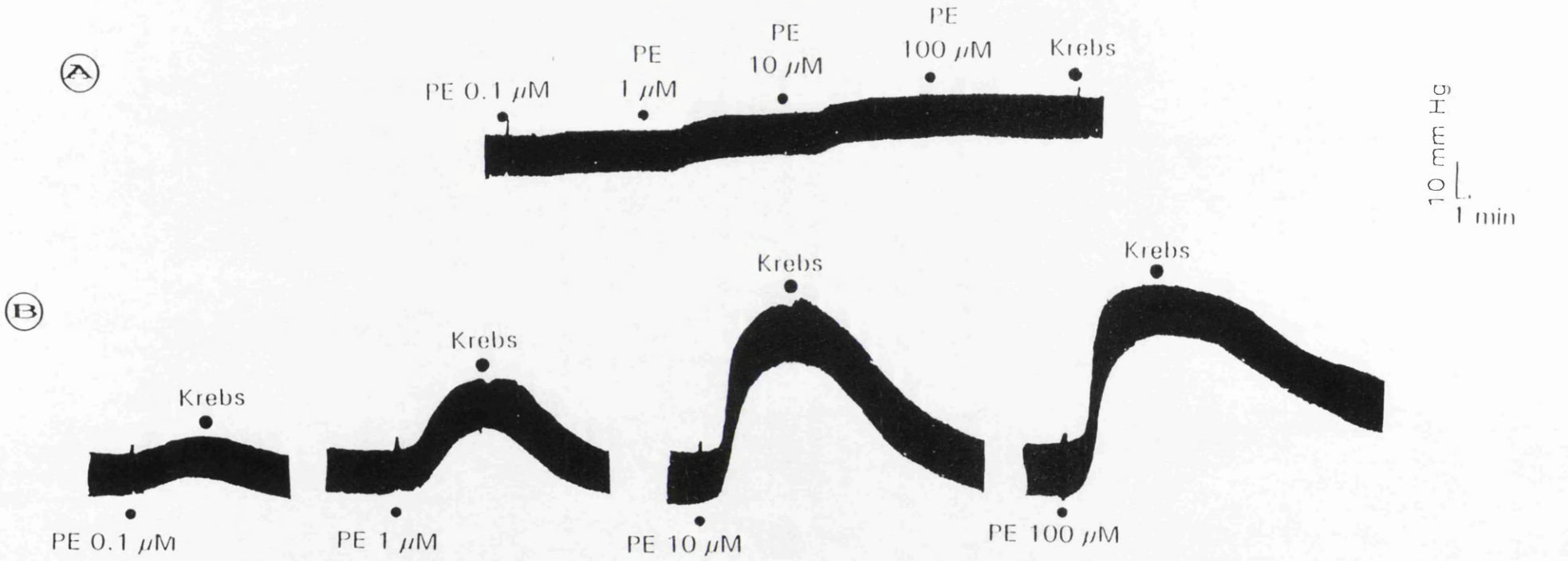


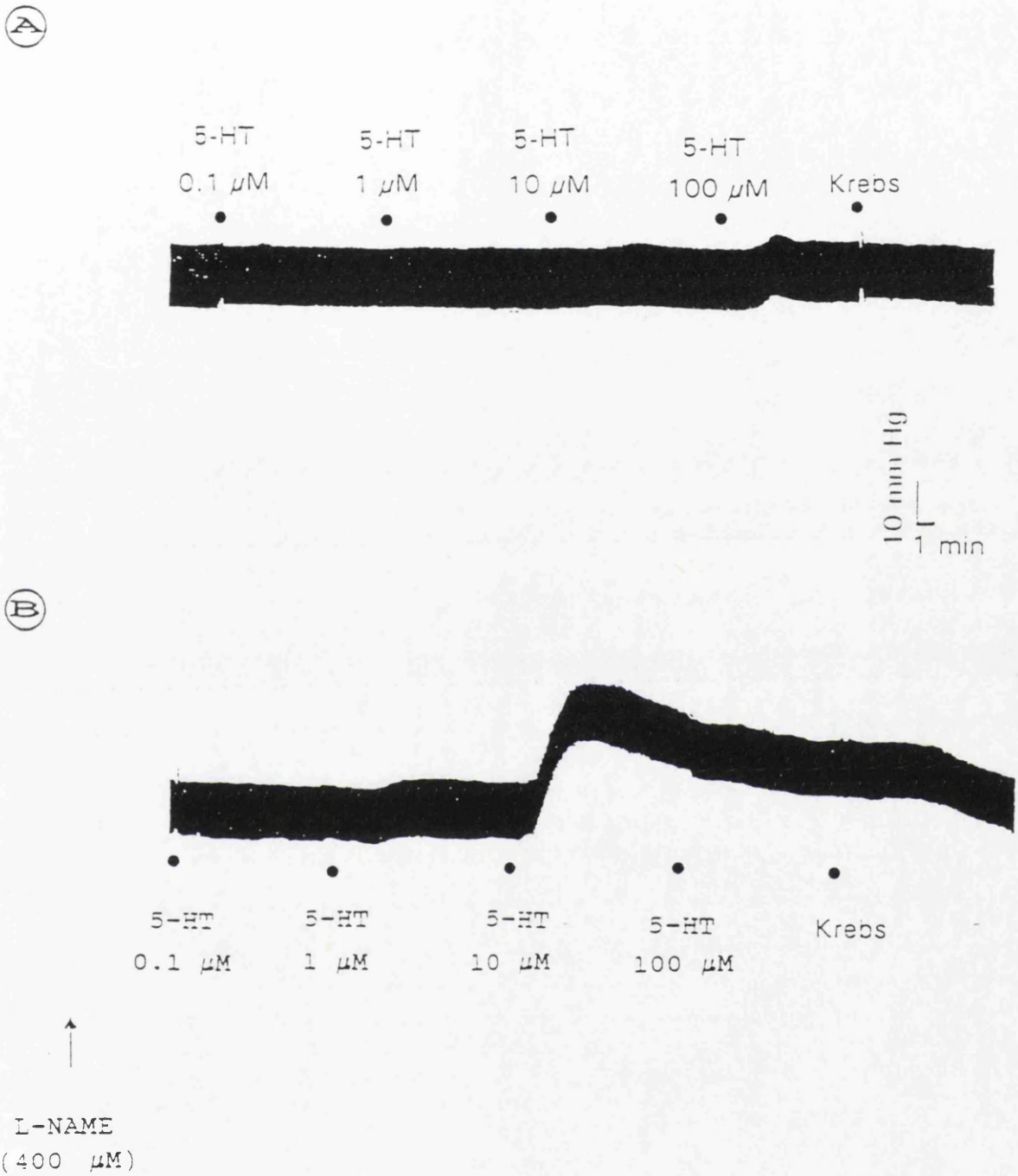
Diagram of the assembly used to perfuse the pulmonary circulation, illustrating from left to right the Krebs-buffer solution reservoir, pulsatile perfusion pump, Statham pressure transducer, heating coil, in situ lungs with the attached heart and waste reservoir. PA (Pulmonary Artery).

Fig-33 Pressor responses to cumulatively perfused and singly perfused PE in the isolated, Krebs-buffer perfused pulmonary vasculature of the Wistar rat



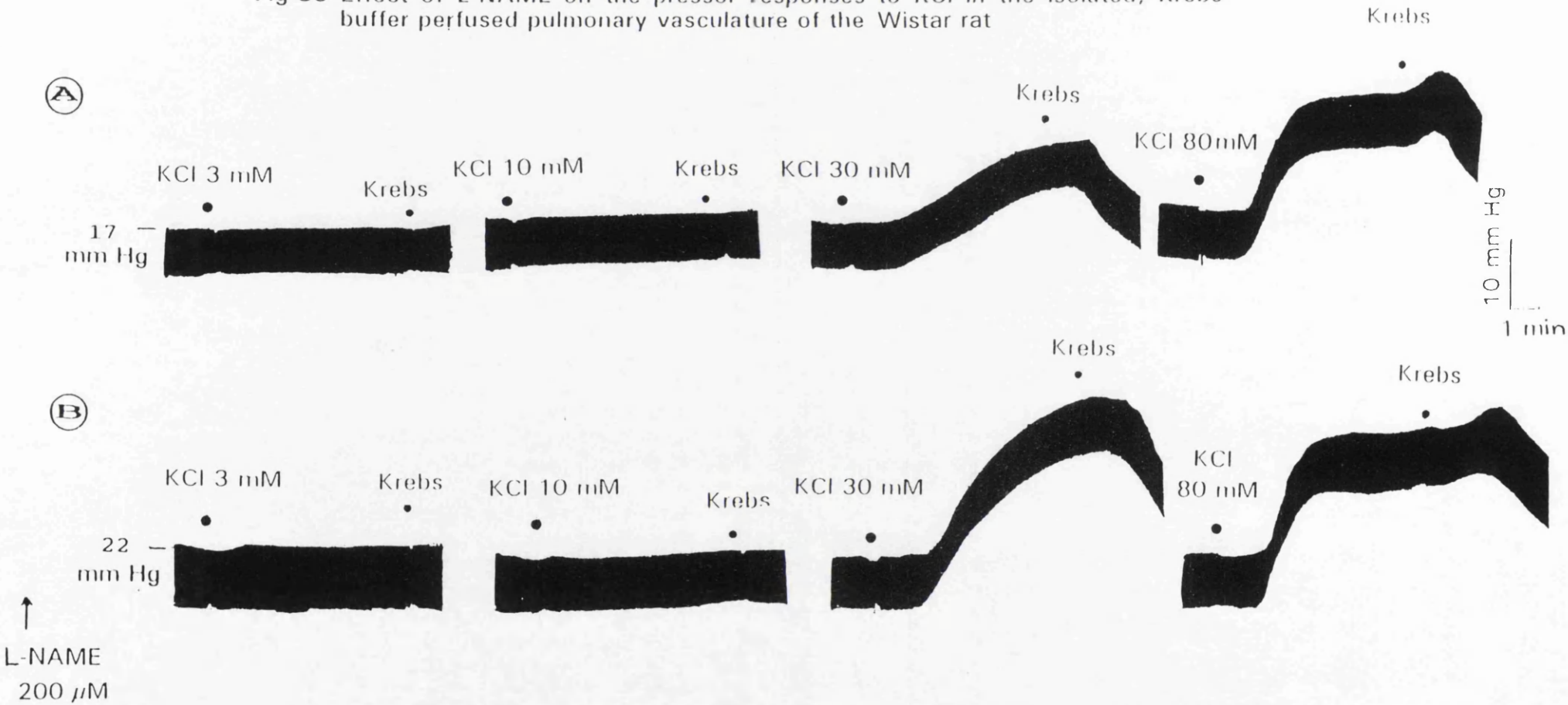
Traces in panels A and B showing pressor responses to increasing concentrations of PE (0.1-100 μM) in the Krebs-buffer perfused rats pulmonary circulations. The trace in panel A illustrates, responses to cumulative perfusions of PE but the trace in panel B illustrates, single perfusions of PE (ie. each concentration of PE was perfused until an equilibrium response was obtained and then the perfusion was stopped and the preparation was allowed to recover before the next concentration was perfused). The pressor responses to PE obtained with cumulative perfusion which had no recovery intervals were smaller than single perfusion with recovery intervals.

Fig-34
Effect of L-NAME on the pressor responses to cumulative concentrations of 5-HT in the isolated, Krebs-buffer perfused pulmonary vasculature of the Wistar rat



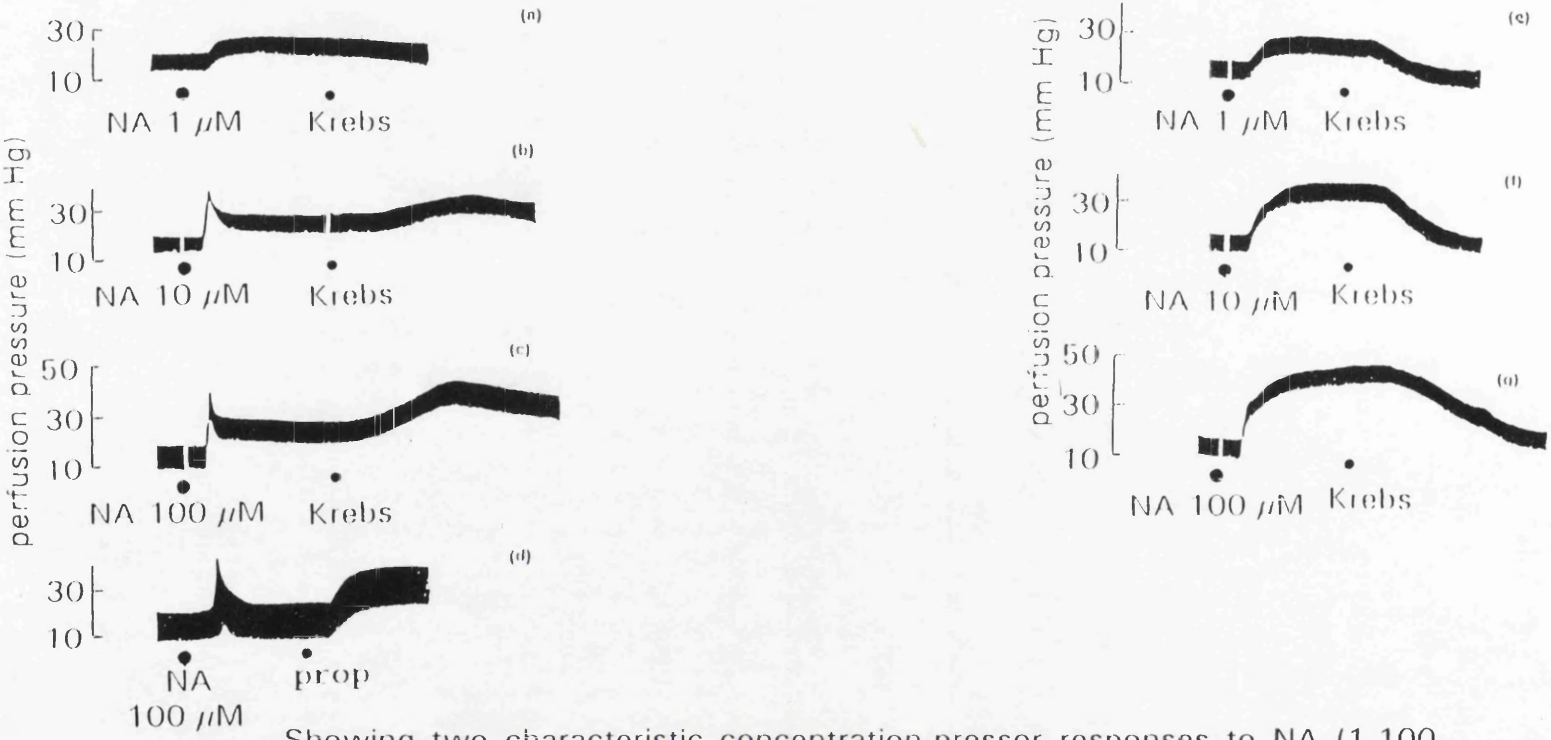
Traces showing pressor responses to cumulatively perfused increasing concentrations of 5-HT (0.1-100 μM) in the Krebs-buffer perfused rat pulmonary circulation in the absence (panel A) and presence (panel B) of L-NAME (400 μM). The trace in panel B is a continuation of the trace in panel A. L-NAME enhance the 5-HT-induced pressor responses at the submaximal concentrations (10-100 μM).

Fig-35 Effect of L-NAME on the pressor responses to KCl in the isolated, Krebs-buffer perfused pulmonary vasculature of the Wistar rat



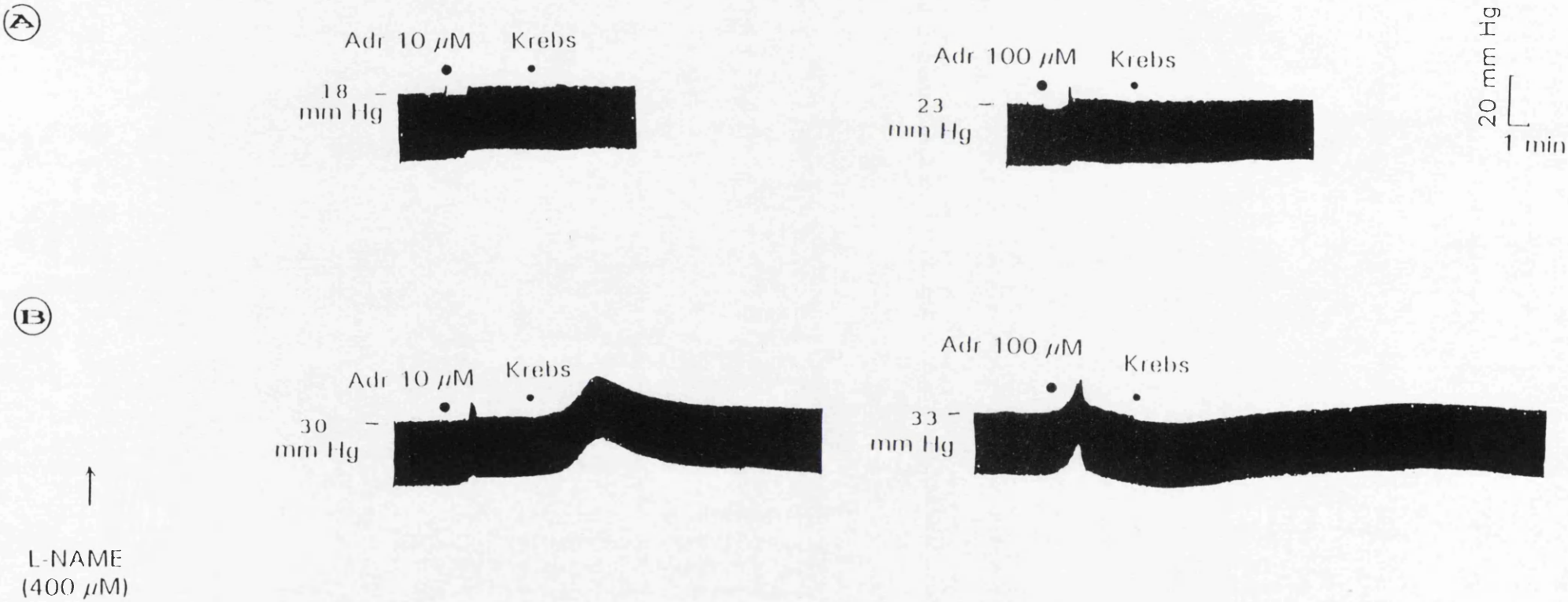
Traces showing pressor responses to increasing concentrations of KCl (3-80 mM) in the Krebs-buffer perfused rat pulmonary circulation, in the absence (Panel A) and presence (Panel B) of L-NAME (200 μM). The trace in panel B is a continuation of the trace in panel A. Each concentration of KCl was perfused until an equilibrium response was obtained and then the perfusion was stopped and the preparation was allowed to recover before the next concentration was perfused.

Fig-36 Two types of pressor responses to NA and the effect of propranolol, administered during the decline in the pressor response to NA, in the isolated, Krebs-buffer perfused pulmonary vasculature of the Wistar rat



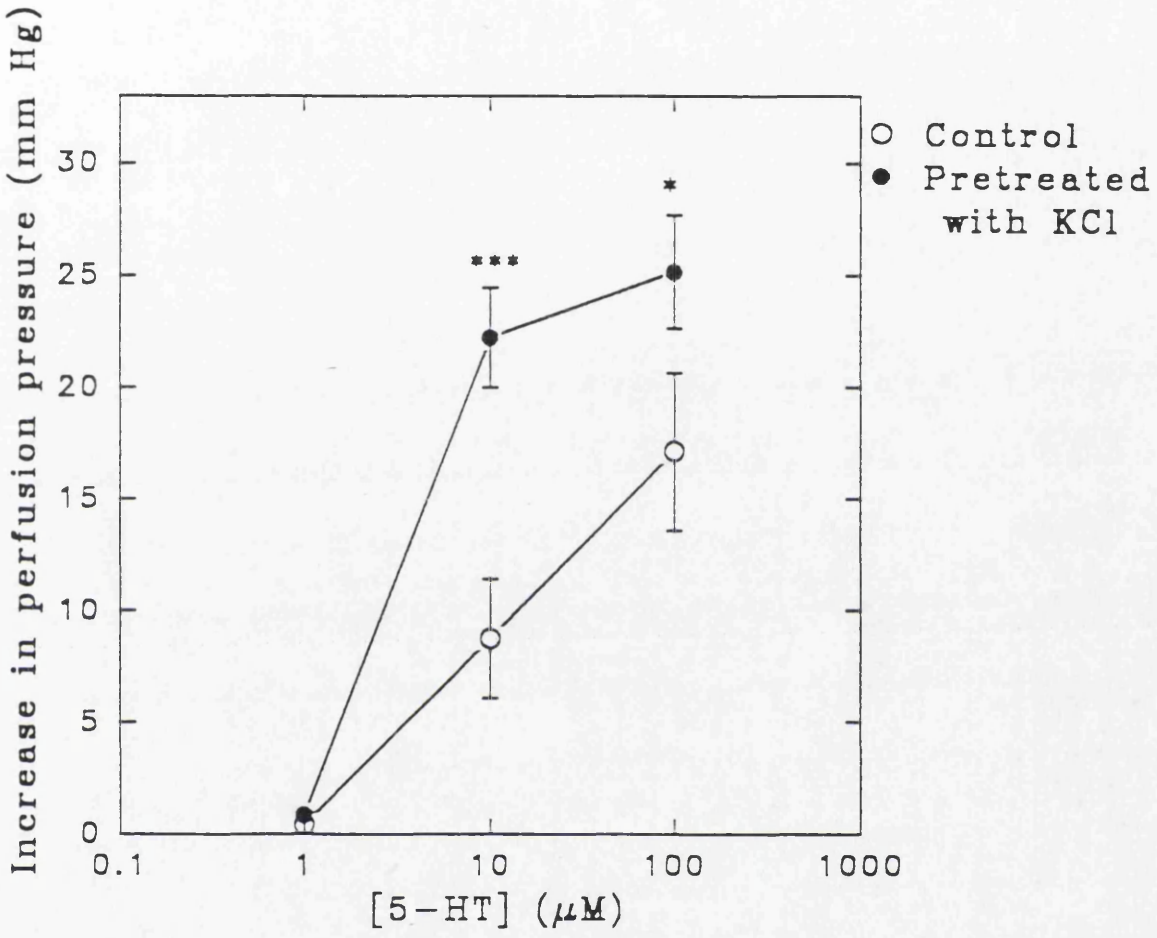
Showing two characteristic concentration-pressor responses to NA (1-100 μM) in the Krebs-buffer perfused rat pulmonary vasculature. Traces (b,c) showing poorly-maintained pressor responses to NA which were the submaximal (10 μM) and maximal (100 μM) concentrations, with developed post-drug increases in perfusion pressure. Propranolol perfusion on the ongoing pressor response to a maximal concentration of NA (100 μM) during its decline, produced a well-maintained pressor response to NA (Trace d). Traces (e,f,g) showing well-maintained pressor responses to increasing concentrations of NA, with quick recovery after washing out the drug.

Fig-37 Effect of L-NAME at 12 ml min^{-1} rate of perfusion on the pressor responses to a submaximal and a maximal concentration of adrenaline in the isolated, Krebs-buffer perfused pulmonary vasculature of the Wistar rat



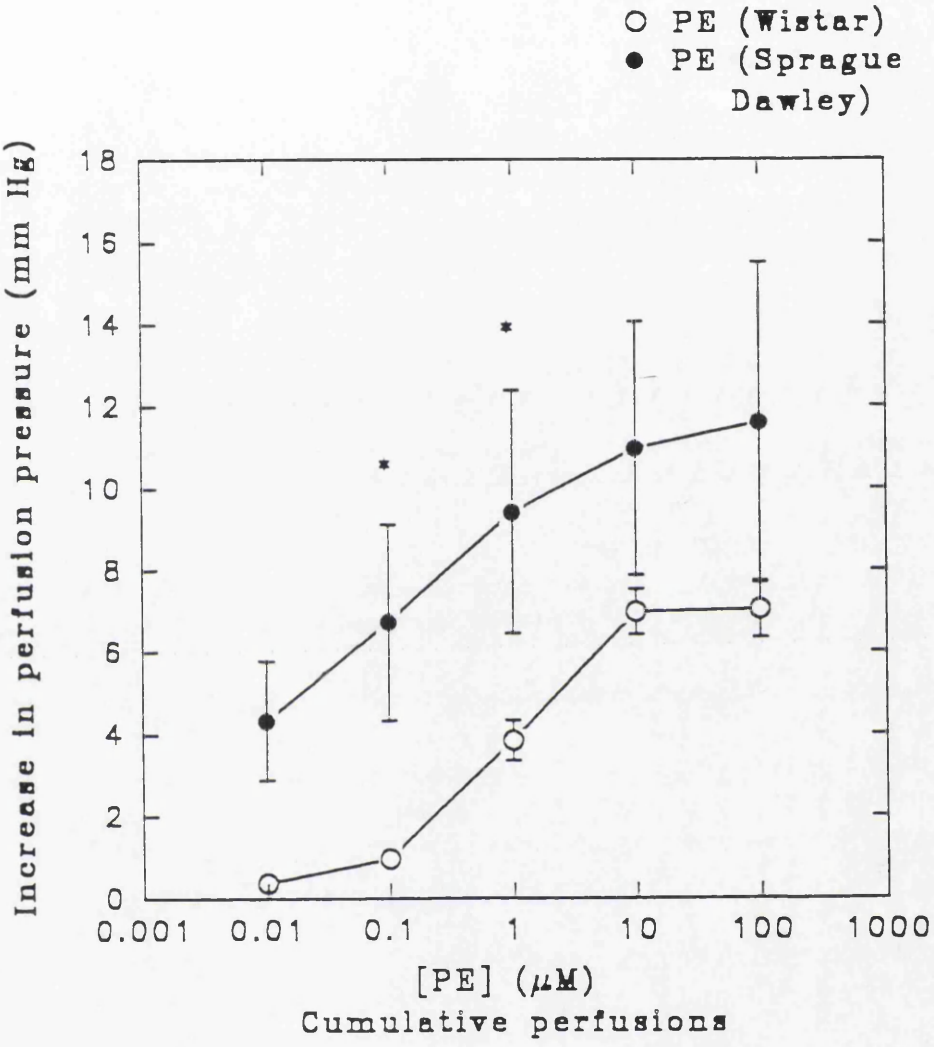
Showing pressor responses to a submaximal (10 μM) and a maximal (100 μM) concentration of adrenaline (Adr) in the absence (Panel A) and presence (Panel B) of L-NAME (400 μM) at a perfusion rate of 12 ml min^{-1} in the Krebs-buffer perfused rat pulmonary vasculature. There is a well-developed post-drug hump after washing out adrenaline (10 μM) in the presence of L-NAME.

Fig-38
Effect of KCl on the concentration-response curve to 5-HT in the presence of L-NAME in the isolated Krebs-buffer perfused pulmonary vasculature of the Wistar rat.



Concentration-response curves to 5-HT (1-100 μM) in the Krebs-buffer perfused rat pulmonary circulation, in the presence of L-NAME (400 μM) and in the absence and presence of an initial equilibrium perfused single submaximal concentration of KCl (40 mM) which was then stopped and the preparation allowed to recover before the concentration-response curve to 5-HT was obtained. Each concentration of 5-HT was perfused to obtain an equilibrium response and then the preparation washed with Krebs-buffer containing L-NAME to allow it to recover before the next concentration was perfused. KCl enhanced the pressor responses to 5-HT (10-100 μM). The results are shown as Means $\pm$ SEM, $n = 3$. The basal perfusion pressure before the start of the control curve was (Mean = 14.67 mm Hg, SEM = 3.93 mm Hg, $n = 3$) and of the test curve was (Mean = 8.33 mm Hg, SEM = 0.33 mm Hg, $n = 3$). (* $0.05 > P > 0.01$, *** $P < 0.001$).

Fig-39
Concentration-response curve to PE in the isolated Krebs-buffer perfused pulmonary vasculature of Wistar and Sprague Dawley rats.



Concentration-response curves to PE (0.01-100 μM), perfused cumulatively, in the Krebs-buffer perfused pulmonary vasculature of Wistar and Sprague Dawley rats. The pressor responses to PE (0.1 and 1 μM) were bigger in Sprague Dawley rats. The results are shown as Means $\pm$ SEM, $n \geq 3$. The basal perfusion pressure before the start of the control curve was (Mean = 13.7 mm Hg, SEM = 0.93 mm Hg, n = 10) and of the test curve was (Mean = 22.0 mm Hg, SEM = 6.0 mm Hg, n = 2). (* $0.05 > P > 0.01$).

Fig-40 Effect of ketanserin on the pressor responses to 5-HT, in the isolated, Krebs-buffer perfused pulmonary vasculature of Sprague-Dawley rat.

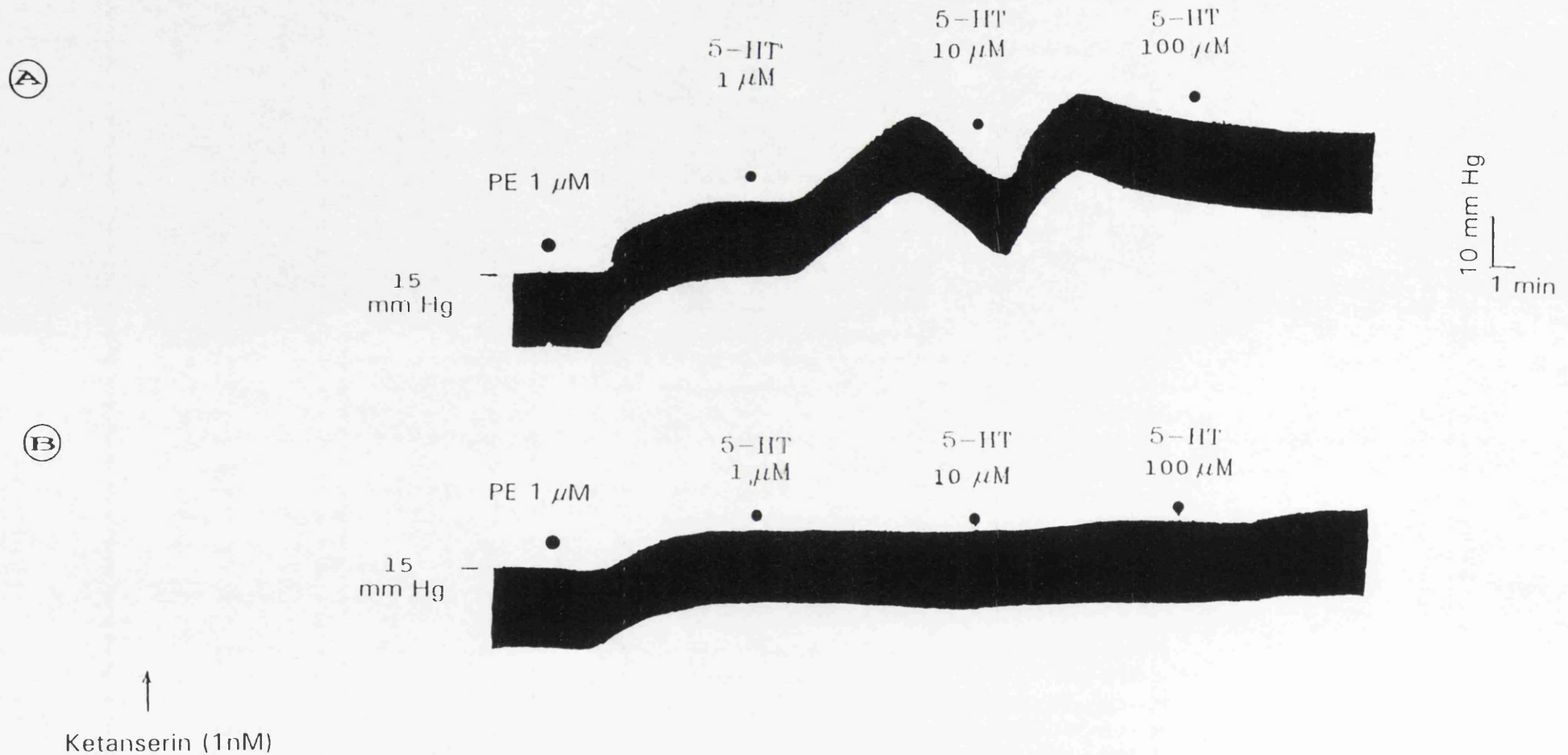
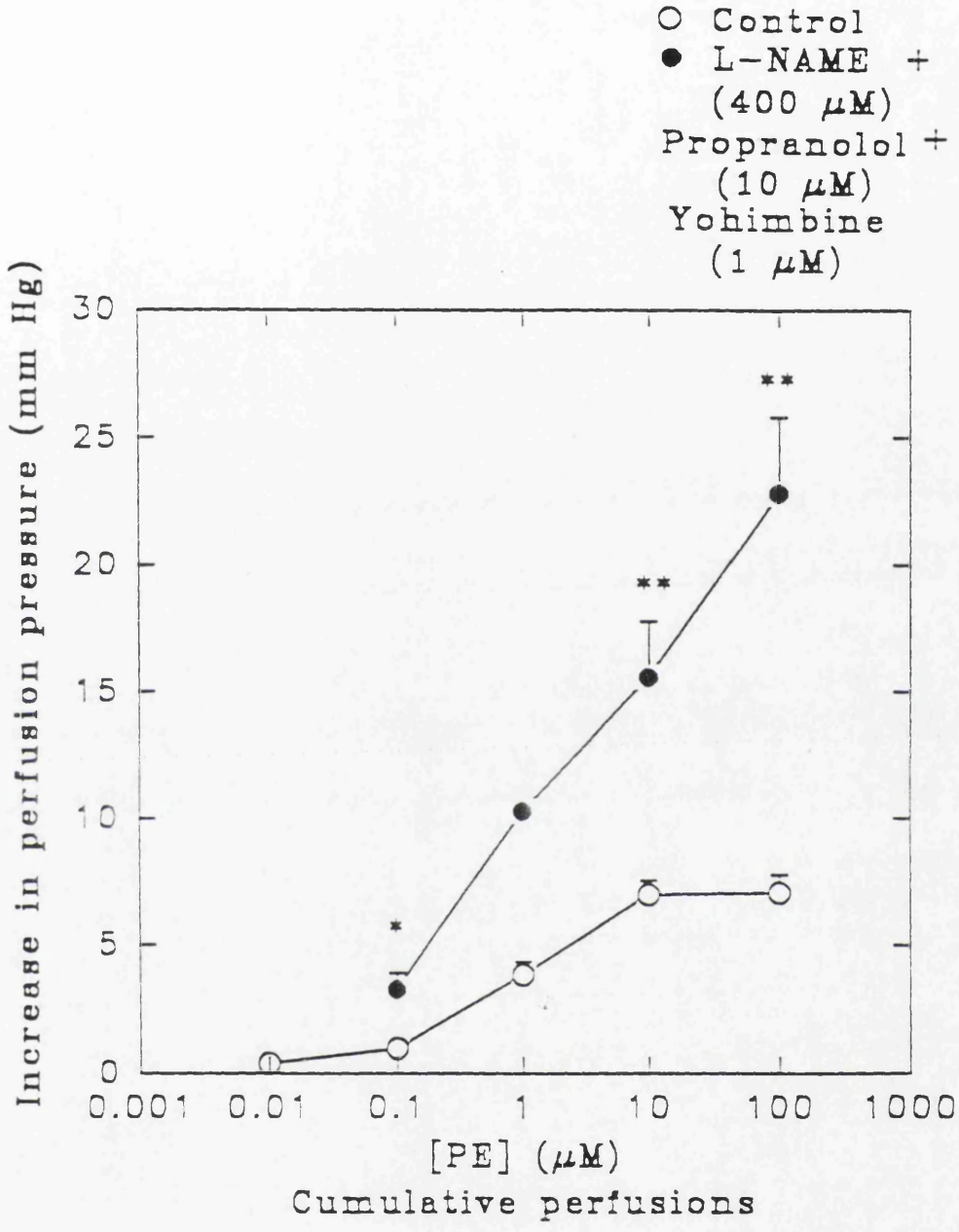


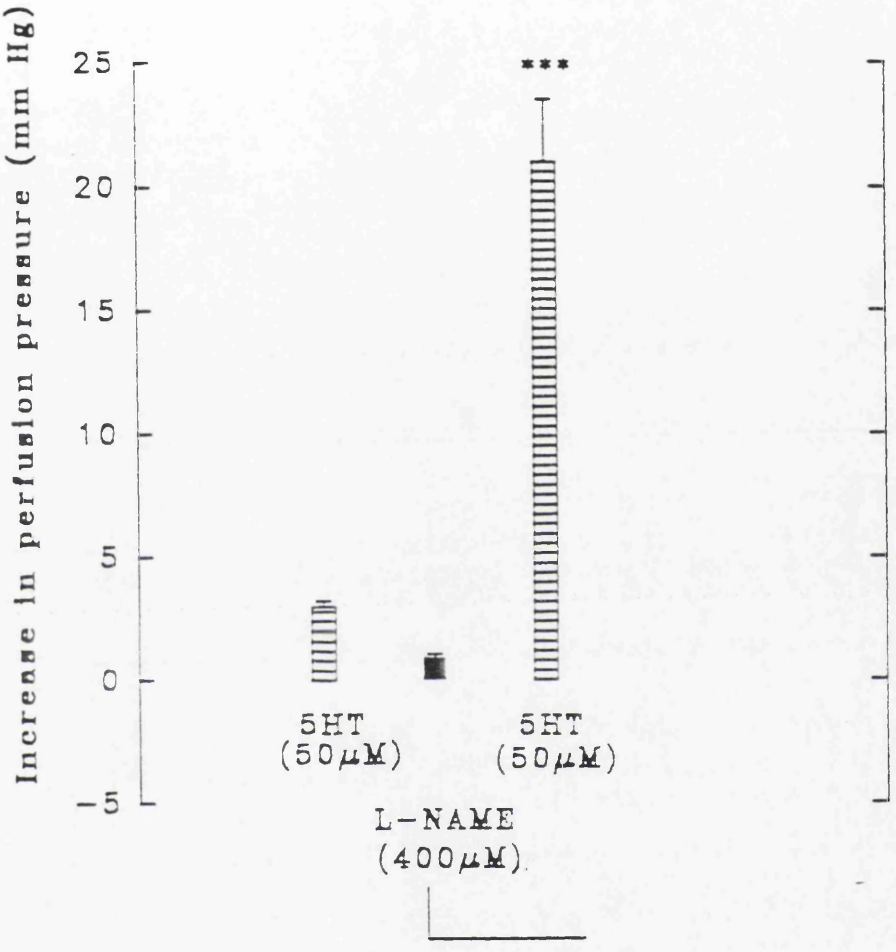
Fig-40
Concentration-responses to 5-HT (1-100 μM), perfused cumulatively, on the ongoing pressor response to a submaximal concentration of PE (1 μM) in the absence (panel A) and presence (panel B) of ketanserin (1 nM). The trace in panel B is a continuation of the trace in panel A. L-NAME (400 μM) was present throughout in the Krebs-buffer that was perfused through the pulmonary vasculature of the Sprague Dawley rat. Ketanserin inhibited the pressor responses to 5-HT.

Fig-41
Effect of a mixture of L-NAME, propranolol and yohimbine on the concentration-response curve to PE in the isolated Krebs-buffer perfused pulmonary vasculature of the Wistar rat.



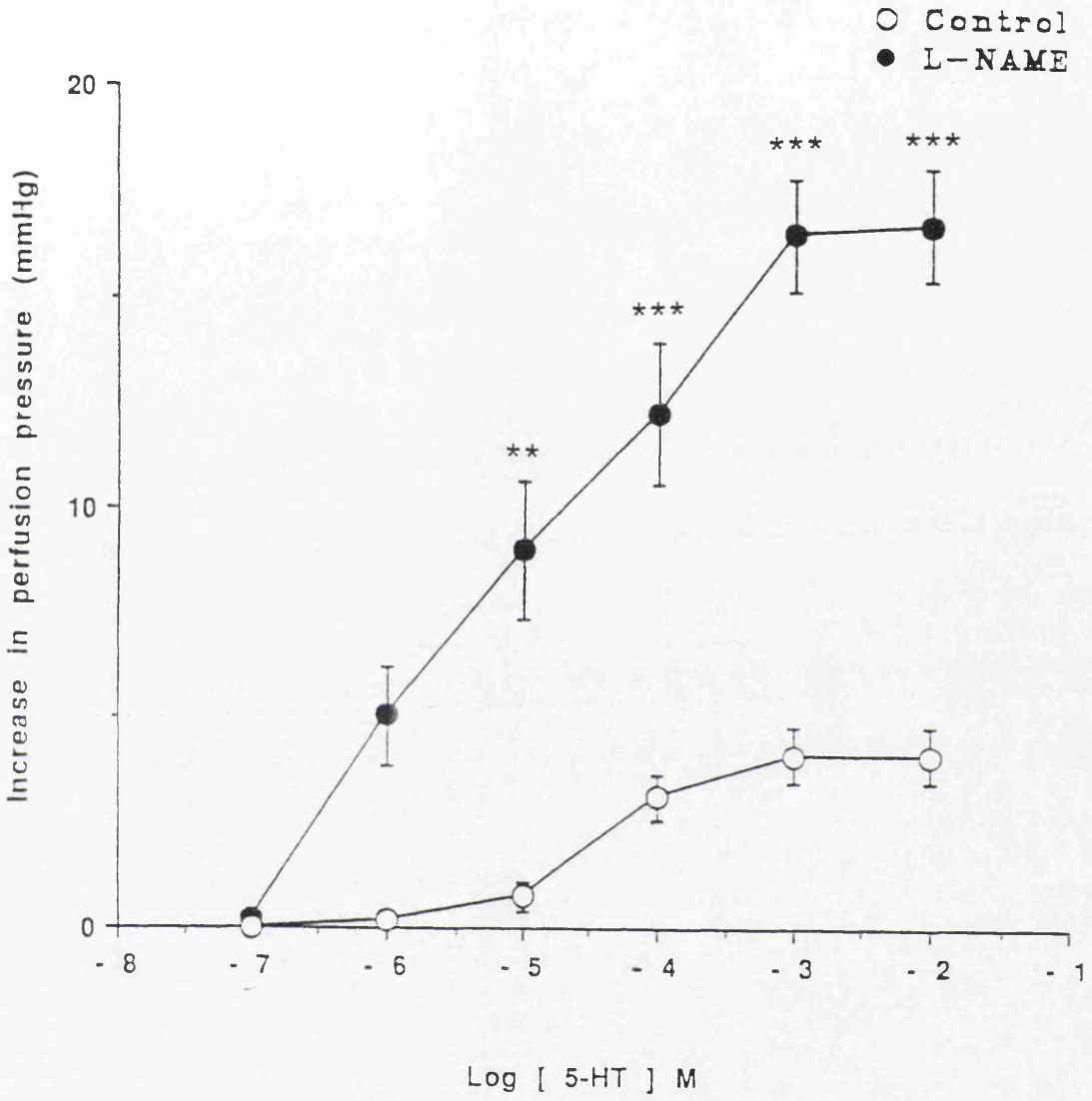
Concentration-response curve to PE (0.01-100 μM), perfused cumulatively, in the absence and presence of a mixture of L-NAME (400 μM), propranolol (10 μM) and yohimbine (1 μM) in the Krebs-buffer perfused rat pulmonary circulation. The mixture of antagonists enhanced the pressor responses to PE. The results are shown as Means ± SEM, $n \geq 3$. The basal perfusion pressure before the start of the control curve was (Mean = 13.7 mm Hg, SEM = 0.93 mm Hg, $n = 10$) and of the test curve was (Mean = 18.0 mm Hg, SEM = 0.0 mm Hg, $n = 1$). (* $0.05 > P > 0.01$, ** $0.01 > P > 0.001$).

Fig-42
Effect of L-NAME on the pressor response to a submaximal concentration of 5-HT in the isolated Krebs-buffer perfused pulmonary vasculature of the Wistar rat.



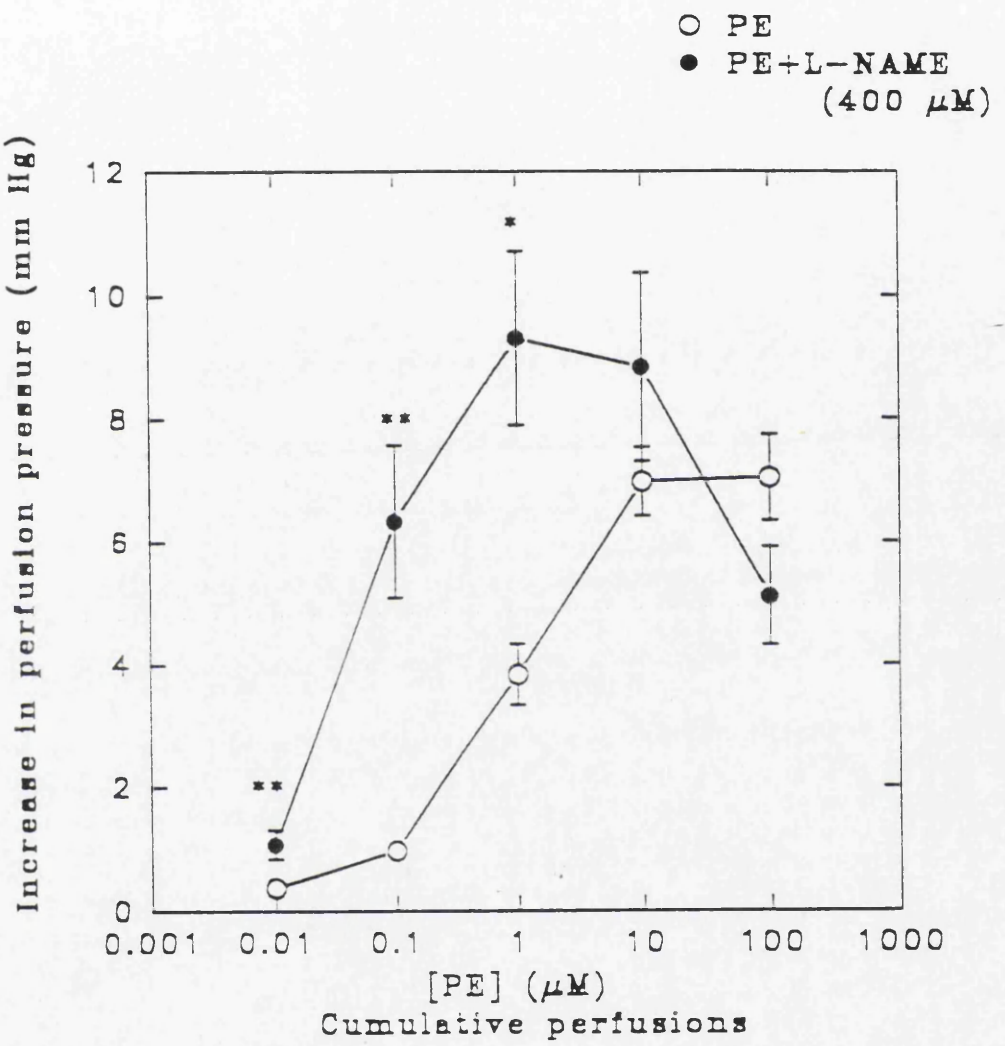
Histograms showing the pressor responses to an equilibrium perfused, single submaximal concentration of 5-HT (50 μM) in the absence and presence of L-NAME (400 μM), in the Krebs-buffer perfused rat pulmonary circulation. L-NAME enhanced the pressor response to 5-HT. The results are shown as Means $\pm$ SEM, $n \geq 14$. The starting, basal perfusion pressure was (Mean = 12.79 mm Hg, SEM = 0.79 mm Hg, n = 14). (*** $P < 0.001$).

Fig-43
Effect of L-NAME on the concentration-response curve to 5-HT in the isolated, Krebs-buffer perfused pulmonary vasculature of the Wistar rat.



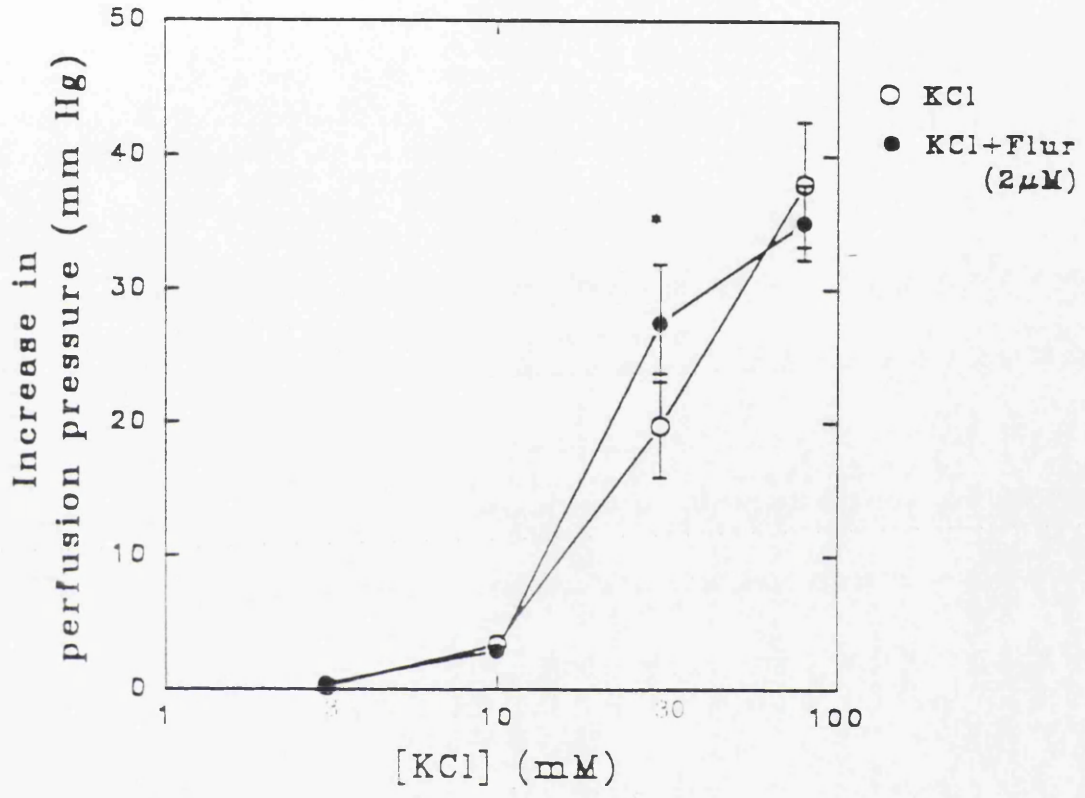
Concentration-response curves for 5-HT in the rat pulmonary vasculature. 5-HT was administered by perfusion until a stable equilibrium response was obtained at each concentration (Means ± SEM, n = 8). This figure shows responses to 5-HT before (○) and after (●) administration of L-NAME (500 μM) to the Krebs-buffer solution. L-NAME increased responses to 5-HT.

Fig-44
Effect of L-NAME on the concentration-response curve to PE in the isolated Krebs-buffer perfused pulmonary vasculature of the Wistar rat.



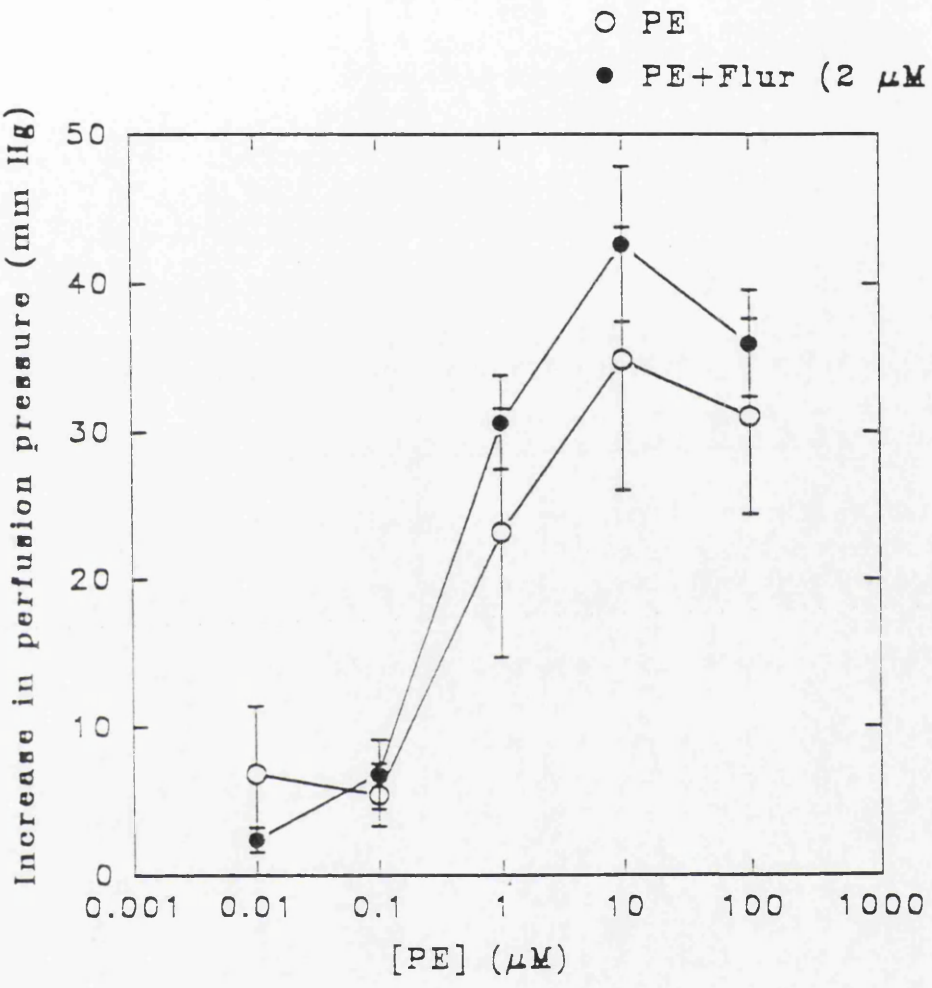
Concentration-response curves to PE (0.01-100 μM), perfused cumulatively, in the absence and presence of L-NAME (400 μM) in the Krebs-buffer perfused rat pulmonary circulation. L-NAME enhanced the pressor responses of PE. The results are shown as Means ± SEM, n≥4. The basal perfusion pressure before the start of the control curve was (Mean = 13.7 mm Hg, SEM = 0.93 mm Hg, n = 10) and of the test curve was (Mean = 20.5 mm Hg, SEM = 1.53 mm Hg, n = 7). (* 0.05 > P > 0.01, ** 0.01 > P > 0.001).

Fig-45
Effect of flurbiprofen on the concentration-response curve to KCl in the isolated Krebs-buffer perfused pulmonary vasculature of the Wistar rat.



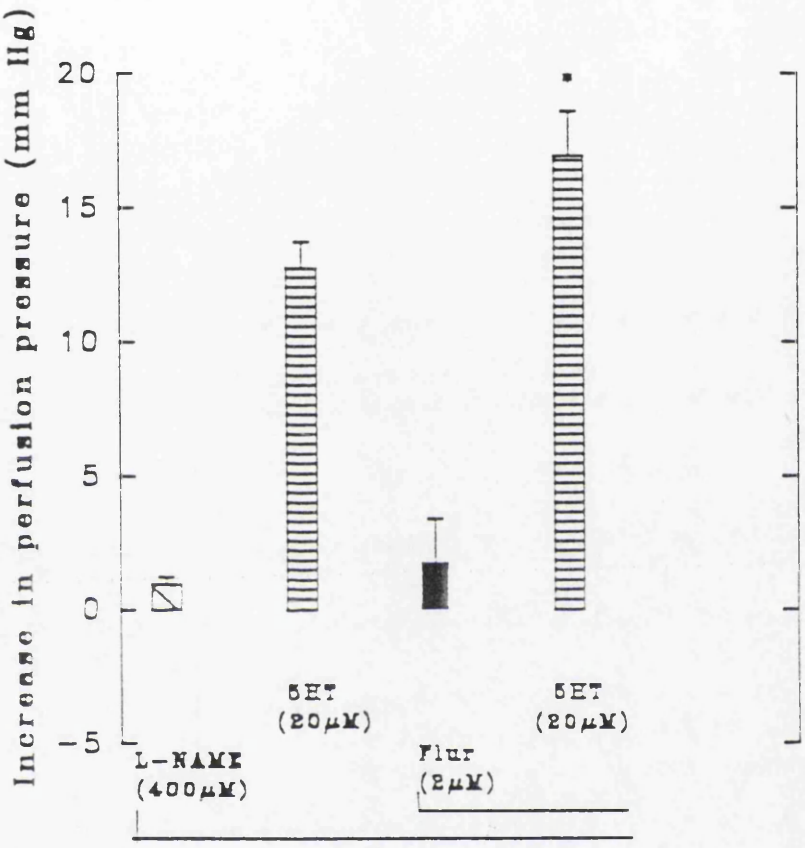
Concentration-response curves to KCl (3-80 mM) in the Krebs-buffer perfused rat pulmonary circulation, in the absence and presence of flurbiprofen (Flur, 2 μM). Each concentration of KCl was perfused until an equilibrium response was obtained and then the perfusion was stopped and the preparation was allowed to recover before the next concentration was perfused. Flurbiprofen (2 μM) enhanced the pressor response to KCl at only one point in the concentration-response curve (30 mM) and did not affect the maximal pressor response to KCl. The results are shown as Means $\pm$ SEM, n = 4. The basal perfusion pressure before the start of the control curve was (Mean = 12.5 mm Hg, SEM = 1.94 mm Hg, n = 4) and of the test curve was (Mean = 14.5 mm Hg, SEM = 1.55 mm Hg, n = 4). (* $0.05 > P > 0.01$).

Fig-46
Effect of flurbiprofen on the concentration-response curve to PE in the isolated Krebs-buffer perfused pulmonary vasculature of the Wistar rat.



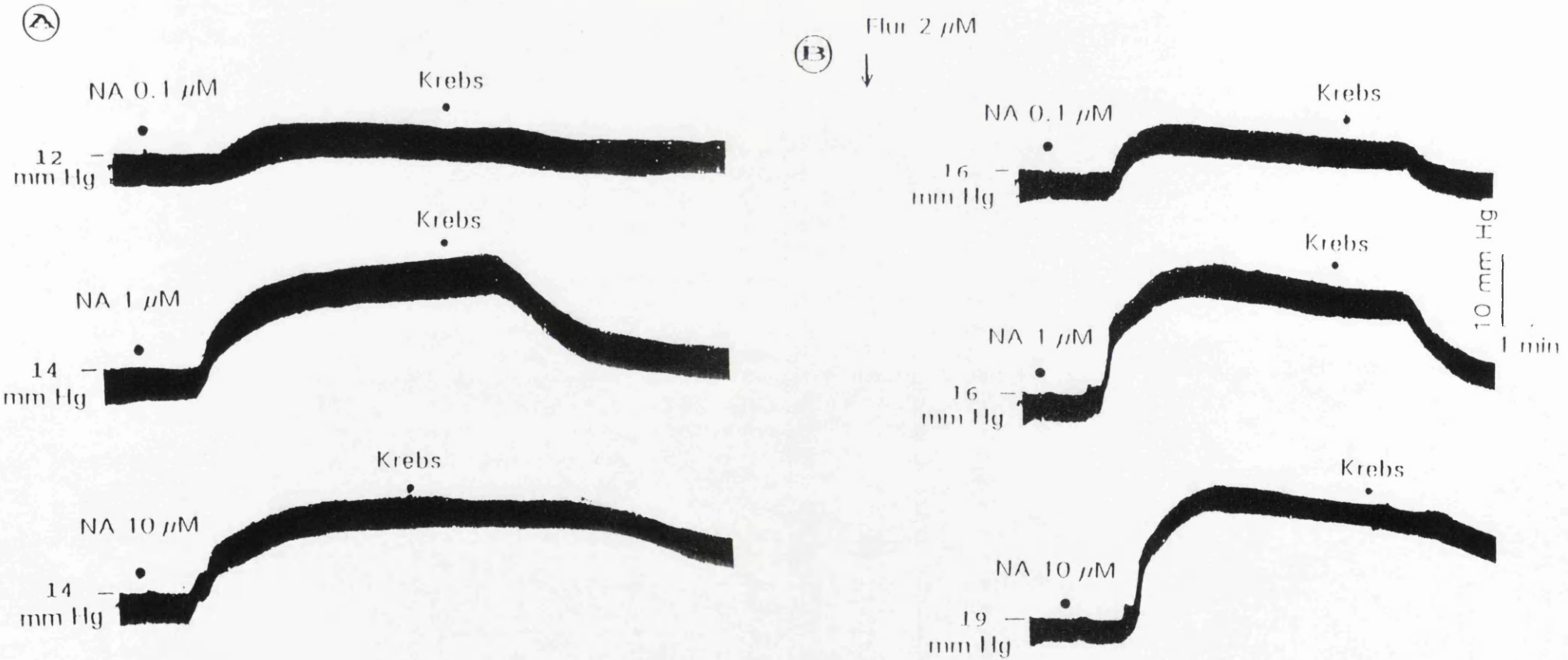
Concentration-response curves to PE (0.01-100 μM) in the Krebs-buffer perfused rat pulmonary circulation, in the absence and presence of flurbiprofen (Flur, 2 μM). Each concentration of PE was perfused until an equilibrium response was obtained and then the perfusion was stopped and the preparation was allowed to recover before the next concentration was perfused. Flurbiprofen had no effect on the PE-induced pressor responses. The results are shown as Means ± SEM, $n \geq 3$. The basal perfusion pressure before the start of the control curve was (Mean = 20.83 mm Hg, SEM = 3.23 mm Hg, n = 6) and of the test curve was (Mean = 16.5 mm Hg, SEM = 1.5 mm Hg, n = 2).

Fig-47
Effect of flurbiprofen on the pressor response to a submaximal concentration of 5-HT in the presence of L-NAME in the isolated Krebs-buffer perfused pulmonary vasculature of the Wistar rat.



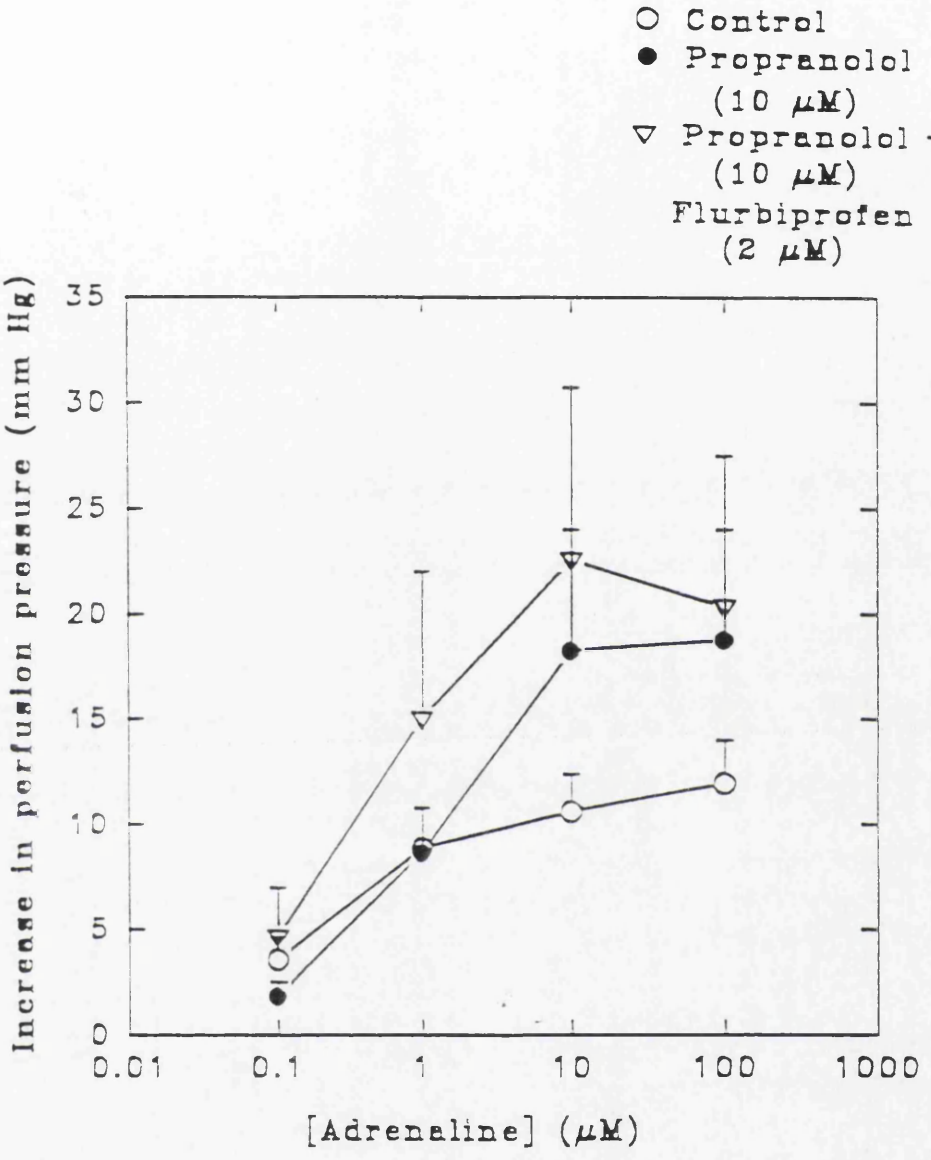
Histograms showing pressor responses to a submaximal concentration of 5-HT (20 μM) in the absence and presence of flurbiprofen (Flur, 2 μM) in the Krebs-buffer perfused rat pulmonary vasculature. L-NAME (400 μM) was present throughout this experiment. Flurbiprofen enhanced the pressor response to 5-HT. The results are shown as Means ± SEM, $n \geq 10$. The starting basal perfusion pressure was (Mean = 13.25 mm Hg, SEM = 0.94 mm Hg, n = 8). (* $0.05 > P > 0.01$).

Fig-48 Effect of flurbiprofen on the pressor responses to NA in the isolated, Krebs-buffer perfused pulmonary vasculature of the Wistar rat



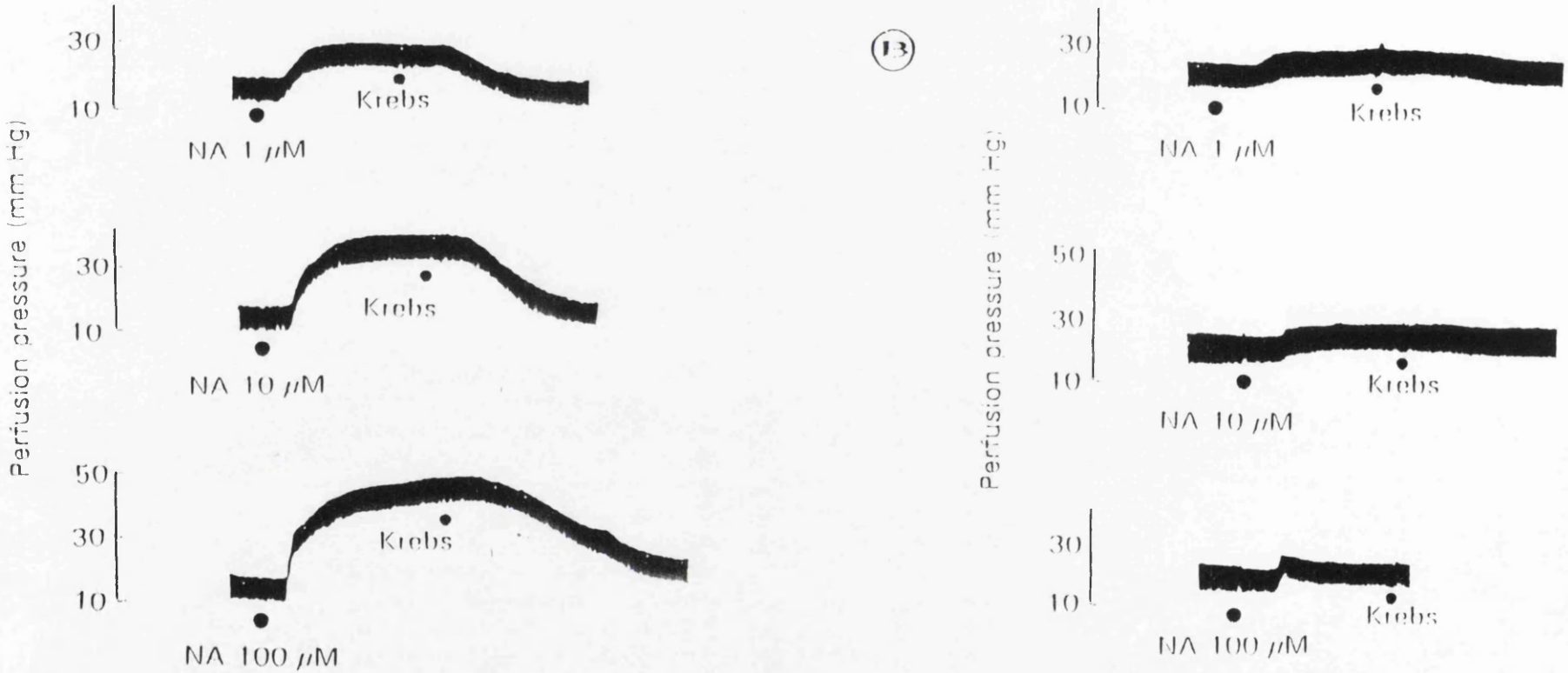
Traces showing pressor responses to increasing concentrations of NA (0.1-10 μM) in the Krebs-buffer perfused rat pulmonary circulation, in the absence (Panel A) and presence (Panel B) of flurbiprofen (Flur, 2 μM). Each concentration of NA was perfused until an equilibrium response was obtained and then the perfusion was stopped and the preparation was allowed to recover before the next concentration was perfused.

Fig-49
Effects of propranolol and flurbiprofen, administered in sequence, on the concentration-response curves to adrenaline in the presence of a mixture of L-NAME and yohimbine in the isolated Krebs-buffer perfused pulmonary vasculature of the Wistar rat.



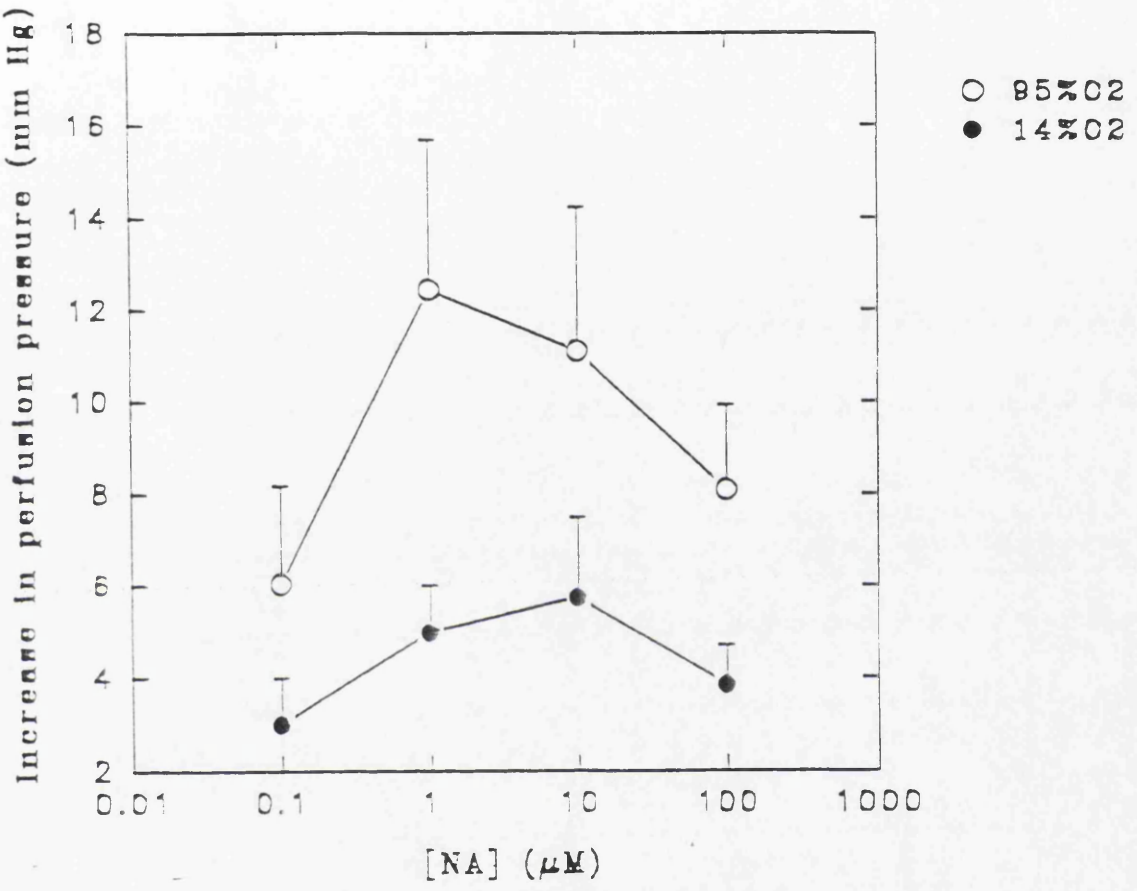
Concentration-response curves to adrenaline (0.1-100 μM) in the absence and presence of propranolol (10 μM) and flurbiprofen (2 μM) while L-NAME (400 μM) and yohimbine (1 μM) being present throughout the experiment in the Krebs-buffer perfused rat pulmonary circulation. Each concentration of adrenaline was perfused until an equilibrium response was obtained and then the perfusion was stopped and the preparation was allowed to recover before the next concentration was perfused. The results are shown as Means ± SEM, $n \geq 2$. The basal perfusion pressure before the start of the control curve was (Mean = 15.5 mm Hg, SEM = 2.9 mm Hg, n = 4), and of the curve in the presence of propranolol was (Mean = 21.6 mm Hg, SEM = 2.98 mm Hg, n = 5) and of the curve in the presence of both propranolol and flurbiprofen was (Mean = 19.5 mm Hg, SEM = 3.5 mm Hg, n = 2).

Fig-50 Pressor responses to NA, gassed with either 95%O_2 or 14%O_2, in the isolated, Krebs-buffer perfused pulmonary vasculature of the Wistar rat



Concentration-pressor responses to NA (1-100 μM) in the Krebs-buffer perfused rat pulmonary circulation. Each concentration of NA was perfused until an equilibrium response was obtained and then the perfusion was stopped and the preparation was allowed to recover before the next concentration was perfused. This experiment examined the effect of reducing the percentage of oxygen in the gas, bubbled through the Krebs-buffer From 95%O_2 (Panel A) to 14%O_2 (Panel B).

Fig-51
Concentration-response curves to NA at 14%O_2 and 95%O_2 gassed-Krebs-buffer, in the isolated, perfused pulmonary vasculature of the Wistar rat.



Concentration-response curves to NA (0.1-100 μM) in the Krebs-buffer perfused rat pulmonary circulation. Each concentration of NA was perfused until an equilibrium response was obtained and then the perfusion was stopped and the preparation was allowed to recover before the next concentration was perfused. This experiment examined the effect of reducing the percentage of oxygen in the gas, bubbled through the Krebs-buffer from 95%O_2 to 14%O_2. The results are shown as Means ± SEM, $n \geq 2$.

Fig-52 Effect of propranolol on the responses to NA, gassed with 14%O_2, in the isolated, Krebs-buffer perfused pulmonary vasculature of the Wistar rat

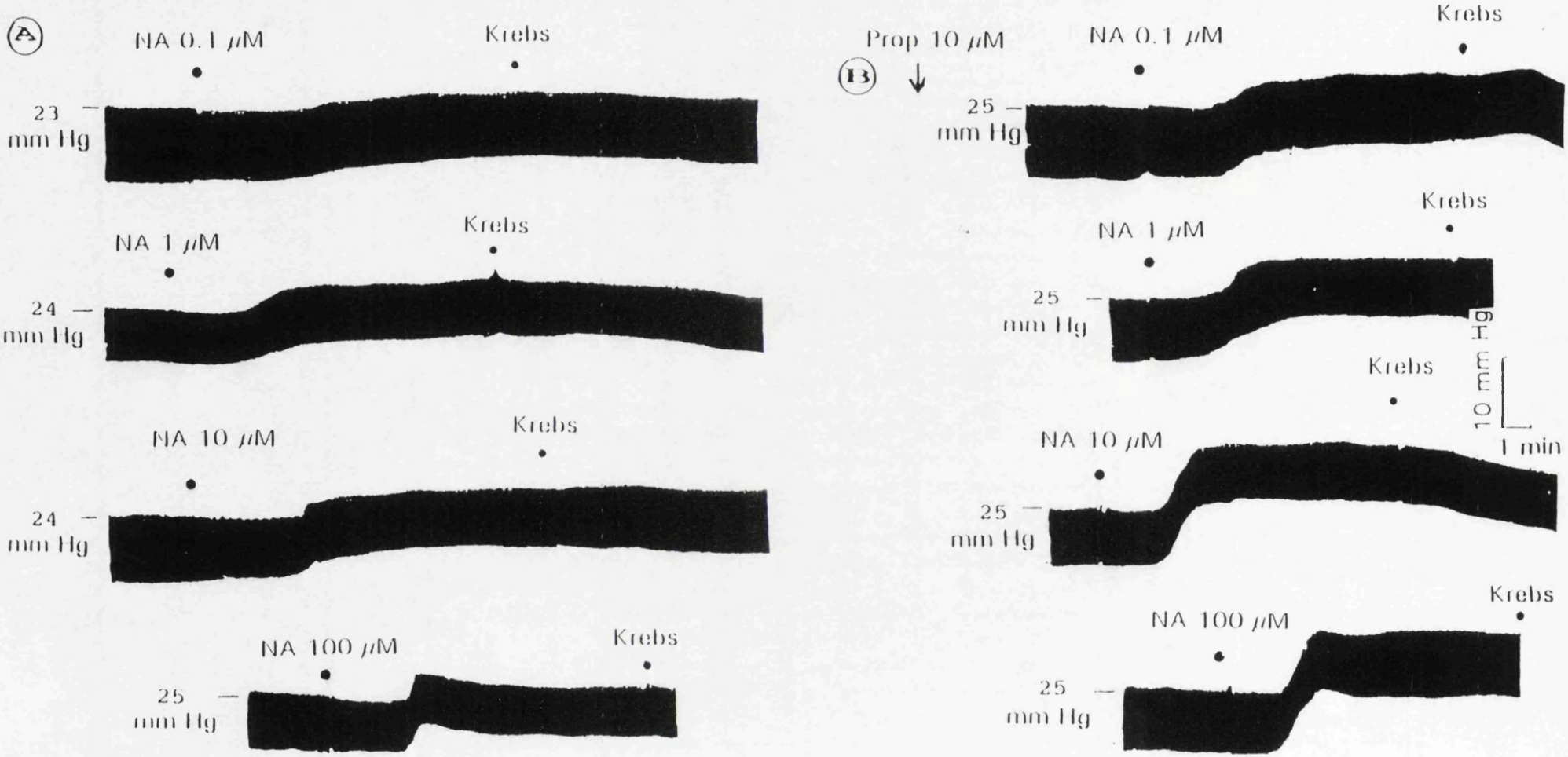
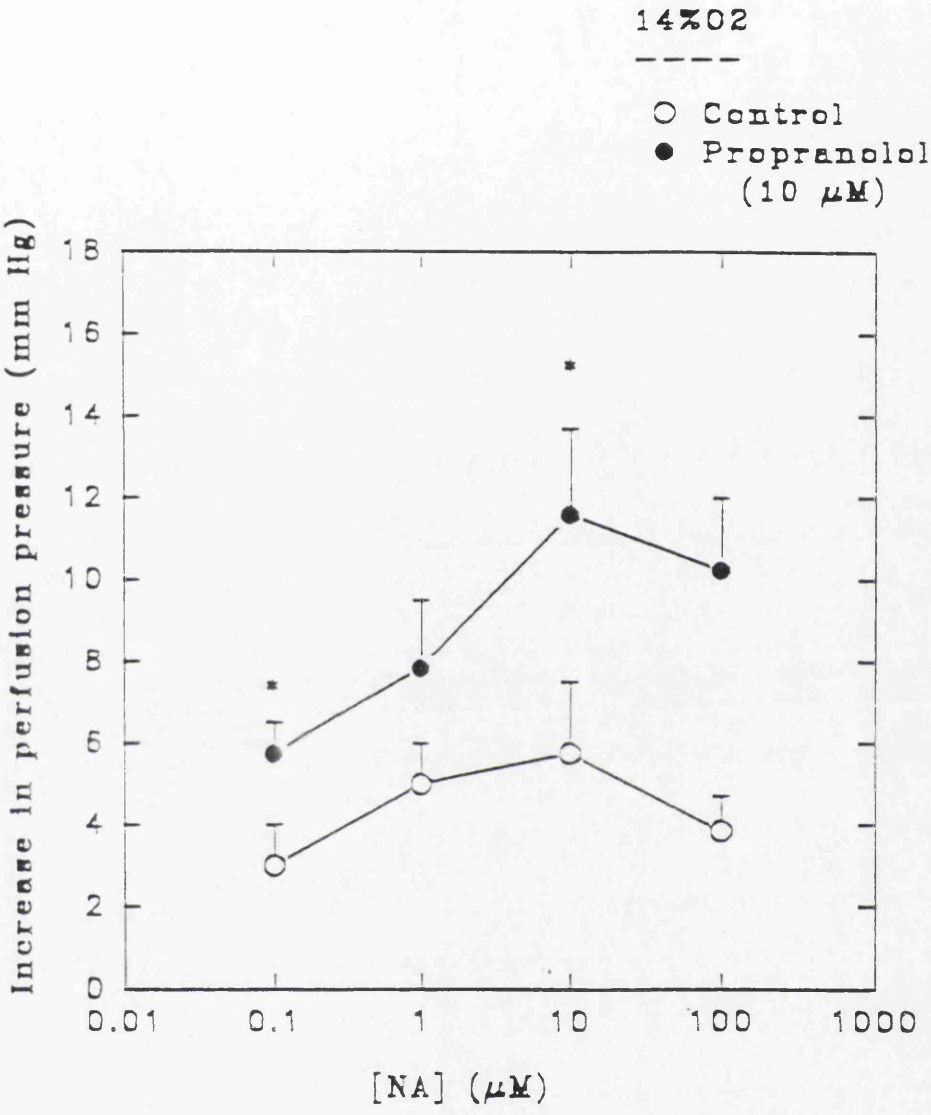


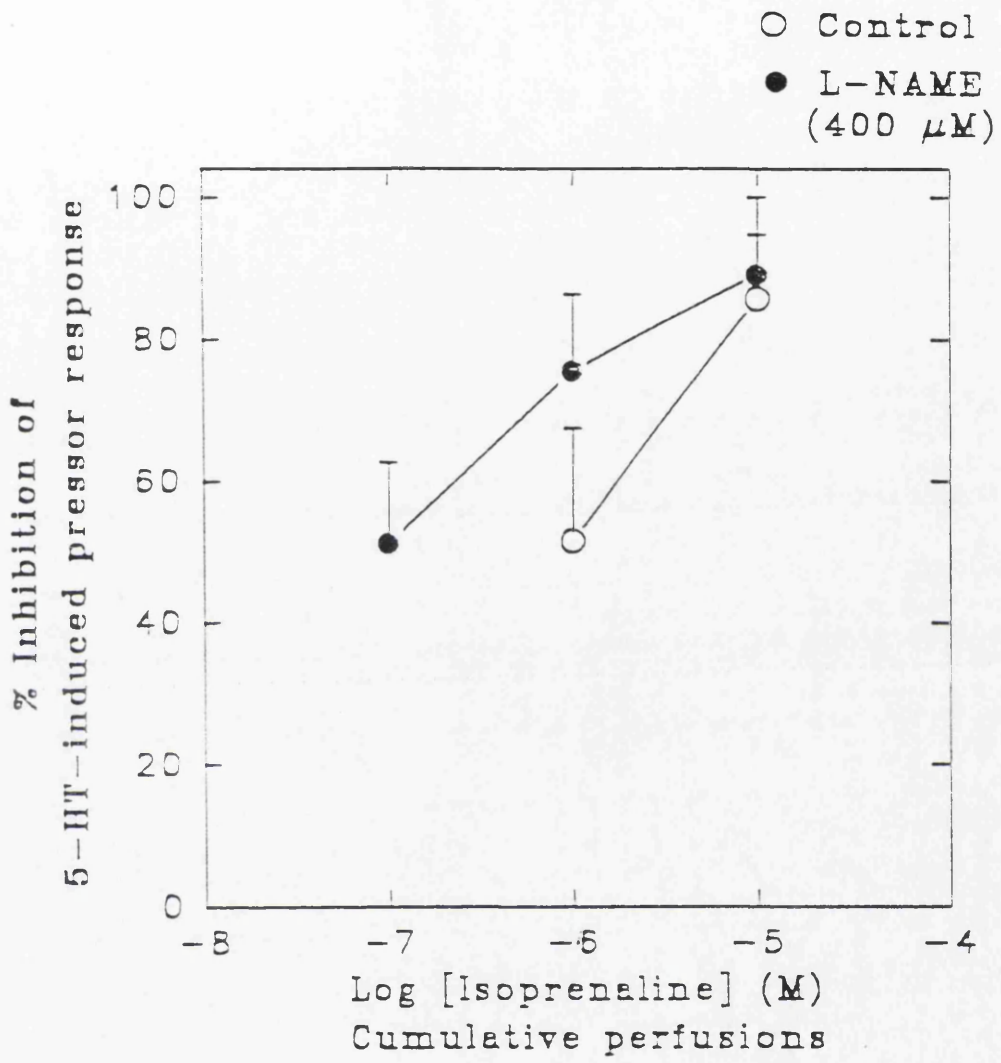
Fig-52
Traces showing pressor responses to increasing concentrations of NA (0.1-100 μM), in the absence (Panel A) and presence (Panel B) of propranolol (Prop, 10 μM). In this experiment, the Krebs-buffer that was perfused through the rat pulmonary circulation, was gassed with 14%O_2, 5%CO_2 and 81%N_2. Each concentration of NA was perfused until an equilibrium response was obtained and then the perfusion was stopped and the preparation was allowed to recover before the next concentration was perfused. Propranolol enhanced the pressor responses to NA.

Fig-53
Effect of Propranolol on the concentration-response curve to NA in 14%O_2 gassed-Krebs-buffer, in the isolated, perfused pulmonary vasculature of the Wistar rat.



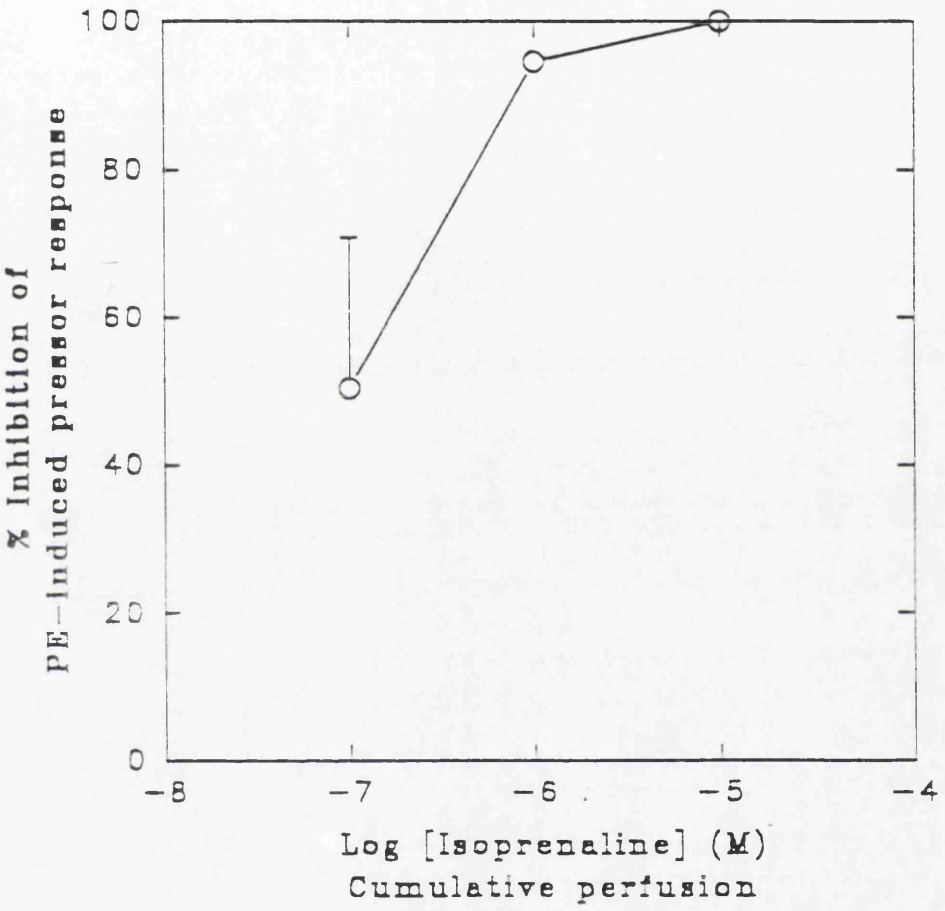
Concentration-response curves to NA (0.1-100 μM) in the absence and presence of propranolol (10 μM) in Krebs-buffer, gassed with a mixture of 14%O_2, 5%CO_2 and 81%N_2 and perfused through the rat pulmonary circulation. Each concentration of NA was perfused until an equilibrium response was obtained and then the perfusion was stopped and the preparation was allowed to recover before the next concentration was perfused. Propranolol enhanced the pressor responses to NA. The results are shown as Means $\pm$ SEM, n = 2. The basal perfusion pressure before the start of the control curve was (Mean = 16.0 mm Hg, SEM = 4.0 mm Hg, n = 2) and of the test curve was (Mean = 21.5 mm Hg, SEM = 1.5 mm Hg, n = 2). (* $0.05 > P > 0.01$).

Fig-54
Effect of L-NAME on the inhibitory concentration-response curve to isoprenaline, perfused on the pressor response to 5-HT, in the isolated Krebs-buffer perfused pulmonary vasculature of the Wistar rat.



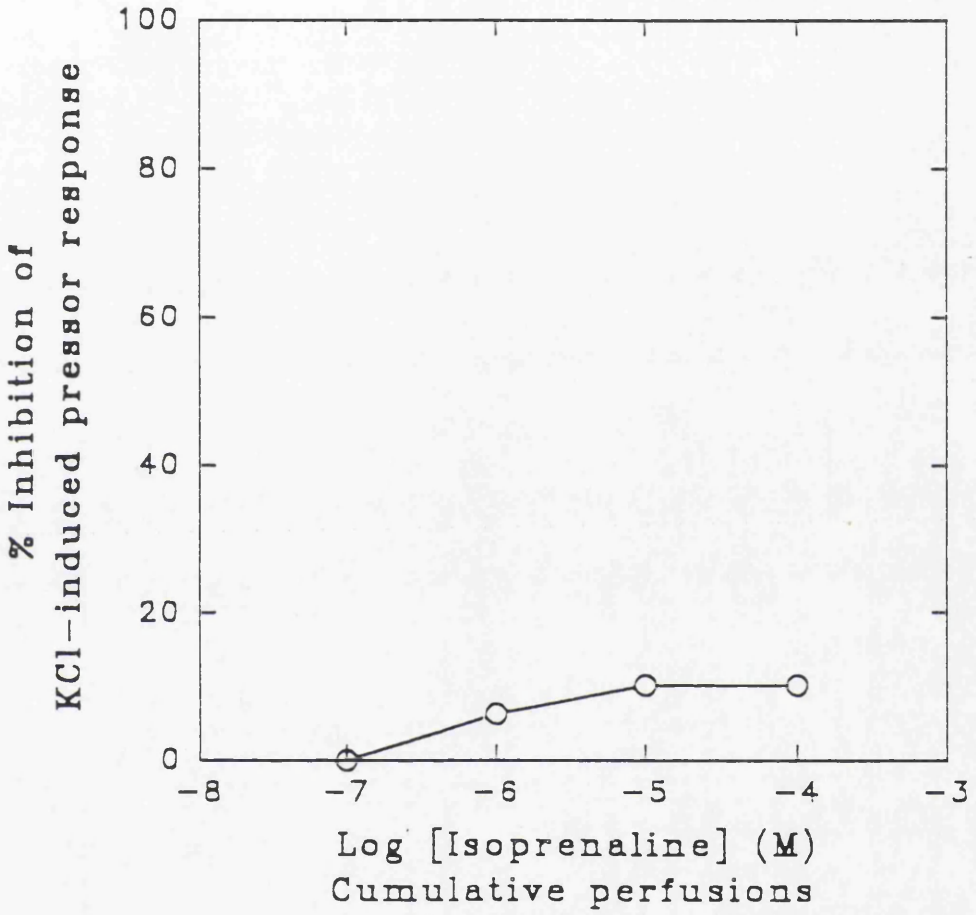
Inhibitory concentration-response curves to isoprenaline (10^{-7}-10^{-5} M), perfused cumulatively, on the ongoing pressor response to a submaximal concentration of 5-HT (50 μM) in the absence and presence of L-NAME (400 μM) in the Krebs-buffer perfused rat pulmonary circulation. Each concentration of isoprenaline was perfused until an equilibrium inhibitory response was obtained. The reason for not having the lower part of the percentage inhibitory effect of isoprenaline was the characteristic pressor response to 5-HT which was not well-maintained. The inhibitory effect of isoprenaline was measured from the peak pressor response to 5-HT and hence it was unclear whether isoprenaline at lower concentrations ($<10^{-7}$ M) had any inhibitory effect as the pressor response to 5-HT had already declined on its own. The results are shown as Means ± SEM, $n \geq 2$.

Fig-55
Inhibitory concentration-response curve to isoprenaline, perfused on the pressor response to PE, in the isolated Krebs-buffer perfused pulmonary vasculature of the Wistar rat.



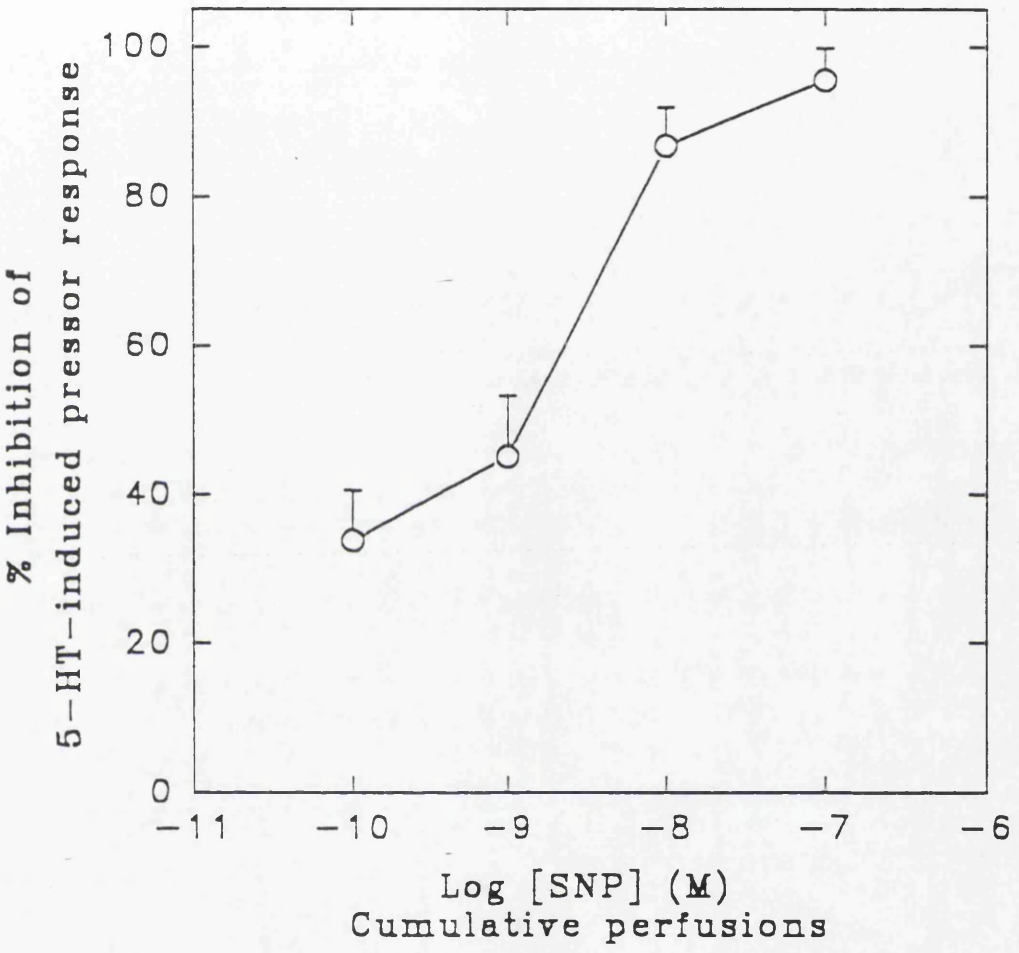
Inhibitory concentration-response curve to isoprenaline (10^{-7}-10^{-5} M), perfused cumulatively, on the ongoing pressor response to PE (100 μM), pretreated with an equilibrium perfused single concentration of KCl (50 mM) in the Krebs-buffer perfused rat pulmonary circulation. Each concentration of isoprenaline was perfused until an equilibrium inhibitory response was obtained. The results are shown as Means ± SEM, n = 2.

Fig-56
Inhibitory concentration-response curve to isoprenaline, perfused on the pressor response to KCl, in the isolated Krebs-buffer perfused pulmonary vasculature of the Wistar rat.



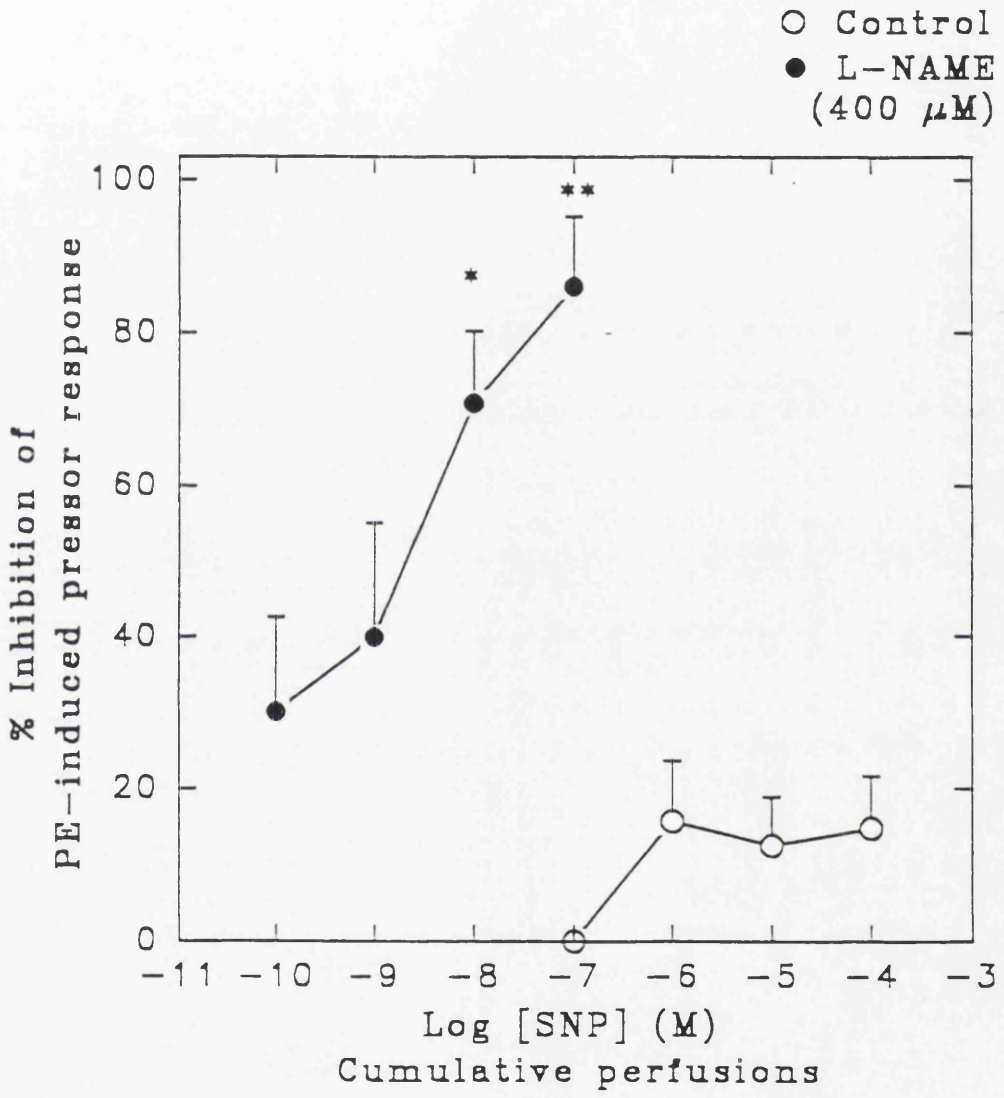
Inhibitory concentration-response curve to isoprenaline (10^{-7}-10^{-4} M), perfused cumulatively, on the ongoing pressor response to a submaximal concentration of KCl (50 mM) in the Krebs-buffer perfused rat pulmonary circulation. Each concentration of isoprenaline was perfused until an equilibrium inhibitory response was obtained. The results are shown as Means ± SEM, n = 3.

Fig-57
Inhibitory concentration-response curve to SNP, perfused on the pressor response to 5-HT, in the presence of L-NAME in the isolated Krebs-buffer perfused pulmonary vasculature of the Wistar rat.



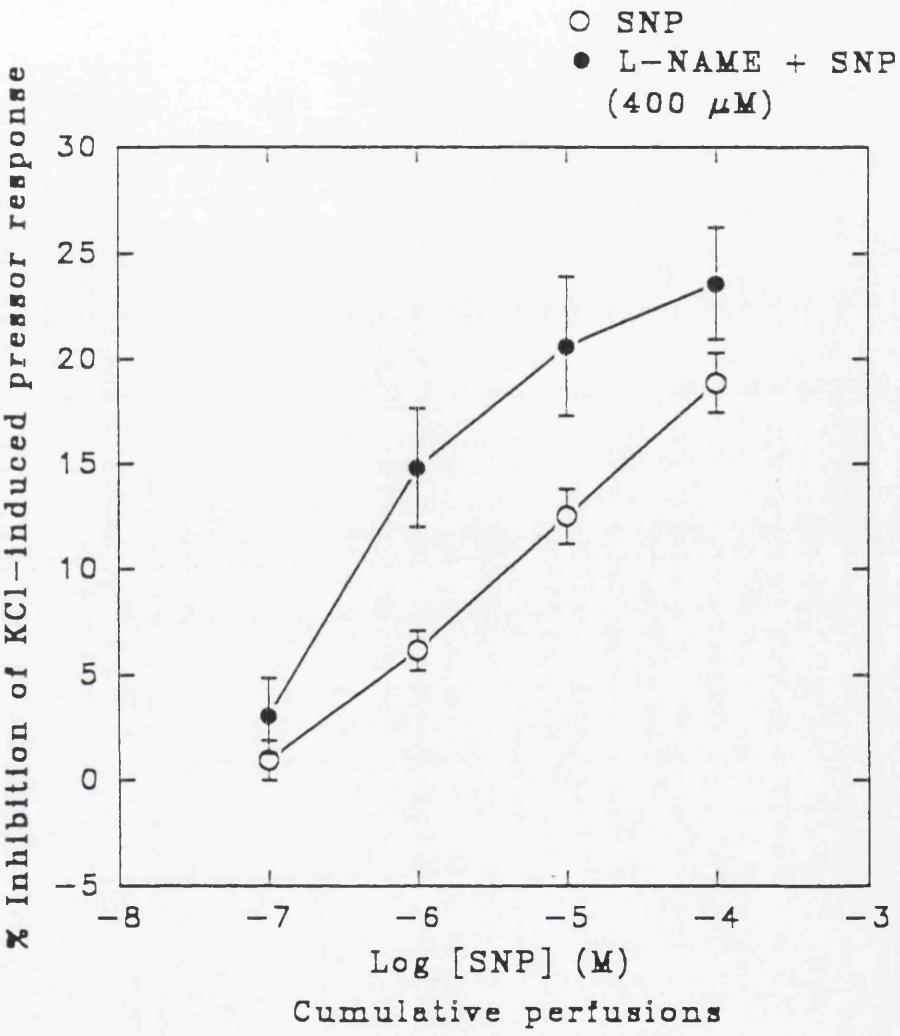
Inhibitory concentration-response curve to SNP (10^{-10}-10^{-7} M), perfused cumulatively, on the ongoing pressor response to a submaximal concentration of 5-HT (20 μM) in the presence of L-NAME (400 μM) in the Krebs-buffer perfused rat pulmonary circulation. Each concentration of SNP was perfused until an equilibrium inhibitory response was obtained. The results are shown as Means ± SEM, $n \geq 6$.

Fig-58
Effect of L-NAME on the inhibitory concentration-response curve to SNP, perfused on the pressor response to PE, in the isolated Krebs-buffer perfused pulmonary vasculature of the Wistar rat.



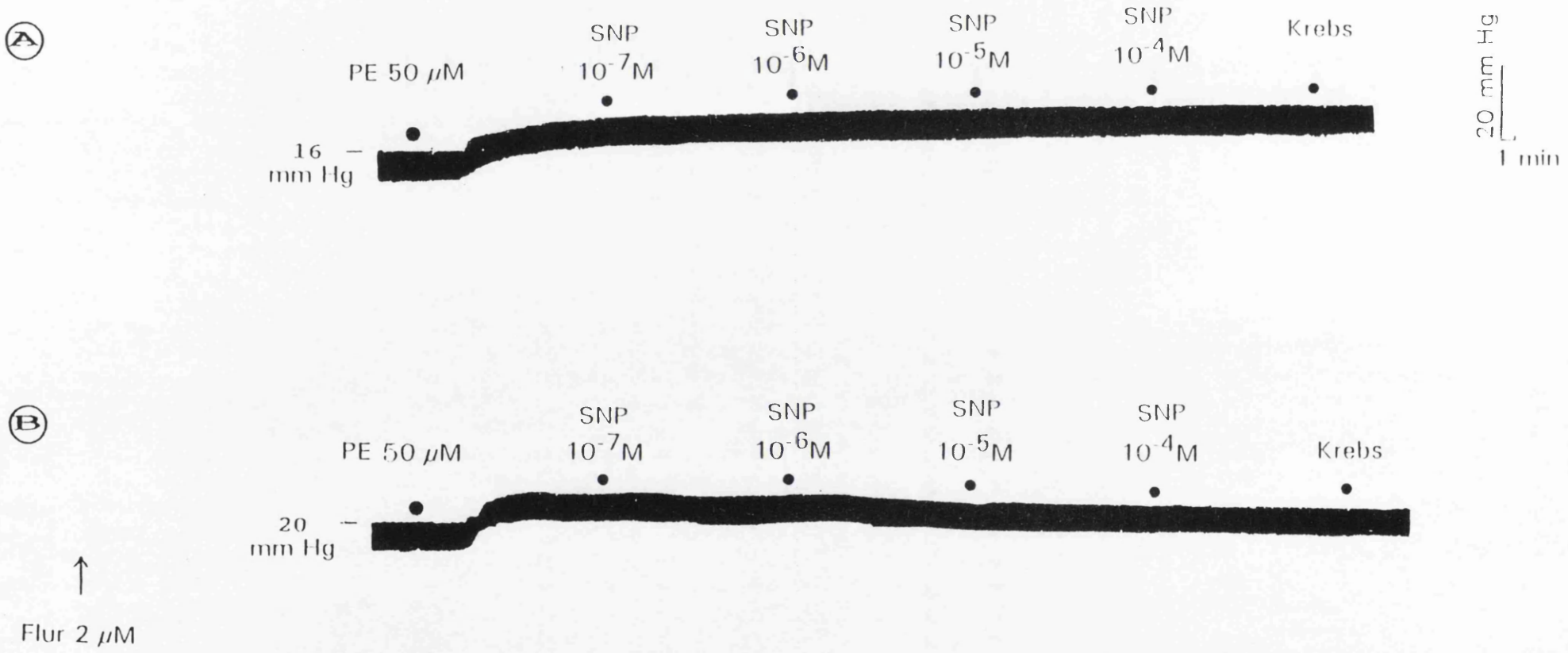
Inhibitory concentration-response curves to SNP (10^{-10}-10^{-4} M), perfused cumulatively, on the ongoing pressor response to a maximal concentration of PE (50 μM) in the absence and presence of L-NAME (400 μM). Each concentration of SNP was perfused until an equilibrium inhibitory response was obtained. L-NAME enhanced the inhibitory effect of SNP on the ongoing pressor response to PE. The results are shown as Means ± SEM, $n \geq 3$, (* $0.05 > P > 0.01$, ** $0.01 > P > 0.001$).

Fig-59
Effect of L-NAME on the inhibitory concentration-response curve to SNP, perfused on the pressor response to KCl, in the isolated Krebs-buffer perfused pulmonary vasculature of the Wistar rat.



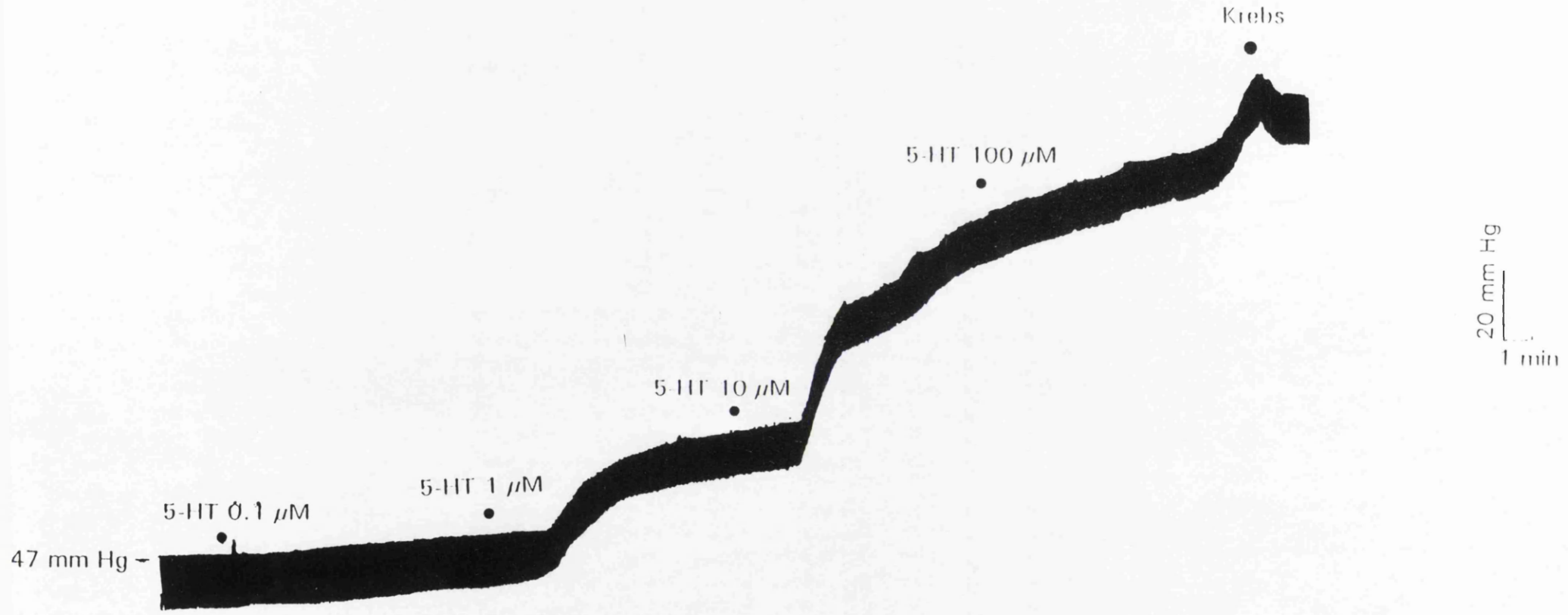
Concentration-response curves to SNP (10^{-7}-10^{-4}M), perfused cumulatively, on the ongoing pressor responses to submaximal concentrations of KCl (40 mM) in the absence and presence of L-NAME (400 μM) in the Krebs-buffer perfused rat pulmonary circulation. L-NAME enhanced the inhibitory effect of SNP on the ongoing pressor response of KCl when the regression lines of the two concentration-response curves were compared according to the method and formulae described in the Bowman and Rand (1980). The results are shown as Means ± SEM, $n=4$, $0.01 > P > 0.001$).

Fig-60 Responses to SNP in the absence and presence of flurbiprofen in the isolated, Krebs-buffer perfused pulmonary vasculature of the Wistar rat



The effects of SNP (10^{-7}-10^{-4}M), perfused cumulatively, on the ongoing pressor response to a maximal concentration of PE (50 μM) in the absence (Panel A) and presence (Panel B) of flurbiprofen (Flur, 2 μM) in the Krebs-buffer perfused rat pulmonary circulation. Each concentration of SNP was perfused until an equilibrium inhibitory response was obtained.

Fig-61 Pressor responses to 5-HT, in the isolated, Krebs-buffer perfused hindquarters vasculature of the wistar rat.



The trace shows pressor responses to cumulative concentrations of 5-HT (0.1-100 μM) in the Krebs-buffer perfused hindquarters vasculature of the rat. Each concentration of 5-HT was perfused until a stable (equilibrium) response was obtained.

Fig-62 Concentration-response curves to the pressor effect of 5-HT and to the inhibitory effect of SNP on submaximal responses to 5-HT in the isolated, Krebs buffer perfused hindquarters vasculature of the Wistar rats.

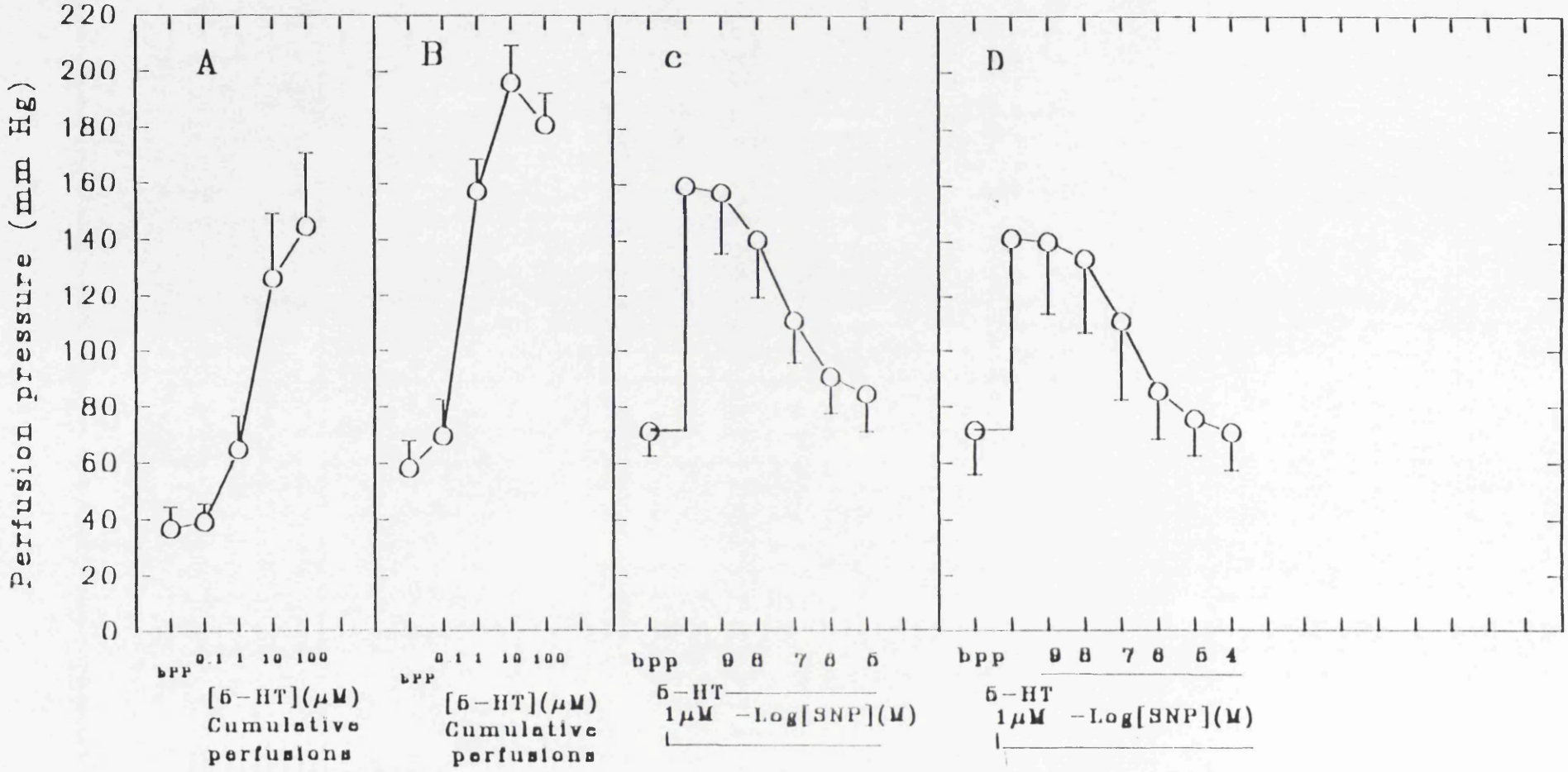
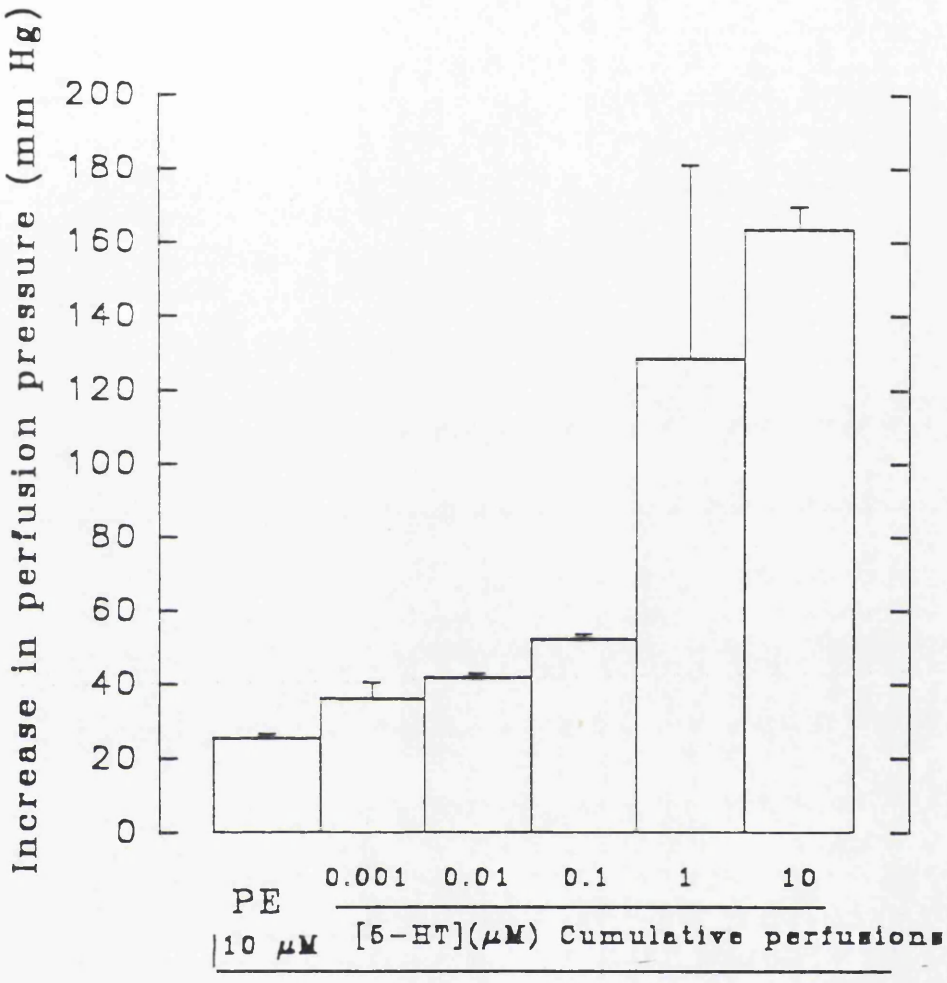


Fig-62
Showing in panels A and B consecutive concentration-response curves to 5-HT (0.1-100 μM) and also showing in panels C and D consecutive concentration-response curves to SNP (10^{-9}-10^{-4} M) perfused cumulatively to produce equilibrium inhibitory responses in hindquarters vasculature in which the perfusion pressure had been raised with 5-HT (1 μM). bPP represents the basal perfusion pressure (mm Hg) for each curve. The results are (Means ± SEM, n = 3).

Fig-63
Pressor responses to 5-HT on the ongoing pressor response to PE, in the isolated, Krebs-buffer perfused hindquarters vasculature of the Wistar rat



Histogram showing the pressor responses to cumulatively perfused, increasing concentrations of 5-HT (0.001-10 μM) on the ongoing pressor response to a submaximal concentration of PE (10 μM) in the rat hindquarters vasculature, perfused with Krebs-buffer. The results are Means $\pm$ SEM, n = 2. The starting basal perfusion pressure was (Mean = 48 mm Hg, SEM = 3 mm Hg, n = 2).

Fig-64 Concentration-response curves to the pressor effect of PE and to the inhibitory effect of SNP on submaximal responses to PE in the isolated, Krebs-buffer perfused hindquarters vasculature of the Wistar rats.

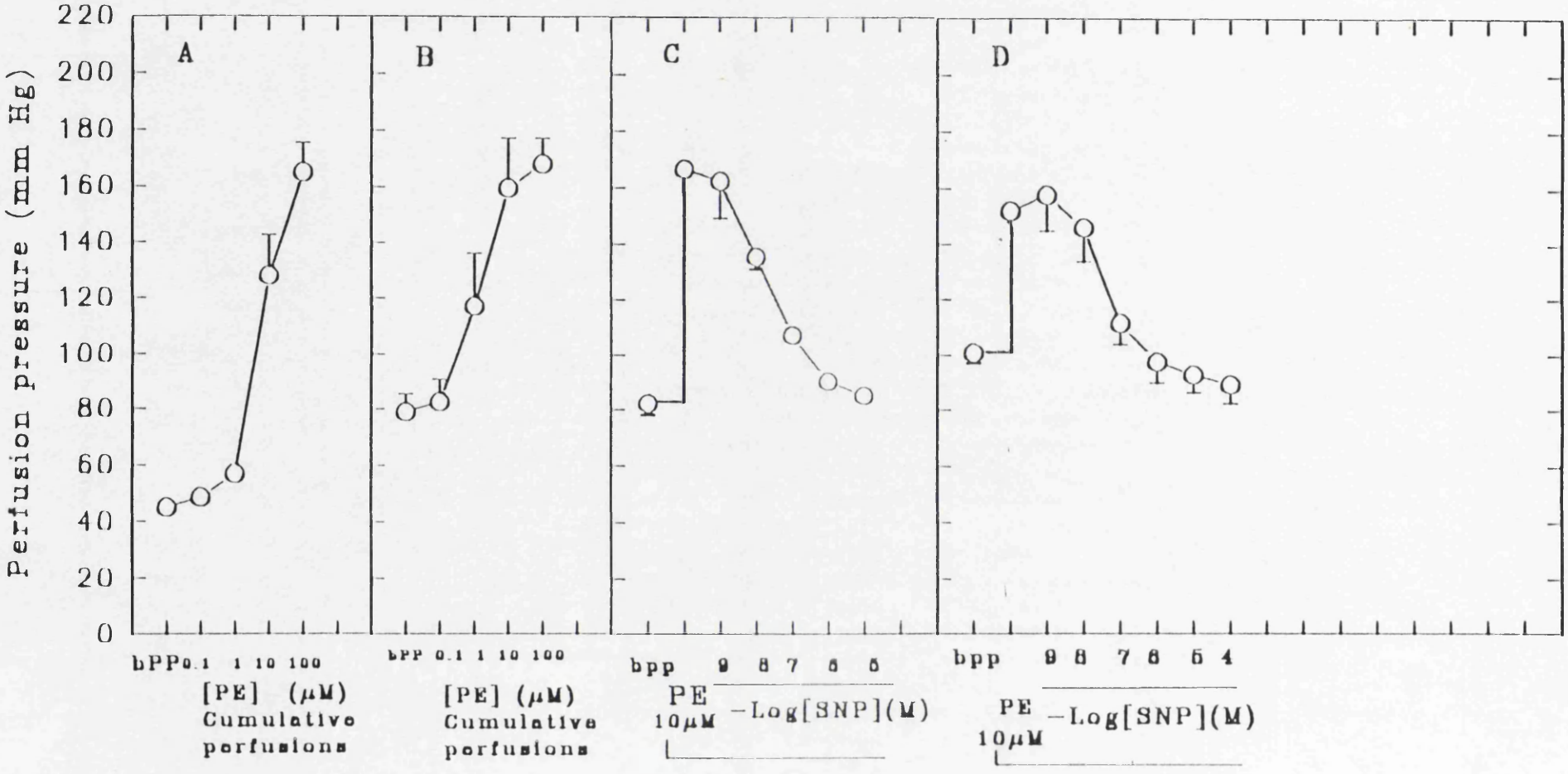
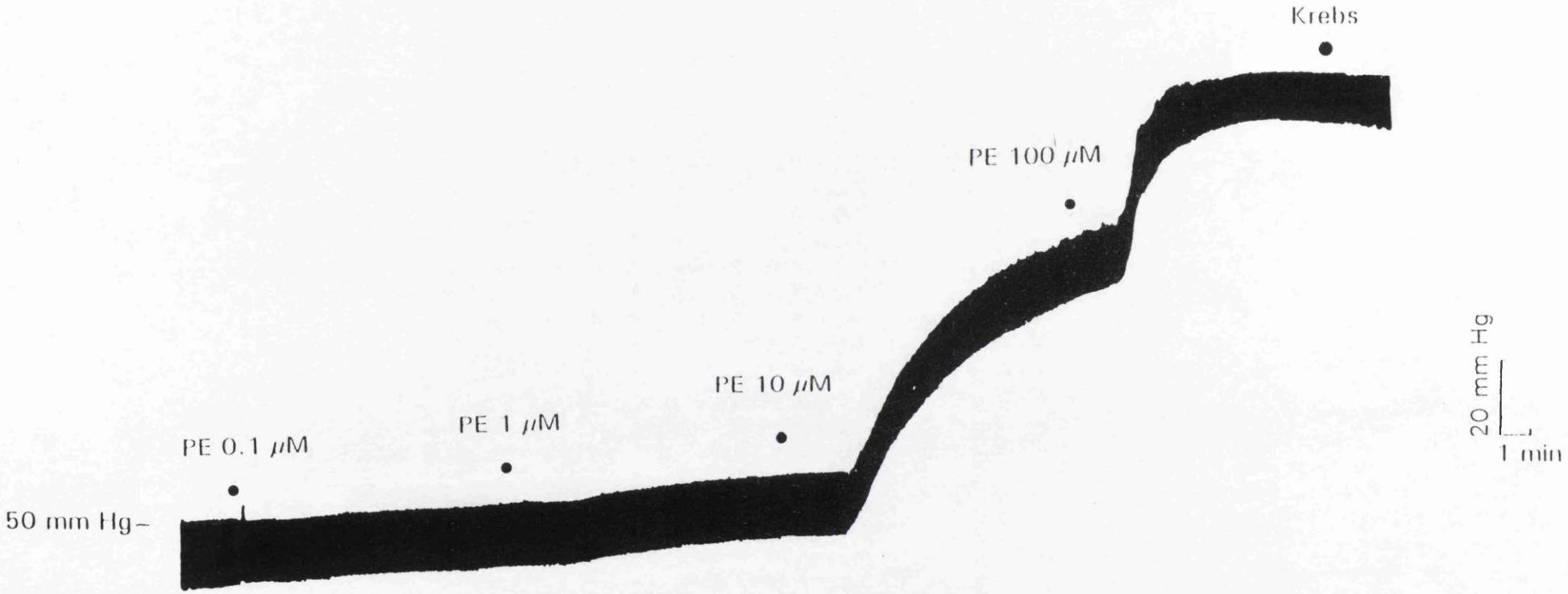


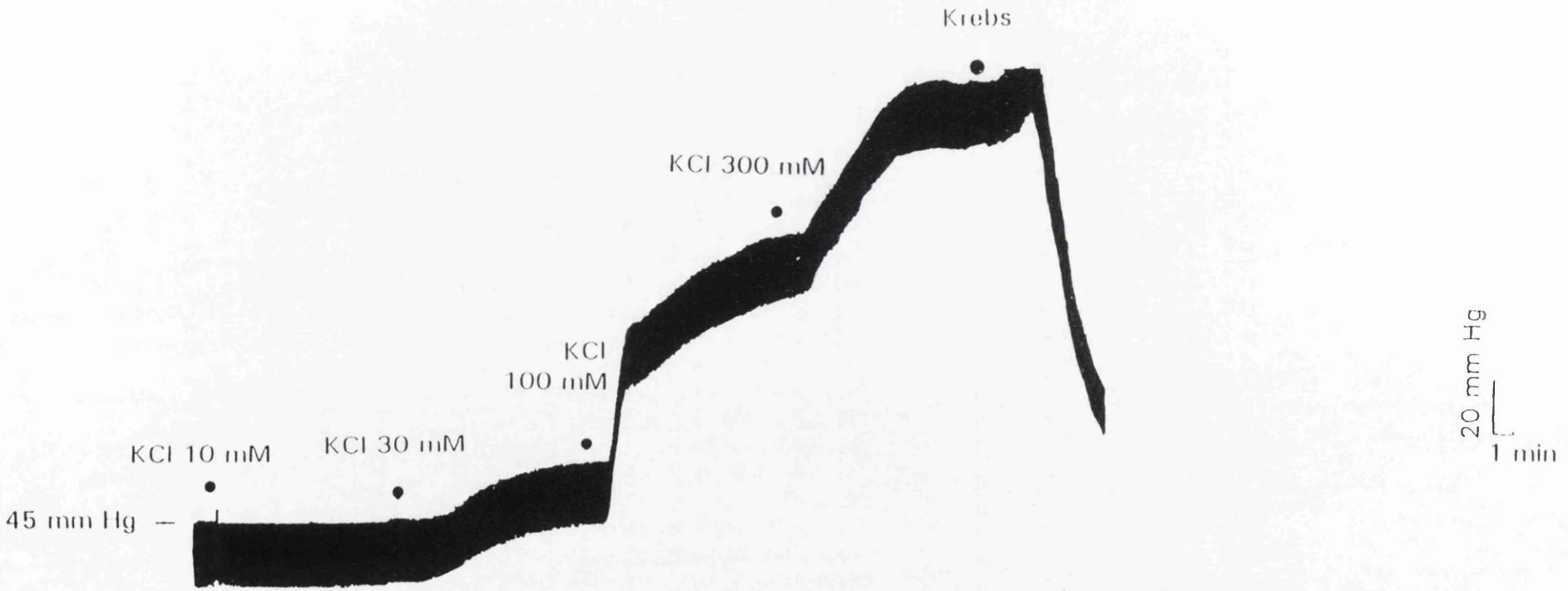
Fig-64
Showing in panels A and B consecutive concentration-response curves to PE (0.1-100 μM) and also showing in panels C and D consecutive concentration-response curves to SNP (10^{-9}-10^{-4} M) perfused cumulatively to produce equilibrium inhibitory responses in hindquarters vasculature in which the perfusion pressure had been raised with PE (10 μM). bPP represents the basal perfusion pressure (mm Hg) for each curve. The results are (Means ± SEM, n = 4).

Fig-65 Pressor responses to PE, in the isolated, Krebs-buffer perfused hindquarters vasculature of the Wistar rat



The trace shows pressor responses to cumulative concentrations of PE (0.1-100 μM) in the Krebs-buffer perfused hindquarters vasculature of the rat. Each concentration of PE was perfused until a stable (equilibrium) response was obtained.

Fig-66 Pressor responses to KCl, in the isolated, Krebs-buffer perfused hindquarters vasculature of the Wistar rat



The trace shows pressor responses to cumulative concentrations of KCl (10-300 μM) in the Krebs-buffer perfused hindquarters vasculature of the rat. Each concentration of KCl was perfused until a stable (equilibrium) response was obtained.

Fig-67 Concentration-response curves to the pressor effect of KCl and to the inhibitory effect of SNP on submaximal responses to KCl in the isolated, Krebs-buffer perfused hindquarters vasculature of the Wistar rats.

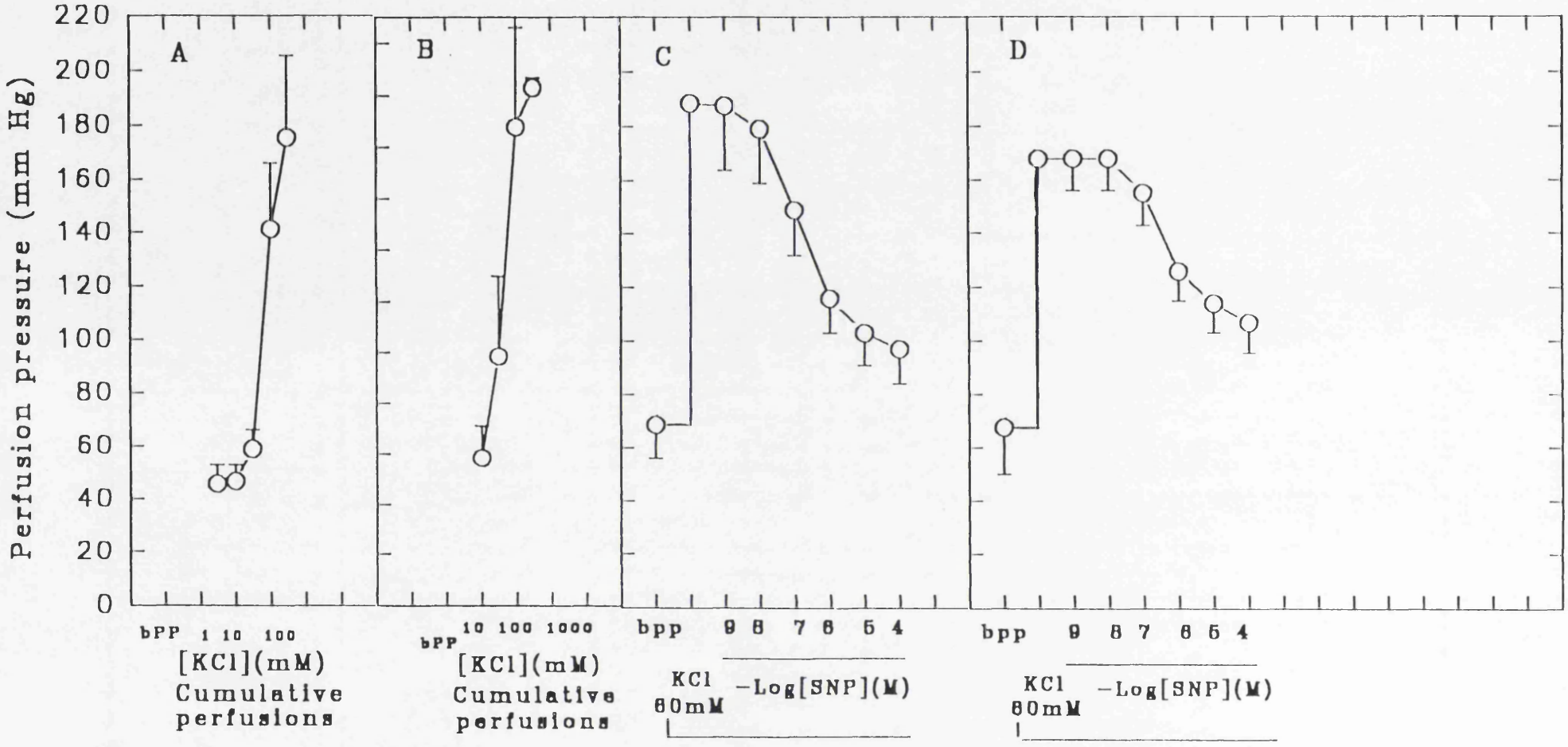
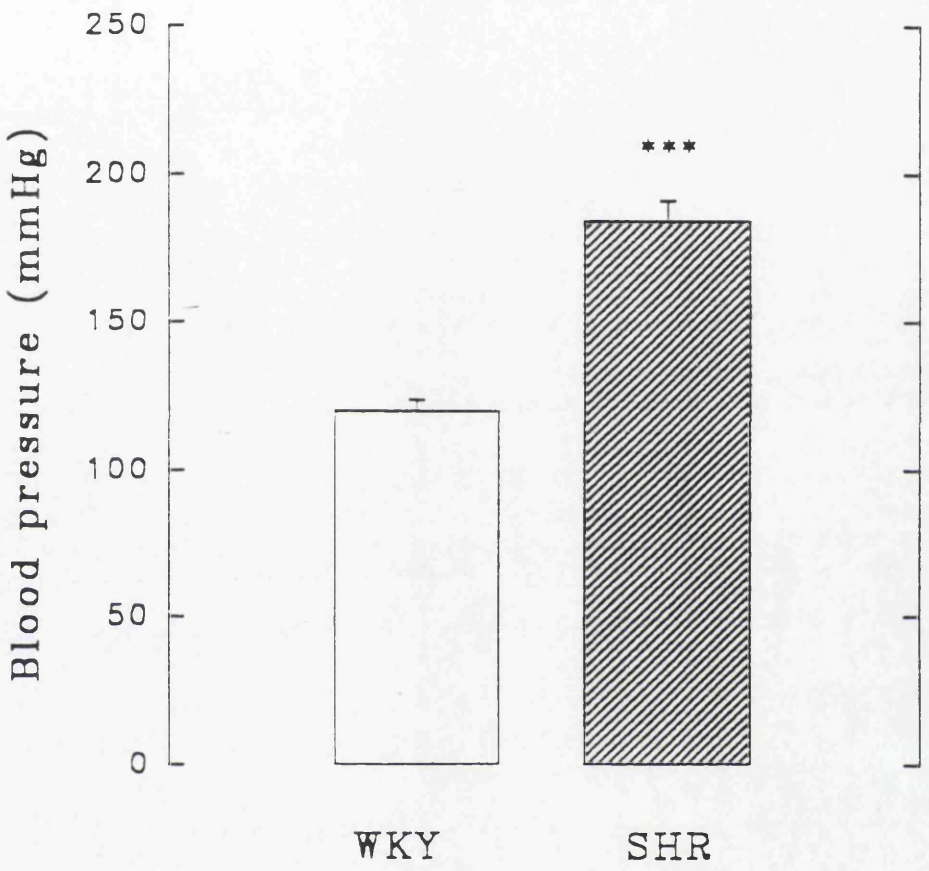


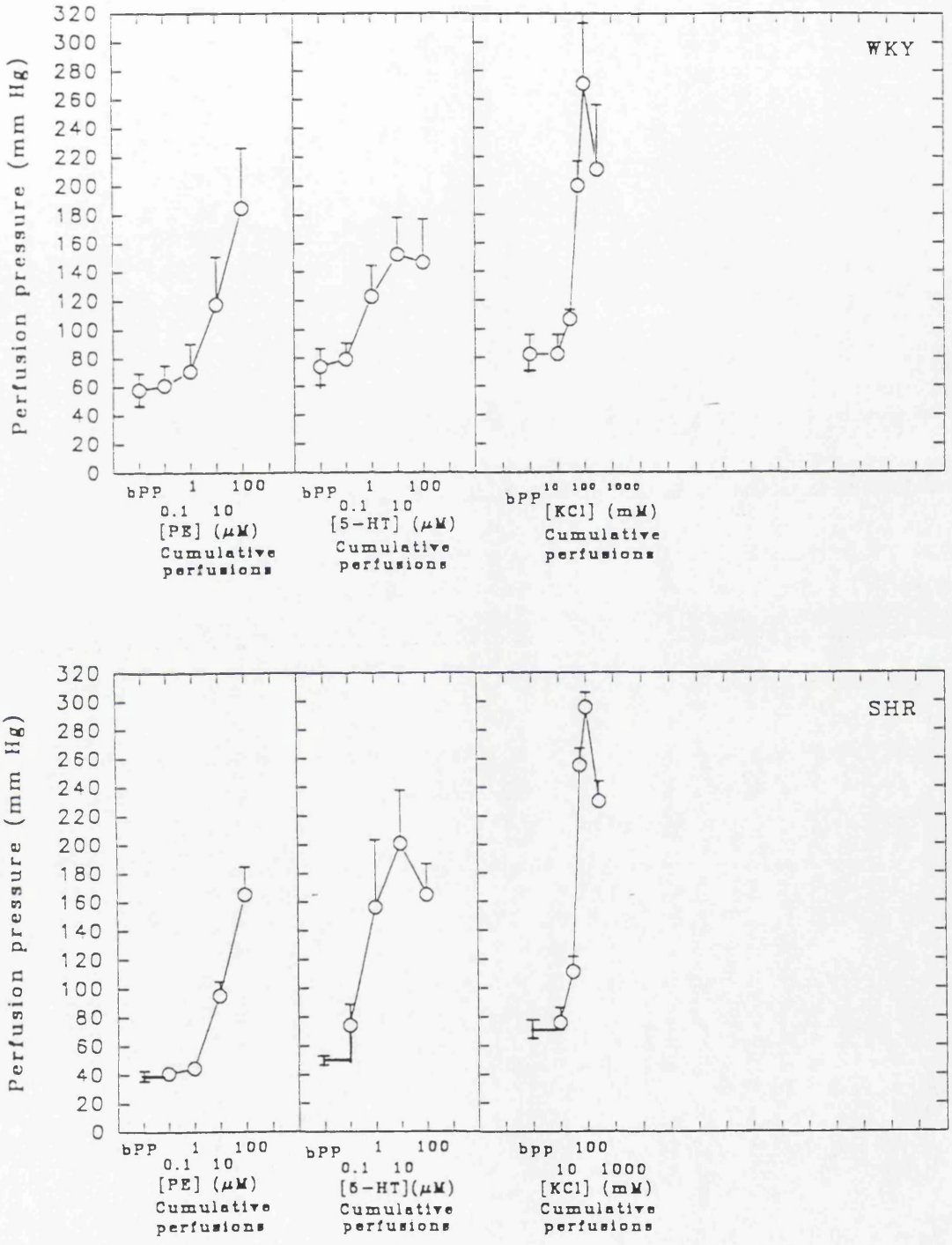
Fig-67
Showing in panels A and B consecutive concentration-response curves to KCl (3-300 mM) and also showing in panels C and D consecutive concentration-response curves to SNP (10^{-9}-10^{-4} M) perfused cumulatively to produce equilibrium inhibitory responses in hindquarters vasculature in which the perfusion pressure had been raised with KCl (60 mM). bPP represents the basal perfusion pressure (mm Hg) for each curve. The results are (Means ± SEM, n = 2).

Fig-68 Blood pressures in SHR and WKY rats.



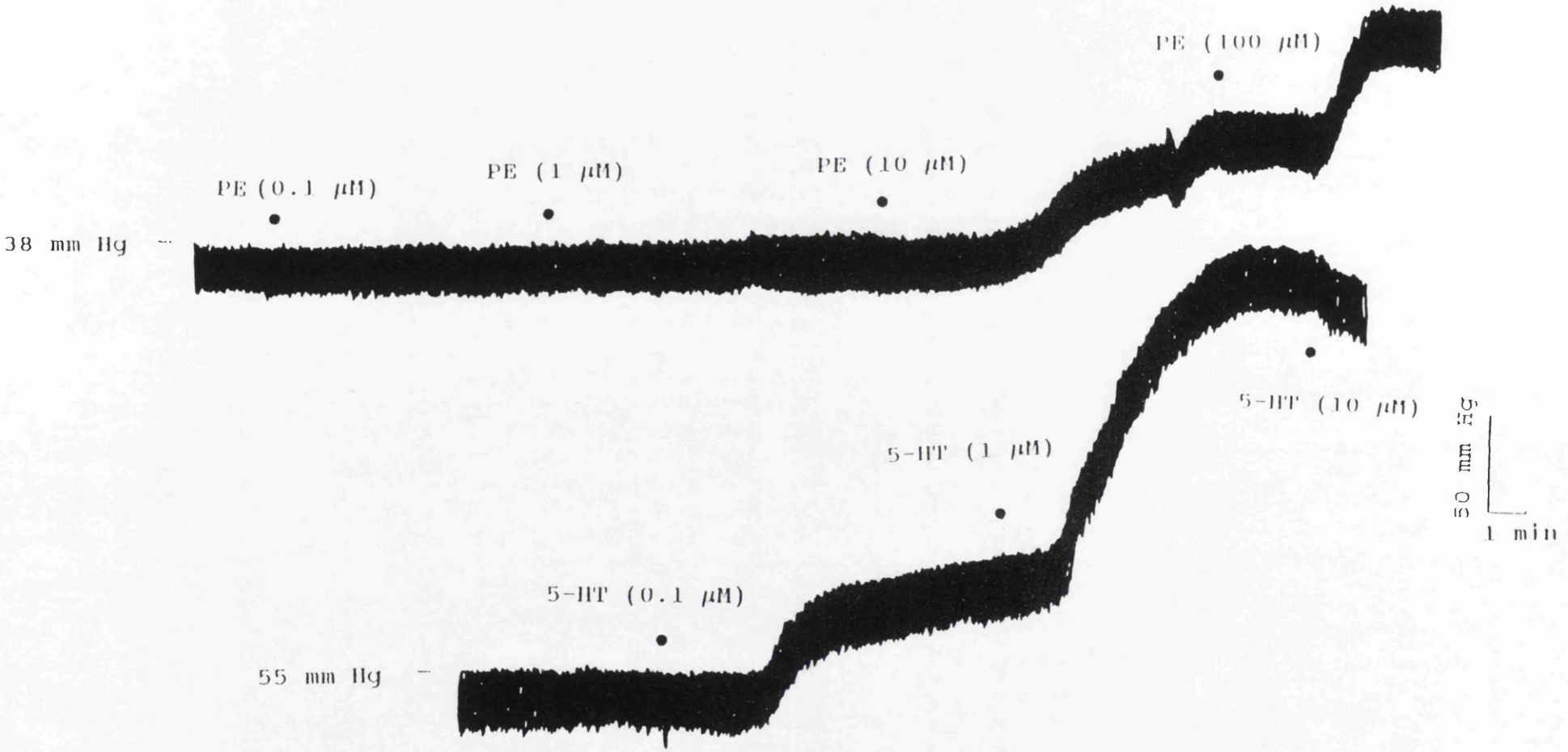
Histogram showing the blood pressures, measured by the tail cuff method in SHR and WKY control rats. The results are Means ± SEM, n=7, *** $P<0.001$.

Fig-69
Concentration-response curves to PE, 5-HT and KCl, administered in sequence in the isolated, Krebs-buffer perfused hindquarters vasculature of SHR and WKY control rats.



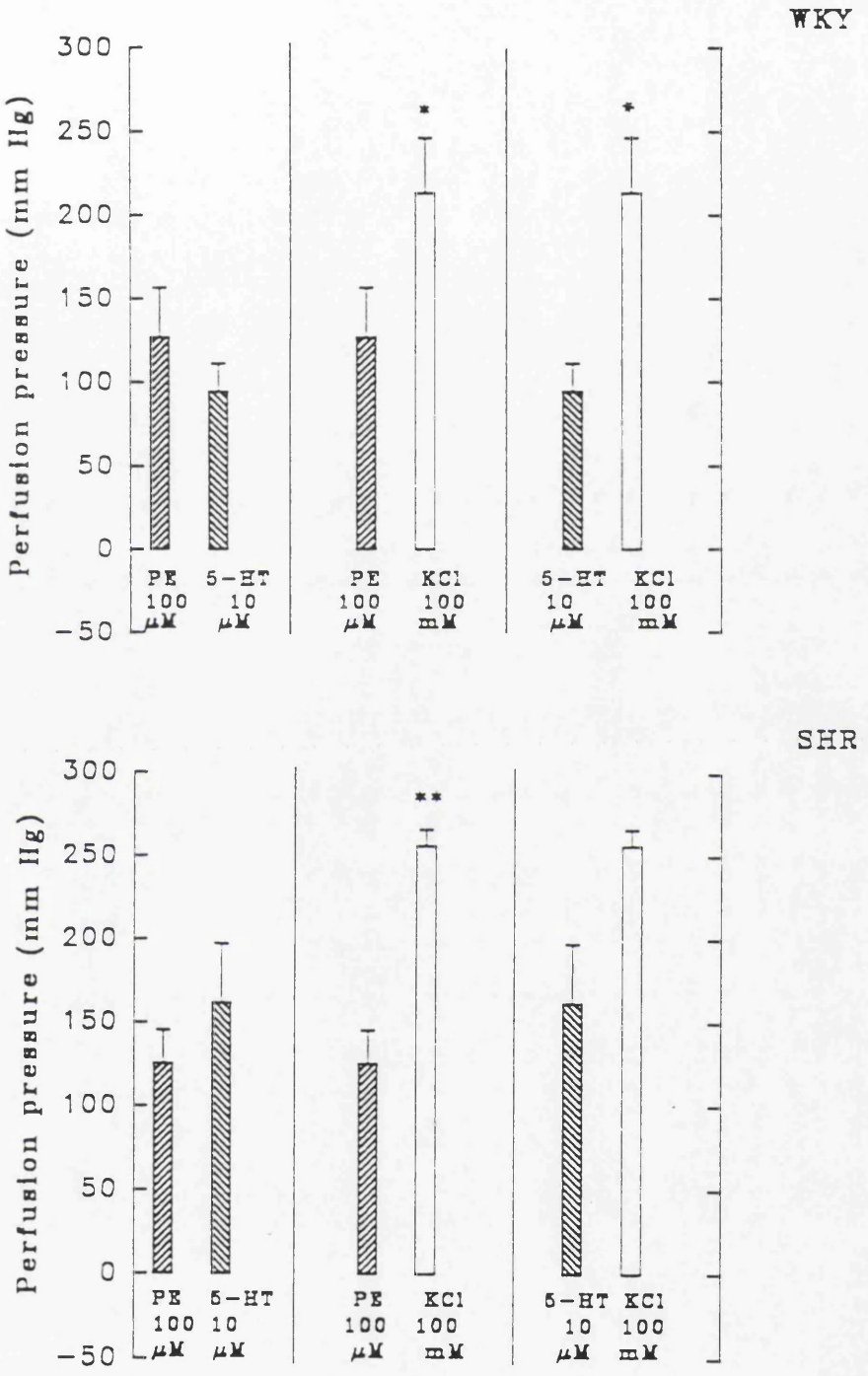
Cumulative concentration-response curves to PE (0.1-100 μM), 5-HT (0.1-100 μM) and KCl (10-300 mM), administered in sequence in Krebs-buffer perfused hindquarters vasculature from SHR and WKY control rats. KCl (100 mM) produced larger maximal pressor responses than PE (100 μM) and 5-HT (10 μM) in both strains, in which the basal perfusion pressure increased throughout the experiments. bPP represents the basal perfusion pressure (mm Hg) for each curve. The results are Means ± SEM, n = 4.

Fig-70 Concentration-responses to PE and 5-HT in the isolated, Krebs-buffer perfused hindquarters vasculature of a SHR rat.



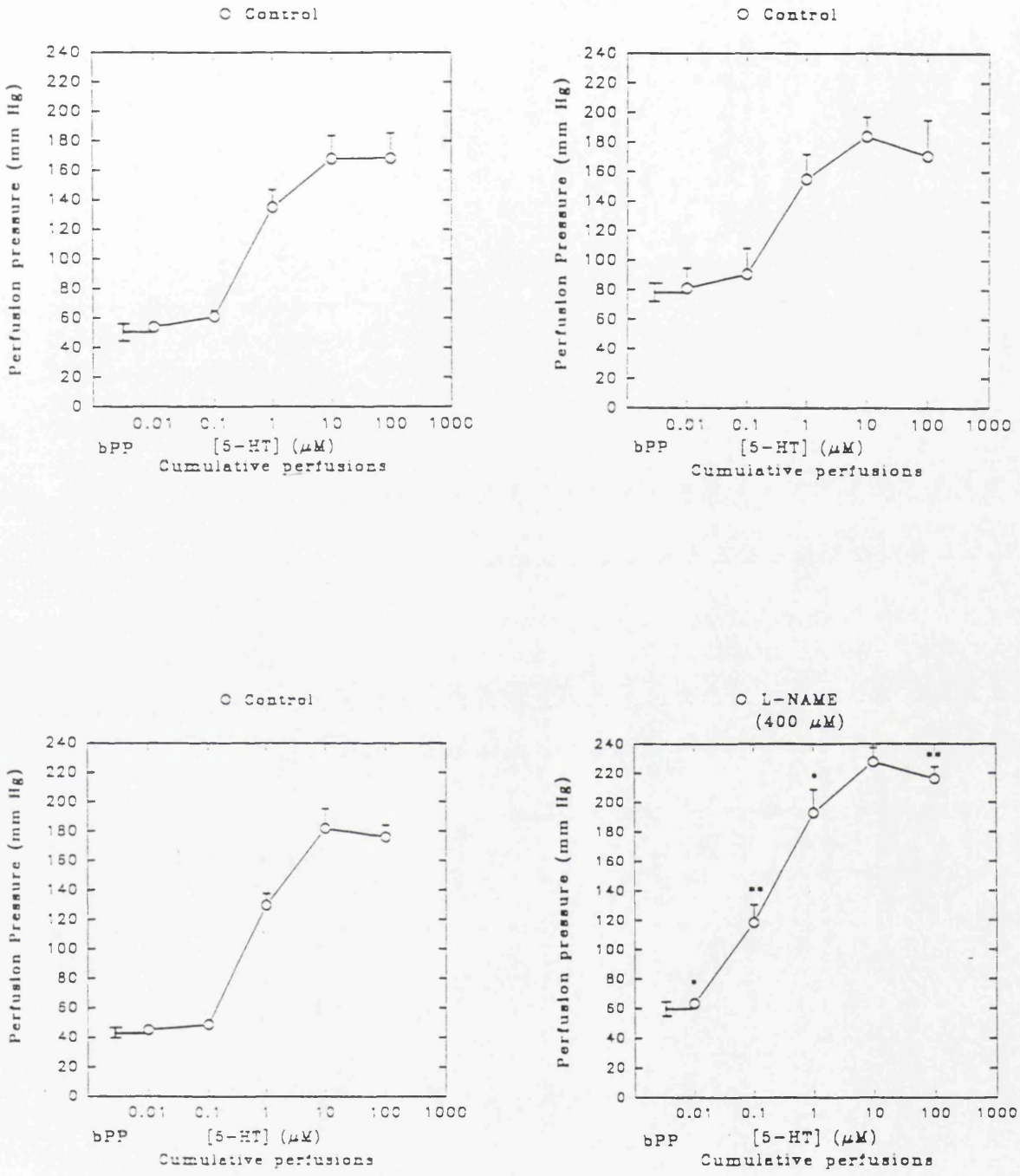
Cumulative concentration-responses to PE (0.1-100 μM, upper trace) and 5-HT (0.1-10 μM, lower trace), administered in sequence in Krebs-buffer perfused hindquarters vasculature of a SHR rat. The lower trace is a continuation of the upper trace.

Fig-71
Comparisons of the maximal pressor responses to PE, 5-HT and KCl in the isolated, Krebs-buffer perfused hindquarters vasculature of SHR and WKY control rats.



Histograms, showing maximal pressor responses to 5-HT (10 μM), PE (100 μM) and KCl (100 mM) in the Krebs-buffer perfused hindquarters vasculature of WKY (upper panel) control and SHR (lower panel) rats. There was a significant difference (** $0.01 > P > 0.001$) between the peak pressor responses to PE and KCl in SHR rats, whereas, in WKY rats, significant differences were found between the peak pressor responses to KCl and PE (* $0.05 > P > 0.01$); and between peak pressor responses to KCl and 5-HT (* $0.05 > P > 0.01$). The results are Means $\pm$ SEM, $n = 4$.

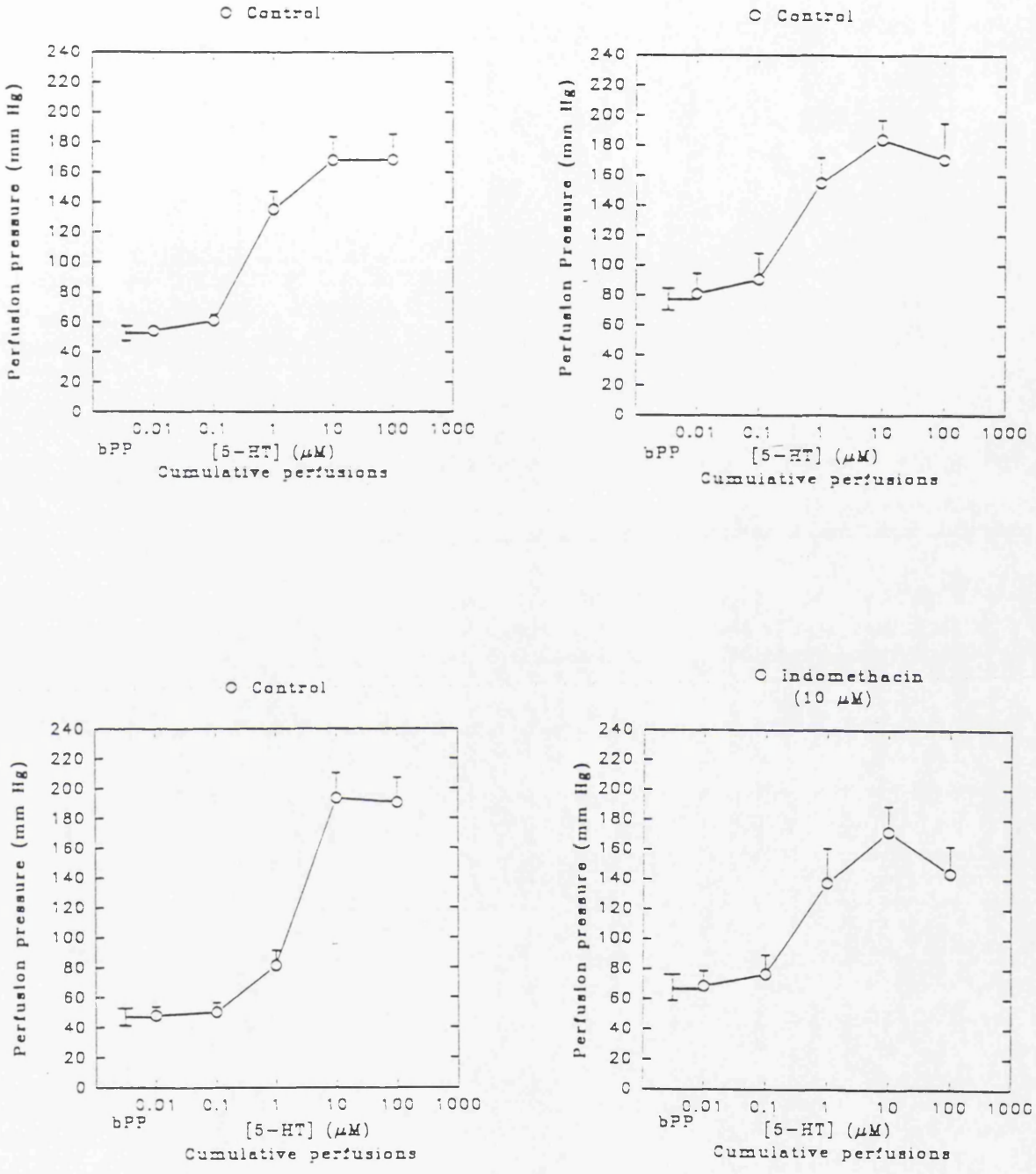
Fig-72
Effect of L-NAME on the concentration-response curve to 5-HT in the isolated Krebs-buffer perfused hindquarters vasculature of the Wistar rats.



Cumulative concentration-response curves to 5-HT (0.01-100 μM) in the absence and presence of L-NAME (400 μM) in the Krebs-buffer perfused hindquarters vasculature of the rat. Each concentration of 5-HT was perfused until a stable (equilibrium) response was obtained. The upper panel shows two consecutive (control) concentration-response curves to 5-HT only. The lower panel shows a control concentration-response curve to 5-HT alone and a subsequent concentration-response curve obtained in the presence of L-NAME (400 μM). bPP represents the basal perfusion pressure (mm Hg) for each curve. The results are Means ± SEM, n = 3 (upper panel) and n = 6 (lower panel), * $0.05 > P > 0.01$, ** $0.01 > P > 0.001$.

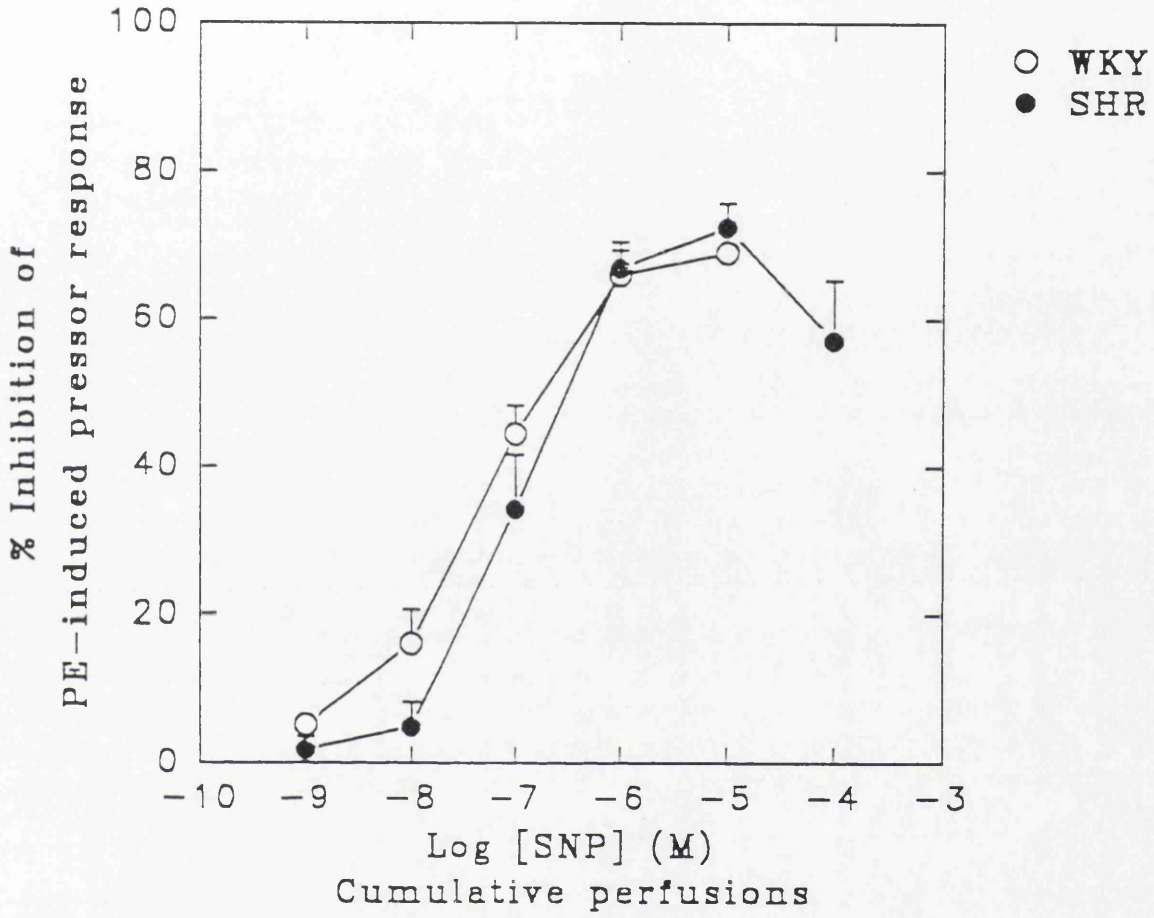
Fig-73

Effect of indomethacin on the concentration-response curve to 5-HT in the isolated, Krebs-buffer perfused hindquarters vasculature of the Wistar rats.



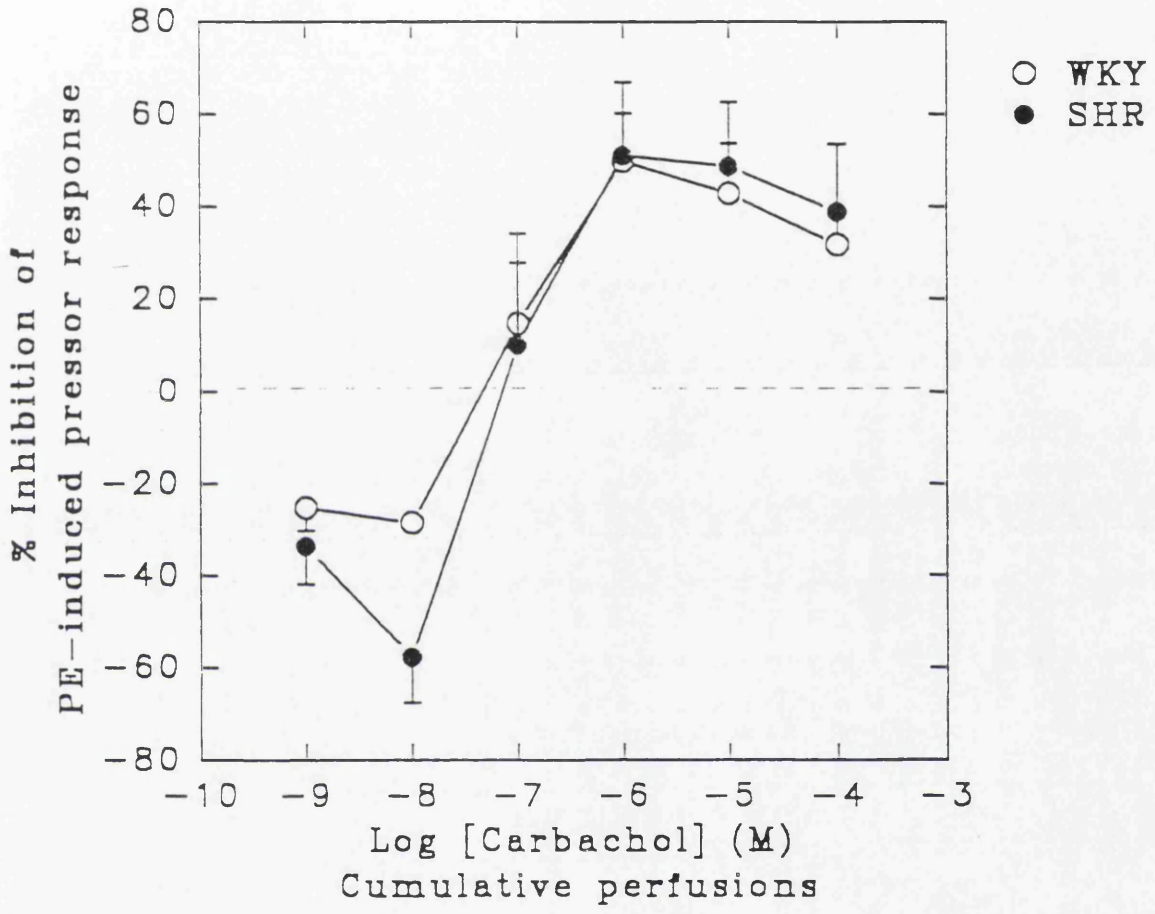
Cumulative concentration-response curves to 5-HT (0.01-100 μM) in the absence and presence of Indomethacin (10 μM) in the Krebs-buffer perfused hindquarters vasculature of the rat. Each concentration of 5-HT was perfused until a stable (equilibrium) response was obtained. The upper panel shows two consecutive (control) concentration-response curves to 5-HT only. The lower panel shows a control concentration-response curve to 5-HT alone and a subsequent concentration-response curve obtained in the presence of Indomethacin (10 μM). bPP represents the basal perfusion pressure (mm Hg) for each curve. The results are Means $\pm$ SEM, n = 3 (upper panel) and n = 4 (lower panel).

Fig-74
Inhibitory concentration-response curves to SNP in the isolated, Krebs-buffer perfused hindquarters vasculature of SHR and WKY control rats.



Inhibitory concentration-response curves to SNP (10^{-9}-10^{-4} M) in the Krebs-buffer perfused hindquarters vasculature of SHR and WKY control rats, in which the perfusion pressure had been raised by PE (10 μM). Each concentration of SNP was perfused until an equilibrium inhibitory response was obtained. The results are Means ± SEM, n = 4.

Fig-75
Inhibitory Concentration-response curves to carbachol in the isolated, Krebs-buffer perfused hindquarters vasculature of SHR and WKY control rats.



Concentration-response curves to carbachol (10^{-9}-10^{-4} M) in the hindquarters vasculature of SHR and WKY control rats, perfused with Krebs-buffer containing PE (10 μM). Initially, at low concentrations, carbachol produced additional constrictor responses which were replaced by vasorelaxant responses at higher concentrations. The results are Means ± SEM, $n \geq 3$.

DISCUSSION

General

The pithed rat preparation was developed by Shipley and Tilden (1947) and was later refined to allow selective stimulation of the spinal autonomic outflows (Gillespie and Muir, 1967; Gillespie, Maclaren and Pollock, 1970). This preparation has been used to examine autonomic neurotransmission and the nature of the receptors involved in the sympathetic regulation of blood vessels. It is a very convenient preparation since it allows the autonomic nerves to be stimulated electrically at the point where they leave the spinal column (Fig-4). In this preparation, by altering the amount of electrode exposed within the spinal canal, it is possible to stimulate either the entire sympathetic outflow or selected fractions of it, so that particular vascular beds can be affected. Since the spinal autonomic outflow is stimulated as the nerves leave the spinal column and before they mingle, the stimulation achieved is specific and avoids the problems that may arise when nominally pure sympathetic nerves are stimulated adjacent to the organ they innervate. However, as this study has demonstrated, the pithed rat is a very complex preparation and although it is easy to set up and readily provides cardiovascular responses to spinal autonomic nerve stimulation, the results obtained must be interpreted with caution.

Advantages of the pithed rat preparation:

1. It is possible to stimulate the sympathetic outflow to the vasculature easily

2. Different levels of the sympathetic outflow can be specifically stimulated
3. The Central Nervous System (CNS) is destroyed and so does not influence the pressor responses to spinal nerve stimulation
4. There are no cardiovascular reflexes present to modify the pressor responses
5. There is no anaesthetic present to influence the response to nerve stimulation

Disadvantages of the pithed rat preparation:

1. The resting blood pressure is abnormally low
2. Temperature regulation is difficult
3. Because of the low resting blood pressure and lack of CNS, endogenous substances may be released to modify and complicate the pressor responses
4. Respiration is artificial and very different from that in the intact animal
5. Repeated injections of aqueous solutions containing drugs may alter the blood volume

This study re-examined the cardiovascular responses of the pithed rat to spinal autonomic nerve stimulation. In addition, since previous work in this preparation has provided evidence that co-transmission, involving both NA and ATP, occurs in sympathetically-innervated blood vessels (Bulloch and McGrath, 1988), this study re-investigated this possibility. This study also examined the role of the adrenals in the pressor response to spinal nerve stimulation and the contribution of the ß-adrenoceptors to this response. This study also considered the possibility that other locally-released

vasoactive agents might affect the pressor response to spinal nerve stimulation.

This study also examined the effects of 5-HT in the cardiovascular system of the anaesthetised and pithed rats and in the isolated perfused lungs, in which the pressor effects of 5-HT were masked by locally-released NO, which has diverse biological activities, since it is a vasoactive, a cytotoxic, a platelet regulatory and a neurotransmitter agent (Furchgott & Zawadzki, 1980; Moncada et al, 1991). Accumulating evidence also indicates that NO has a significant role in cardiovascular disease (Gordon et al, 1989; Drexler et al, 1991).

Studies in the pithed and anaesthetised rats

Stimulation of the spinal autonomic outflow produced characteristic pressor responses and cardio-acceleration, the magnitude of which was determined by the location of the electrode within the spinal canal (Fig-5) and by the stimulation parameters used. At most levels of stimulation, the pressor response was biphasic, with one component that occurred during stimulation and a secondary response that persisted after stimulation ceased. This occurred during stimulation of the spinal sympathetic outflow at position T_{8-9} which was used in this study because stimulation here produced the biggest pressor response to spinal nerve stimulation (Fig-6). In addition, the cardio-acceleration that occurred usually also persisted when stimulation ceased. These observations suggest that the secondary pressor response and the cardio-acceleration may be caused by circulating catecholamines released during stimulation from the adrenals. This was confirmed during the experiments in which the adrenals were removed after

having obtained standard pressor responses to spinal nerve stimulation (Fig-7). In those circumstances, the secondary pressor response was abolished. In addition and perhaps more importantly, the pressor response obtained during stimulation was greatly reduced. This observation contrasts with that of MacLean et al (1994), who reported that the initial component of the pressor response to nerve stimulation was purely neuronal. The discrepancy between their results and those obtained in the present study may be partly explained by the fact that the simulation parameters used in this study were different from those used by MacLean at el (1994), who used fewer pulses. However, it is still surprising that if electrical stimulation was effective in releasing catecholamines from the adrenals, that this did not occur or was not detectable during stimulation. In this study, there certainly was evidence that catecholamines were released during electrical stimulation, since adrenalectomy greatly reduced the size of the pressor response obtained during stimulation, as well as reducing the secondary pressor response (Figs-7,10).

The adrenal medulla and the catecholamines released from it, have important physiological roles in the regulation of the cardiovascular system (Bevan et al, 1980; Clutter et al, 1980). The release of these vasoactive catecholamines from the adrenals could further complicate the responses to drugs investigated in the pithed rat. If catecholamines are released from the adrenals during stimulation of the spinal outflow, as the present results indicate, then this would have to be taken into consideration if this preparation is used to analyse the nature of neurotransmission in the sympathetic nerves that innervate blood vessels. It would also be important to be aware of this possibility if this preparation was used to examine presynaptic mechanisms, which regulate transmitter release, since circulating adrenaline might act on presynaptic ß-adrenoceptors to

counteract the auto-inhibition caused by neuronally-released NA (Majewski and Rand, 1981; Rand et al, 1975 and Langer, 1979). Moreover, the potentiating effect of circulating catecholamines on the cardiac output would also be a contributory factor to the pressor response, especially since in the pithed rat there would be no reflex mechanism to restrain the effect of adrenaline on the heart.

The cardiovascular effects produced by stimulation of the adrenal glands in the pithed rat, were similar to those evoked by exogenous adrenaline (Flavahan & McGrath, 1980), which is the main catecholamine secreted by the adrenals (Yamaguchi & Kopin, 1979). In this study, evidence indicating that adrenaline is released during spinal nerve stimulation, was obtained with propranolol, which increased the pressor response obtained both during and after spinal nerve stimulation at 2 Hz as well as increasing the secondary pressor response obtained after stimulation at 20 Hz (Fig-8). The inability of propranolol to potentiate the pressor response to stimulation at 20 Hz during stimulation may have been because stimulation at this frequency, produced even before administration of propranolol, a maximal response so that the vasodilator effect of adrenaline (acting on vascular $ß_2$-adrenoceptors) was completely overcome by the vasoconstrictor effect of very large amounts of neuronally-released NA. The effect of propranolol on the pressor responses to the spinal nerve stimulation was in agreement with the results obtained by Flavahan et al (1985). These results suggest that when the pressor response to spinal nerve stimualtion is used, for example, to study the nature of neurotransmission in blood vessels, it is necessary to remove at least the effect of circulating adrenaline on ß-adrenoceptors with a non-selective ß-antagonist such as propranolol, which would antagonise the effect of catecholamines on the heart and on ß-adrenoceptors elsewhere. The effects of this treatment on the pressor response are

certainly complex and potentially conflicting. This was apparent in those experiments in which propranolol reduced rather than enhanced pressor responses to spinal nerve stimulation (Fig-9). In these experiments, in which characteristically the basal blood pressure was very low (<18-22 mmHg), it is possible that when the effects of catecholamines on the heart were blocked by propranolol, the heart was unable to continue to pump the blood into the constricted blood vessels and so the pressor response was minimal. These observations make it clear that the role of the heart in the pressor responses to spinal nerve stimulation is easy to overlook. If the contributions of the heart and the adrenals are not taken into account, then the interpretation of the results will be difficult and may lead to serious misconceptions.

An important purpose of this study was to determine whether there was any evidence that co-transmission occurred in sympathetically-innervated blood vessels in the pithed rat. Previous work had shown that stimulation of the sympathetic outflow in the pithed rat caused a pressor response that was partly noradrenergic and partly purinergic (Flavahan et al, 1985; Grant et al, 1985). The evidence for this was derived from experiments in which stimulation of the sympathetic fibres to the blood vessels produced a pressor response that had a component which was not blocked by the α-adrenoceptor antagonist, prazosin, but was abolished by α,β-mATP (Burnstock & Warland, 1987; Ramme et al, 1987; Bulloch & McGrath, 1988), which desensitizes P_{2X}-purinoceptors to ATP. This study rexamined this hypothesis but took account of other factors that might be expected to influence the responses to stimulation of the sympathetic outflow via the spinal electrode.

The results obtained in this study with α,ß-mATP do not support the view that there is a purinergic component in the pressor response to stimulation of the sympathetic outflow. This can be concluded from the experiments in which repeated administration of α,ß-mATP caused desensitization of the pressor response to α,ß-mATP (Fig-12). In these experiments, responses to spinal nerve stimulation also declined but not to a greater extent that did the control response to exogenous NA. Moreover, although interruption of the administration of α,ß-mATP restored the sensitivity to α,ß-mATP, it did not restore the sensitivity to NA or to nerve stimulation. These results do not support the view that purinergic co-transmission contributes to the pressor response to spinal nerve stimulation at T_{8-9} in the pithed rat. These results conflict with those of Bulloch and McGrath (1988) who also examined the effect of α,ß-mATP on pressor responses to spinal nerve stimulation in the pithed rat. There certainly were differences between the methods used in the present study and those used by Bulloch and McGrath (1988) but it is difficult to see how these could explain the discrepancies between the results obtained in the two studies. For example, in this study, the pithed rats were respired artificially with air, whereas Bulloch and McGrath (1988) used 40% oxygen and 60% nitrogen. In addition, there was a slight difference in the location of the electrode during stimulation, which was achieved in this study with the electrode being exposed at T_{8-9} but in the study carried out by Bulloch and McGrath, the electrode was exposed at T_{6-8}. It is difficult to avoid the conclusion that if there is a purinergic component in the sympathetically-mediated pressor response to spinal nerve stimulation in the pithed rat, then it must be very small, since most of the pressor response is the result of stimulation of the fibres to the adrenal medulla, from which catecholamines are released.

The pithed rat has a high circulating renin activity (De Jonge et al, 1982) due to its low resting blood pressure. There are many reports indicating a facilitatory action of angiotensin II (AII) on peripheral noradrenergic transmission (Rand et al, 1990; Zimmerman, 1978; Majewski et al, 1984; Starke, 1977). In this study, the results obtained with captopril provided further evidence about the complexity of the pressor response to stimulation of the spinal autonomic outflows. Captopril had little effect on the resting blood pressure (BP), only occasionally producing a slight fall in BP (Fig-16). However, captopril inhibited pressor responses to injected NA and also inhibited responses to spinal nerve stimulation at both 2 Hz and 20 Hz (Figs-16,17). This observation suggests that endogenous angiotensin II, whose synthesis captopril inhibits, enhances the responses to NA and to spinal nerve stimulation in this preparation. Majewski in 1989 also reported that captopril decreased NA release in the pithed rat when the sympathetic outflow was stimulated. Another more complex explanation is based upon the fact that angiotensin converting enzyme (ACE) which is inhibited by captopril not only converts angiotensin I (AI) to angiotensin II (AII) but also inactivates the vasodilator, bradykinin (Vanhoutte et al, 1993; Busse et al, 1993; Erdos, 1990). The inhibitory effect of captopril on pressor responses to nerve stimulation and NA may therefore be due to inhibition of the synthesis of AII and/or to prolongation of the effect of bradykinin, which releases EDRF (NO), whose action would oppose that of constrictors, such as NA.

The results considered so far indicate that the pressor response to spinal nerve stimulation in the pithed rat is very complex. α_1-Adrenoceptors contribute a great deal to the vascular pressor responses and the characterization of the postjunctional α_1-adrenoceptor-mediated pressor response was made with prazosin, a selective α_1-adrenoceptor antagonist

(Timmermans & Van Zwieten, 1982; McGrath, 1982; Drew & Whiting, 1979). In this study in the presence of propranolol and captopril, prazosin, inhibited the pressor response to spinal nerve stimulation and the extent of the inhibition produced was no less than was the inhibition of the standard pressor response to exogenous NA (Figs-16,17). This observation does not support the view that there is a prazosin-resistant component in the pressor response to spinal nerve stimulation at T_{8-9}.

Several interesting observations were obtained with L-NAME. Intravenous injections of L-NAME produced a prolonged rise in the basal blood pressure in both anaesthetised and pithed rats. The rise in the baseline blood pressure was about four and a half fold higher in the anaesthetized rats than in the pithed rats in the presence of propranolol (2 mg kg^{-1}) (Fig-18 panel D). The main reason why the basal blood pressure was increased by L-NAME to a greater extent in anaesthetised rats than in pithed rats, was probably because in the former there was a tonic discharge in the autonomic sympathetic pathway and consequently a controlled release of NA to the effector cells in the blood vessels in these animals. NO synthesis was inhibited in both pithed and anaesthetised rats but the major difference would be this sympathetic tone which when unopposed by endogenous NO, caused the blood pressure to rise to a greater extent in the anaesthetised rats. Shear stress could be another reason for the difference since in the anaesthetised rat, blood pressure was higher and hence the shear stress on the intraluminal surface of the vasculature would probably also be higher and thus could release more NO into the blood circulation (Forstermann et al, 1994). The fact that the L-NAME-induced increase in the basal blood pressure was the same, in the absence of propranolol (2 mg kg^{-1}), in both pithed and anaesthetised rats (Fig-18 panel C), was surprising, not least because the basal blood pressure prior to the administration of L-NAME was

higher in the anaesthetised rats than in the pithed rats. It may be that the pressor response to L-NAME in the anaesthetised rat was restrained by cardiovascular reflexes, despite the evidence to the contrary in intact animals (Gardiner et al, 1990; MacLean, MacMillan, 1993). On the other hand, a more likely explanation of the similar pressor responses to L-NAME in pithed and anaesthetised rats is that in the anaesthetised rats, L-NAME produced a maximum pressor response and no higher response was possible.

In pithed rats, L-NAME increased the blood pressure and the size of this response was directly proportional to the resting blood pressure both in the absence and presence of propranolol (2 mg kg^{-1}) (Fig-19). This was reflected in the positive correlation between the L-NAME-induced increment in blood pressure and the basal blood pressure prior to the administration of L-NAME in the absence (correlation coefficient $R=0.96$, $n=5$, $0.01>P>0.001$) and presence (correlation coefficient $R=0.64$, $n=19$, $0.01>P>0.001$) of propranolol (2 mg kg^{-1}). This may indicate that the higher the basal blood pressure, the more NO is produced, perhaps as a result of increased shear stress on the endothelium. However, in the anaesthetised rats, there was no significant correlation between the L-NAME-induced increment in the blood pressure and the basal blood pressure either in the absence or in the presence of propranolol (2 mg kg^{-1}) (Fig-20). This may reflect merely the variability of the data or it may reflect the greater complexity of the anaesthetised rat, which still had an intact nervous system that would be capable of reflexly modifying, the L-NAME-induced pressor responses, which might have been diminished, so that the possibilty of seeing a positive correlation between the pressor responses to L-NAME and the basal blood pressure would also be diminished. However, this interpretation of the results is contradicted by observations obtained in

other studies in conscious rats, in which L-NAME caused only a slight, inconsistent bradycardia with an increase in blood pressure (Gardiner et al, 1990). Their results suggests that, slowing of the heart due to cardiovascular reflexes is unlikely to oppose the increase in the resting blood pressure produced by L-NAME. Furthermore, chronic oral treatment with L-NAME can also cause hypertension in Wistar rats, with no major effect on the heart rate (MacLean, MacMillan, 1993). The observations in this study in the pithed and anaesthetised rats, therefore indicate that the normal sympathetic tone is not an important prerequisite for the release of endogenous NO in vivo. These results contradict those of some workers (Vargas et al, 1990; Lacolley et al, 1991) but are consistent with those obtained by others (Pegoraro et al, 1992). L-NAME (45 $mgkg^{-1}$) did not enhance pressor responses to α,β-mATP but increased the toxicity of this pressor agent in the pithed rat (Fig-25). The lethal effect of α,β-mATP may be due to its ability to constrict blood vessels that no longer have the capacity to produce NO because of the presence of L-NAME. Vascular beds such as the pulmonary vascular bed may be involved in this toxic effect of α,β-mATP in the presence of L-NAME. In this study, L-NAME enhanced pressor responses to injected 5-HT but in most experiments, had no such effect on pressor responses to spinal nerve stimulation. This is surprising, especially since L-NAME did increase basal blood pressure in both anaesthetised and pithed rats. However, the inability of L-NAME in this study to potentiate pressor responses to spinal nerve stimulation (Fig-22) conflicts with the observations of MacLean et al (1994), who reported that L-NAME potentiated pressor responses to spinal nerve stimulation. An important difference between the results reported here and those of MacLean et al (1994) is that, in this study, the effect of L-NAME on pressor responses to spinal nerve stimulation were investigated in the presence of propranolol whereas in the study by MacLean et al (1994), the rats received

no propranolol. In these circumstances it is likely that catecholamines would be released from the adrenals by spinal nerve stimulation and a substantial vascular ß-mediated vasodepressor component would oppose the vasoconstrictor effects of neuronally-released NA. In the present study, paradoxically, the vasopressor component of the response to spinal nerve stimulation might have been limited by propranolol, which would act presynaptically to antagonize the action of circulating adrenaline on the ß-adrenoceptors, thereby inhibiting the ability of adrenaline to promote NA release. The nature of these prejunctional ß-adrenoceptors is not clear. There are some reports which indicate that these are $ß_2$-adrenoceptors (Stjarne and Brundin, 1976) yet other reports suggest that these are $ß_1$-adrenoceptors (Majewski et al, 1980; Dahlof et al, 1975). This may be an indication that in different tissues the prejunctional ß-adrenoceptors may be of either the $ß_1$- or $ß_2$-subtype. The discrepancy between the lack of effect of L-NAME in this study on the nerve-mediated pressor response and the ability of L-NAME to enhance such pressor responses to spinal nerve stimulation in the absence of propranolol in the experiments of MacLean et al (1994), suggests that there may be a connection between the lack of effect of L-NAME in the presence of propranolol and the enhancement caused by L-NAME in the absence of propranolol. One possibility is that a large part of the vascular ß-adrenoceptor-mediated vasorelaxation caused by circulating adrenaline released from the adrenals by spinal nerve stimulation, is associated with the synthesis and release of NO within the vasculature. If activation of vascular ß-adrenoceptors leads ultimately to release of NO, which is responsible for the vasodilatation (Gray & Marshall, 1992), then this vasodilator mechanism could be inhibited either by blocking the ß-adrenoceptors with propranolol or by blocking the synthesis of NO with L-NAME. Either drug would potentiate the pressor response to the nerve stimulation but potentiation by one would preclude potentiation by the

other. In some experiments, L-NAME apparently did enhance the pressor response to spinal nerve stimulation at T_{8-9} at both 2 Hz and 20 Hz, but this was only after exogenous NA (2 μg kg^{-1}) had inhibited this response (Figs-26,27). In these experiments, the apparent ability of L-NAME to potentiate the nerve-mediated pressor response may merely have been due to recovery of the nerve-mediated pressor response from the inhibitory effect of NA. L-arginine the precursor for the biosynthesis of the endogenous vasodilator, NO (Moncada et al, 1989) lowered the basal blood pressure in the presence of L-NAME (Figs-26,27). The dose of L-arginine (45 mg kg^{-1}) which was the same as the dose of L-NAME (45 mg kg^{-1}), only inhibited the first component of the pressor response to spinal nerve stimulation at 2 Hz and had no such effect on the post-stimulation component of this response. L-arginine (45 mg kg^{-1}) did not affect either component of the pressor response to stimulation at 20 Hz and did not affect the NA (2 μg kg^{-1})-induced pressor response.

Receptor-mediated modulation of peripheral NA release in various in vitro and in vivo preparations has been described extensively (reviews by Gillespie, 1980; Langer, 1981; Starke, 1981a,b, 1987). In this study, in the pithed rat, NA (0.1, 2 and 15 μg kg^{-1}) produced dose-dependent pressor responses in the absence and presence of L-NAME (45 mg kg^{-1}) with propranolol (2 mg kg^{-1}) being present throughout the experiment (Fig-23). The first dose of NA (0.1 μg kg^{-1}) inhibited the pressor responses to nerve stimulation at 2 Hz but further administration of higher doses of NA did not inhibit the neuronal pressor responses further. The inhibitory effect of exogenous NA could be mediated through activation of α_2-adrenoceptors on the sympathetic nerve terminals. Such a mechanism would hinder the Ca^{2+}-dependent sympathetic neurotransmitter release during electrical stimulation of the sympathetic nerve roots at T_{8-9} of the spinal vertebrae. It

was surprising that higher doses of NA did not produce larger inhibitions. This may have been because the inhibitory effect of the lowest dose of NA (0.1 μg kg^{-1}), together with the endogenous feedback inhibition of neuronally-released NA, had a maximal effect. Antagonism of these prejunctional α_2-adrenoceptors can increase the stimulation-induced release of NA (Gillespie, 1980; Starke, 1981a) and there was some evidence in this study that the α_2-adrenoceptor antagonist yohimbine, potentiated pressor responses to spinal nerve stimuation (Figs-14,15).

The responses to 5-HT, which is normally inactivated to a large extent by the pulmonary vasculature (Furchgott, 1984; Said, 1982), were different in the pithed and anaesthetized rats (Figs-29 panel B, 30). In the pithed rat, 5-HT only produced a pressor response but in the anaesthetised rat, 5-HT usually produced a triphasic response in the blood pressure. This response consisted of an initial brief depressor component, followed by a secondary pressor component, which was then follwed by a final sustained depressor component. The triphasic response to 5-HT obtained in anaesthetised rat was similar to that in other studies (Kalkman et al, 1984; Page, McCubbin, 1953). The triphasic nature of the response to 5-HT in anaesthetised rats became more evident as the doses were increased. The initial, brief depressor component was similar to that associated with the Von Bezold-Jarisch-like reflex (Saxena and Villalon, 1990). In both preparations, 5-HT produced dose-related responses in the absence and presence of L-NAME (Figs-28,29;31,30) (Moncada et al, 1991). In the pithed rat, 5-HT inhibited the pressor component to the spinal nerve stimulation at T_{8-9} at 2 Hz and 20 Hz (Fig-29) and the significance of this inhibitory effect of 5-HT on the sympathetic nerve terminals can be seen in Fig-22. These results are consistent with an inhibitory effect of 5-HT on the release of NA from the sympathetic nerves, and suggest the presence of inhibitory 5-HT receptors

on the peripheral sympathetic nerves. In the pithed rat, L-NAME enhanced the pressor responses and prolonged recovery from these responses to 5-HT, particularly at low (6 μg kg^{-1}), medium (12 μg kg^{-1}) and submaximal (25 μg kg^{-1}) doses (Figs-28,24). In the anaesthetized rat, L-NAME reduced the initial brief depressor component, increased the secondary pressor component, prolonged recovery from this component, and reduced and shortened the final sustained depressor component of the response to 5-HT (Figs-31,30). The latter effect is similar to that reported in rat kidney, where L-NAME also inhibited vasorelaxation induced by 5-HT (Verbeuren et al, 1991). These results are consistent with the hypothesis that 5-HT can release NO, which would tend to oppose the vasoconstrictor effects of 5-HT in both the pithed and anaesthetized rats, as well as adding to the vasodepressor components of 5-HT in anaesthetized rats. This mechanism would resemble that in the pulmonary vasculature, where the vasoconstrictor effects of 5-HT may be masked by the vasodilator effects of endogenous NO, which may be involved in maintaining the low blood pressure in the pulmonary circulation, perhaps by opening collaterals so that a larger fraction of the pulmonary endothelium is available to inactivate the 5-HT. If this occurs then the blockade of the synthesis of NO by L-NAME would then increase the circulating levels of 5-HT which might then exert a toxic effect on the heart. If NO is produced either directly in response to 5-HT acting on its receptors or indirectly, as a result of its vasopressor activity, then there must be a very substantial amount released, since it is likely that much of it would normally be taken up by erythrocytes and inactivated by haemoglobin (HbO_2) via stoichiometric conversion to methaemoglobin (MetHb) and nitrate (Gillespie & Sheng, 1988; Wennmalm et al, 1992 and Martin et al, 1985).

Since there were three components (an initial brief depressor component, a secondary pressor component and a final sustained depressor component) in the 5-HT-induced response in blood pressure in the anaesthetized rats, it is likely that different mechanisms would be involved in producing these opposing effects.

The initial brief depressor component to 5-HT could have been due to activation of 5-HT_3 receptors on the sensory vagal nerve endings in the heart, consequently causing a short-lasting bradycardia and reduction in cardiac output (Saxena and Villalon, 1990). This initial component of the 5-HT-induced response resembled that associated with the Von Bezold-Jarisch-like reflex. However, there is some evidence that a short lasting vasodilatation may be obtained via 5-HT_3 receptors, which probably invoke an axon-like reflex (Blauw et al, 1988).

The secondary, pressor component of the 5-HT-induced response, would probably be produced by an increase in the free $[Ca^{2+}]_i$ ions in the vascular smooth muscle cells to activate the contractile mechanism (5-HT_1-like and 5-HT_2 receptor mediation). In isolated large arteries, the receptor mediating contraction belongs to the 5-HT_2 subtype (Van Nueten et al, 1981; Cohen et al, 1981; Cohen et al, 1983a). However, in other blood vessels, the receptors mediating contraction resemble the 5-HT_1-like subtype (Peroutka et al, 1983; Van Nueten et al, 1985; Houston, Vanhoutte, 1986). No attempt was made to identify the 5-HT receptor subtype involved in this study. Not all the blood vessels in the body have the same pattern of response to 5-HT and this probably reflects differences in the populations of 5-HT receptor subtypes. The secondary pressor component to 5-HT obtained in anaesthetised rats in this study could be related to the vasoconstriction of large blood vessels and was probably

mediated via 5-HT_2 and 5-HT_1-like receptors (Van Nueten et al, 1985; Feniuk and Humphrey, 1989). This 5-HT-induced vasoconstriction in the large blood vessels would increase the shear stress on the endothelial cells of the blood vessels and this could lead to the production and release of the endogenous vasodilator, NO and perhaps other relaxing factors, such as PGI_2, which is released in response to increased intraluminal pressure. In the microvasculature, 5-HT has more complex effects. It causes arteriolar vasodilatation and lowers the peripheral resistance. 5-HT also increases the permeability of the capillaries and hence increases the level of plasma leaving the blood vessels and accumulating in the interstitial spaces (ie. oedema). Vasoconstriction of the venules is another important response of the microvasculature to 5-HT (Houston, Vanhoutte, 1986).

The mechanisms underlying the predominant sustained depressor response to 5-HT seen in this study in anaesthetised rats are likely to be very complex. It has been reported that 5-HT can release vascular endothelial NO. This response is mediated by 5-HT_1-like receptors (Cohen et al, 1983a,b; Cocks, Angus, 1983; Martin et al, 1987; Houston, Vanhoutte, 1988). The biosynthesis of vascular endothelial NO is a Ca^{2+}-dependent process and is catalyzed by the enzyme, NO synthase (Moncada et al, 1991). This NO-releasing effect of 5-HT may have been partially responsible for the final, sustained depressor component response to 5-HT, since it was reduced by L-NAME (Fig-30). NO released from the vascular endothelium would activate guanylate cyclase in the vascular smooth muscle and increase the synthesis of cGMP, causing vasodilatation. NO may also be synthesised in vascular smooth muscle where it occurs as a result of a Ca^{2+}-independent process (Moncada et al, 1991) and has been suggested to be a major source of endogenous NO (Gonzalez et al, 1992). Therefore, the increase in the synthesis of both endothelial and non-endothelial NO,

resulting perhaps from the increase in the vascular tone, could contribute to the prolonged depressor effect of 5-HT. Vasodilator responses to 5-HT also occur in the canine and porcine coronary artery and are mediated by 5-HT_1-like receptors on endothelial cells and inhibitory 5-HT receptors on vascular smooth muscle (Cohen et al, 1984; Vanhoutte et al, 1986; Vanhoutte, 1987; Luscher, Vanhoutte, 1990). The sustained depressor component of the 5-HT-induced response could also be due to activation of some specific 5-HT_1-like receptors perhaps located in the nervous system. Some of these 5-HT_1-like receptors are located in the CNS, where 5-HT_1 binding sites were originally described in the rat brain (Peroutka & Snyder, 1979) on the basis of radioligand binding studies. These binding sites have been subdivided into subtypes, termed 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} (Pedigo et al, 1981; Pazos et al, 1984; Hoyer et al, 1985; Heuring & Peroutka, 1987). The functional receptors which correspond to these binding sites are well established in brain tissue (De vivo & Maayanic, 1986; Engel et al, 1986; Conn et al, 1986; Schoeffter et al, 1988). These 5-HT_1-like receptors are not only located in the CNS, but also occur in the peripheral nervous system including autonomic neurons, enteric neurons and also in blood vessels. 5-HT_1-like receptors have an inhibitory effect on the central and peripheral sympathetic neurons through inhibition of neuronal adenylate cyclase. Activation of these receptors reduces the level of the neuronal cAMP and reduces transmitter release. Thus, activation of 5-HT_1-like receptors decreases the sympathetic discharge and increases vagal nervous activity (Cohen et al, 1983a,b; Cocks and Angus, 1983; Martin et al, 1987; McGrath, 1977; Houston and Vanhoutte, 1988; Mylecharane and Phillips, 1989; Saxena and Villalon, 1990; Verbeuren, 1990). This complex interaction of 5-HT with different components of the vascular and neuronal systems, together with its action on cardiac $5HT_3$ receptors (Fozard, 1989), results in it having a multiphasic influence on

blood pressure in the intact organism (Vanhoutte, 1985; Vayssettes-Courchay et al, 1990).

In the anaesthetised rat, the maximal dose of 5-HT (55 μg/kg) injected after L-NAME produced an irregular pressor response (Fig-30). As the doses of 5-HT increased and in the absence of any endogenous NO, cardiac irregularities occurred and interfered with the outcome of the 5-HT-induced pressor response. These irregularities could perhaps result from reduced inactivation of 5-HT by the lungs as well as an excessive 5-HT-induced release of catecholamines from the adrenal medullae, from which catecholamines can be released by activation of 5-HT receptors (ie. 5-HT2 receptors) (Feniuk et al, 1981; Kimura, Satoh, 1983; Saxena and Villaton, 1990). Such a release of catecholamines from the adrenal medullae into the blood circulation could complicate and add to the pressor response to 5-HT in the vasculature of the vital organs such as heart, lungs and brain. The result would be an excessive vasoconstriction and perhaps interruption in the normal blood flow and hence cardiac and pulmonary irregularities.

Since 5-HT and its receptors are involved in several cardiovascular diseases, the development of new drugs that act on 5-HT receptors and its subtypes have potential therapeutic benefits in the treatment of some cardiovascular pathologies such as hypertension, peripheral vascular diseases, cardiac failure and migraine (Saxena and Villalon, 1990).

The results obtained in this study in pithed and anaesthetised rats are complex and agree only in part with previous studies. They conflict with observations which indicated that spinal nerve stimulation in the pithed rat produced a biphasic component, which consisted of a neuronal component that occurred during stimulation and an adrenal component that occurred

only after stimulation had ceased (MacLean et al, 1994). Adrenalectomy not only abolished the post-stimulation, sustained pressor response but also greatly reduced the pressor response that occurred during stimulation. If spinal nerve stimulation releases adrenal catecholamines and particularly adrenaline during nerve stimulation, then this will have profound effects not only on the performance of the heart but also on the pressor responses to NA and its hypothetical co-transmitter, ATP, whose constrictor effects would be opposed by the circulating adrenaline. This study has drawn attention to the fact that stimulation of the spinal outflow in the pithed rat causes release of catecholamines from the adrenal medulla and these contribute in a complex manner not only to the pressor response but probably also influence the heart and perhaps also affect, via presynaptic receptors, the release of the sympathetic transmitter(s) (Rand et al, 1975; Majewski and Rand, 1981). Unless the effects of neurally-released, circulating, adrenaline from the adrenal medulla are taken into account, analysis of the nature of neurotransmission in the vasculature is likely to be fraught with difficulties.

In this study the effects of adrenaline on ß-adrenoceptors was blocked with propranolol so that the complications arising from the effects of neurally-released adrenaline would be avoided. However, the use of propranolol led to some difficulties. For example, in some rats with very low blood pressure, propranolol did not increase the pressor responses to spinal nerve stimulation but instead reduced these responses. This may have been because in these rats the heart was already inadequate to maintain a high blood pressure and the blockade of the effects of catecholamines on the heart further impaired the capacity of the heart to pump blood into the constricted blood vessels.

The results also conflict with previous observations, which apparently indicated that the sympathetic neurotransmission in the blood vessels in the pithed rat involves co-transmission, in which both NA and ATP are released together to cause peripheral vasoconstriction. In this study, although α,ß-mATP produced pressor responses and these showed tachphylaxis with repeated administration, there was no evidence that the pressor responses to spinal nerve stimulation was similarly affected. In fact, pressor responses to spinal nerve stimulation did decline throughout experiments in which α,ß-mATP-induced tachyphylaxis occurred but not to a greater extent than did a standard response to NA, which was used as a control. Moreover, when administration of α,ß-mATP was discontinued to allow the sensitivity to α,ß-mATP to recover, the sensitivity to exogenous NA and spinal nerve stimulation did not recover. These results together with the observation that most of the pressor response to spinal nerve stimulation is produced by adrenal cathecholamines rather than being neuronally mediated, cast some doubt on the idea that the sympathetic neurotransmission in the vasculature of the rat involves co-transmission, in which NA and ATP are released together and are jointly responsible for the vasopressor response seen in this preparation during spinal nerve stimulation.

This study has also demonstrated that other factors influence the response of the pithed rat to spinal nerve stimulation and probably make it an inappropriate preparation for characterising vascular receptor mechanisms or for studying neurotransmission. In particular, it is clear that AII influences the response to spinal nerve stimulation and this study has provided indirect evidence for the presence of AII in the pithed rat. This is not a new observation but it is a reminder of the complexity of the preparation, in which responses to NA and to nerve stimulation can be enhanced by AII. However, even this apparently staightforward observation is capable of

different interpretations since captopril, which was used to inhibit the enzyme that synthesises AII, would also inhibit the destruction of bradykinin, which would be capable of releasing NO from the endothelium and thus acting as a physiological antagonist of NA. It is also possible that captopril inhibited pressor responses to NA and nerve stimulation because it inhibited both the synthesis of AII and the destruction of bradykinin.

The role of NO in the pithed rat is also complex. The results obtained in this study conflict with those of MacLean et al (1994), who reported that L-NAME enhanced pressor responses to spinal nerve stimulation. The results obtained in this study show no potentiation of the pressor responses to nerve stimulation or to NA. This may have been because propranolol was present throughout this study. Since MacLean et al (1994) did not block the ß-adrenoceptors with propranolol, it is likely that the catecholamines released from the adrenals in their experiments would be available to act on vascular ß-adrenoceptors and cause vasorelaxation but this would not be possible in the experiments described in this thesis. There arises the possibility that activation of vascular ß-adrenoceptors might lead ultimately to release of NO which might then be responsible for the vascular relaxation (Gray and Marshall, 1992). If this occurs then the lack of any potentiation of the pressor responses to spinal nerve stimulation might be explained by the fact that the effects of the circulating adrenaline were already blocked by propranolol and the subsequent administration of L-NAME could have no further effect, since the inhibitory mechanism had already been blocked at an earlier stage by propranolol.

Intravenous injection of 5-HT produced a triphasic response in the blood pressure in the anaesthetised rat which was more marked at higher doses of 5-HT. This consisted of an initial brief depressor response which was

followed successively by a short-lived vasopressor and then a sustained vasodepressor response. In contrast, in the pithed rat, 5-HT produced a pressor response but also inhibited pressor responses to spinal nerve stimulation. Unlike pressor responses to NA or spinal nerve stimulation, which were unaffected by L-NAME, pressor responses to 5-HT were potentiated and prolonged by L-NAME, suggesting that NO normally plays a part in regulating responses to and terminating the effects of intravenously-administered 5-HT. Whether the NO involved in opposing the vasoconstrictor effects of 5-HT is basally-released or is released in response to the pressor effect of 5-HT or is released directly by 5-HT remains to be determined. However, since L-NAME increased the resting blood pressure in the anaesthetised rat, it appears that there is a basal release of NO. Since the depressor components of the triphasic response to 5-HT were reduced but not abolished by L-NAME, it appears that these responses were not entirely mediated by NO. Another posibility is that the residual, 5-HT-induced depressor responses that persist in the presence of L-NAME in the anaesthetised rat may be due to the presynaptic inhibitory effects of 5-HT on the sympathetic nerves and to central stimulation of the parasympathetic nerves (Saxena and Villalon, 1990). Another possibility is that the endogenous NO, which opposes the pressor effects of exogenous 5-HT, might be released in a particular vascular bed where 5-HT is inactivated. In those circumstances, inhibition of the synthesis of NO would interfere with the destruction of 5-HT, whose pressor effects would therefore be prolonged, whilst any direct or indirect ability of 5-HT to release NO would be blocked.

PERFUSED LUNGS PREPARATION

This study examined the effects of drugs in the perfused pulmonary circulation, which is very unlike the systemic vasculature in a number of ways (Murray & Nadel, 1986). For example, it is a low pressure circuit, in which the resistance to flow does not increase even when the cardiac output increases. An important feature of the pulmonary circulation and one that may explain to some extent its ability to remain a low pressure system even when cardiac output increases, is the fact that there is considerable redundancy in the system, with the possibility that additional vessels may come into operation as required (Murray and Nadel, 1988). A very important difference between the systemic and the pulmonary circulation is the fact that in the latter, hypoxia causes vasoconstriction (Staub, 1985), whereas in the systemic circulation, hypoxia causes vasodilatation (Murray and Nadel, 1988). Surprisingly, The mechanism underlying this important phenomenon, which diverts blood from hypoxic regions of the lungs to normoxic areas, is still poorly understood. Other important features of the pulmonary circulation include its importance as a site where vasoactive substances are activated (angiotensin II) or inactivated (5-HT) (Furchgott, 1984; Said, 1982). Clearly, the pulmonary circulation is vital, not only in respiration but also in synthesising and removing important substances that have important and in some cases toxic effects elsewhere (Ganong, 1989c). In this respect, the pulmonary circulation has a protective effect on the heart and other organs.

This study examined the effects of 5-HT and other vasoconstrictors in the pulmonary circulation and sought to determine whether their effects were modified by endogenous NO. 5-HT is present in the lungs in neuroepithelial bodies, from which it may be released in response to neuronal and chemical

stimuli. It may also be released by hypoxia and may therefore be involved in the hypoxic vasoconstriction that diverts blood to better ventilated areas of the lungs (Lauweryns & van Lommel, 1982; Johnson, 1991). This study demonstrated that responses of the pulmonary circulation to pressor agents are influenced by the release of NO, which may therefore have a role in regulating blood flow in the pulmonary circulation and in modifying the effects of drugs in this vascular bed. In particular, NO may have a role in maintaining the low resistance of the pulmonary circulation to perfusion. A comparison was made between the responsiveness to vasoactive drugs of the pulmonary circulation and the vasculature of the hindquarters. It appears that NO and perhaps prostaglandins play a larger part in modifying responses of the pulmonary circulation to drugs than they do in the hindquarters. One reason for the differences between the responsiveness of the two vascular beds may be the existence of collateral vessels in the lungs (Maseri et al, 1972).

In order to investigate the effects of vasoactive drugs in the pulmonary vasculature, it was necessary to obtain control pressor responses to vasopressor agents. The normal pressor responses to 5-HT (0.1-100 μM) and to PE (0.1-100 μM), which act through PI-turnover were small when perfused cumulatively and were not well-maintained, especially with 5-HT (Fig-34 Panel A). The pulmonary vasculature was less sensitive to pressor agonists, such as PE (Fig-33) and 5-HT when they were perfused cumulatively than when these drugs were perfused in separate, single concentrations, with recovery intervals between each dose. These vasoconstrictors may cause desensitization which may be in part due to some adaptation in the coupling mechanism or to the production of NO or PGI_2, produced by the increased shear stress. This seems likely since secretion of EDRF (NO) occurs spontaneously and is accelerated by

increased shear stress on the vascular endothelial cells (Angus, Cocks, 1989; Griffith et al, 1988; Vanhoutte et al, 1986). The modulatory effect of NO on the pulmonary vascular tone become even more important when the pulmonary circulation is perfused with Krebs-buffer, since there are no erythrocytes present to inactivate NO (Gillespie & Sheng, 1988).

5-HT has different mechanisms of action since it acts on various receptor subtypes which mediate distinctive and opposing effects. 5-HT has a direct vasopressor effect and an indirect sympathomimetic effect (Hollenberg, 1988). NO may also be involved in the vasodilator activity of 5-HT, which may release NO from the vascular endothelial cells (Newby and Henderson, 1990 and Angus, 1990, 1989).

Increasing, cumulative concentrations of 5-HT (0.1-1000 μM) produced only small pressor responses in the pulmonary vasculature in Wistar rats. However, if only basally-released NO was responsible for suppressing pressor responses to 5-HT, then 5-HT-induced responses to low concentrations of 5-HT should have been potentiated by L-NAME to a greater extent than responses to higher concentrations, because a constant basal level of NO would have been a less effective physiological antagonist of high concentrations of 5-HT. Instead, L-NAME potentiated responses to high concentrations of 5-HT to greater extent, suggesting that 5-HT either directly or indirectly increased NO release. In contrast, pressor responses to phenylephrine in the pulmonary vasculature were not enhanced to a greater extent at high concentrations by L-NAME and so there was less evidence that phenylephrine released NO than there was that 5-HT did.

L-NAME has been shown to have some selectivity of action on different agonists that act on subtypes of α-adrenoceptors in different species. For

example, in rabbit pulmonary arteries L-NAME appears to increase the α_2-adrenoceptor mediated responses but in the rat pulmonary arteries, it increases responses to both α_1- and α_2-adrenoceptor agonists (MacLean et al, 1993; Shaw et al, 1992c). There is some evidence that at least part of the adrenaline-induced vasorelaxation in vivo in the rat is mediated by endogenous NO since L-NAME inhibited the vasodilator response to adrenaline (Gardiner et al, 1991a,b). In this study a submaximal (10 μM) and a maximal (100 μM) concentration of adrenaline added to the Krebs-buffer used to perfuse the lungs, produced small and poorly-maintained pressor responses (Fig-37). L-NAME enhanced the peak pressor response to adrenaline but did not affect the secondary depressor component that occurred during the pressor response. Whether this ability of L-NAME to increase the response to adrenaline indicates that adrenaline releases NO directly or indirectly via ß-adrenoceptor activation or merely because of its ability to cause vasoconstriction increases shear stress and therefore indirectly increases NO release, remains to be determined.

Some of the products of cyclo-oxygenase metabolism including prostaglandins, have vasoactive properties which affect pulmonary vascular resistance (Aiken, 1984; Wood et al, 1981). In this study, flurbiprofen which is a cyclo-oxygenase inhibitor, had no apparent effect on the basal perfusion pressure in the pulmonary circulation. Flurbiprofen only enhanced responses to KCl (30 mM) when it was perfused in a single perfusion to equilibrium in an increasing dose-response curve of KCl (3-80 mM) in the pulmonary vasculature of the rat (Fig-45). Flurbiprofen did not affect responses to PE (Fig-46) and had little effect on responses to 5-HT in the presence of L-NAME (Fig-47) or to NA (0.1-100 μM) (Fig-48). These results indicate that, although flurbiprofen had no effect on the PE-induced pressor responses, its slight ability to enhance pressor responses to 5-HT and KCl at

least at the middle concentration of their respective dose-response curves, indicates that vasodilator prostagandins (eg. PGI_2) may be produced, perhaps as a result of shear stress acting on the vascular endothelial cells. It was surprising that flurbiprofen had so little effect since, it is known that PGI_2 is produced and released in the pulmonary vascular bed and may be responsible for the low resistance of the pulmonary vasculature to perfusion (Pohl et al, 1986a,b; Bhagyalakshmi and Frango, 1989; Nakache, Gaub, 1988).

In pulmonary hypertension there is an augmentation in the pulmonary vascular reactivity and this can be a factor in chronic obstructive lung disease (Reeves & Voelkel, 1989). Alterations in the intimal layer is one of the first signs of the development of pulmonary vascular disease (Magee et al, 1988). In this study, prior exposure of the pulmonary vasculature to KCl (40 mM) enhanced pressor responses to 5-HT (10-100 μM) (Fig-38). This increased pulmonary vasculature reactivity could have been caused by the ability of KCl to produce powerful contractions of smooth muscle and to increase vascular permeability and thus cause oėdema, in which vascular permeability to intraluminal fluid increases and thus more fluid passes through the vascular bed into the pulmonary parenchyma. KCl might increase the size of the gaps in the vascular endothelium and damage the fragile single layer of vascular endothelial cells, hence interfering with the normal production and release of prostacyclin (PGI_2) that is produced in response to increased vascular tone. It is known that endothelial cells metabolise arachidonate to PGI_2 and PGE_2 (Eldor et al, 1983; Ragab-Thomas et al, 1987; Resink et al, 1987; Revtyak et al 1988; Whorton, et al, 1982) as well as to other products. Therefore, if KCl does damage the endothelium, there would be less PGI_2 released to antagonise, physiologically, the pressor responses to 5-HT.

In order to investigate the effects of drugs in the pulmonary vascular bed, it is necessary to have a well-maintained response. Pulmonary blood vessels characteristically responded less to vasopressor agents such as PE and 5-HT when these pressor agents were perfused cumulatively. To enlarge the pressor responses to PE, perfused cumulatively to equilibrium, a mixture of L-NAME (400 μM), propranolol (10 μM) and yohimbine (1 μM) was used to inhibit the production and release of endogenous NO, whose basal release may be responsible for the low resistance of the pulmonary vasculature and to suppress the pressor responses to vasoconstrictor agents (Archer et al, 1989). This mixture also blocked the ß- and α_2-adrenoceptors so that a pure α_1-adrenoceptor-mediated pressor response to PE was obtained (Fig-41). The results suggested that the PE-induced pressor responses were enhanced in the presence of L-NAME, propranolol and yohimbine but even in this situation the pressor responses to PE were much smaller than those obtained in the hindquarters vasculature in the absence of these antagonists.

Humoral substances such as catecholamines, 5-HT, histamine can affect the tone of the pulmonary vasculature. Some of these vasoactive agents produce complex effects since they act on different receptors which mediate opposing effects. In this study, NA produced two different kinds of pressor responses in the perfused pulmonary vasculature of Wistar rats (Fig-36). In some experiments the pressor responses to NA were well-maintained with a rapid recovery after washing the drug out of the circulation with the physiological Krebs-buffer solution. However, in other experiments, NA produced a complex response, which consisted of an initial small pressor response which was unstable and quickly declined, although not to the original baseline. This decline occurred during the period of the NA

perfusion. Interestingly, in these experiments, when the administration of NA was stopped and the NA had been washed out of the system, by perfusing with Krebs-buffer solution, the perfusion pressure rose again. The decline in the pressor response to NA was due to activation of ß-adrenoceptors, since propranolol restored the perfusion pressure almost to the original level. The increase in perfusion pressure that occurred after NA administration could have been due to α_1-adrenoceptor supersensitivity and/or $ß_2$-adrenoceptor subsensitivity, as these post-drug increases in perfusion pressure were observed mostly at higher concentrations of NA.

The vascular endothelium has been suggested by various groups to influence pressor responses to α-adrenoceptor agonists (Carrier & White, 1985; Lues & Schumann, 1984; Egleme et al, 1984) and the contribution of the endothelial cells may vary in different species and strains. In this study, a comparison of the pressor responses obtained to equilibrium concentrations of PE (0.01-100 μM) in the Krebs-buffer perfused pulmonary vasculature of Wistar and Sprague Dawley rats (SDR), showed that the pressor responses to PE (0.1-1 μM) in the middle range concentrations of the dose-response curve were larger in the Sprague Dawley rats than in the Wistar rats (Fig-39). This may indicate, a) larger amount of smooth muscle in the medial layer of the vasculature of Sprague Dawley rats so that when the α_1-adrenoceptors are activated more tone is generated, b) more α_1-adrenoceptors and perhaps higher sensitivity of α_1-adrenoceptors on the smooth muscle cells, c) the coupling mechanism between the α_1-adrenoceptor and the enzyme phospholipase C may be more efficient and hence more PI-turnover and production of IP_3 and DAG occurs, d) higher storage of Ca^{2+} ions in the endoplasmic reticulum and facilitated release of this ion in response to IP_3 activation and also facilitated influx of Ca^{2+} ions through the voltage sensitive Ca^{2+} ion channels in the plasma cell

membrane, e) higher sensitivity of the contractile mechanism to Ca^{2+} ion in the vascular smooth muscle cell, f) vascular endothelium may also have a modulatory effect by producing and releasing various vasoactive agents mainly NO, in response to an increase in the vascular tone and shear stress through α_1-adrenoceptor activation by PE. One or more of these mechanisms may be responsible for the difference in the response to a particular drug in different strains.

The well-known ability of hypoxia to cause vasoconstriction in the pulmonary vasculature (Staub, 1985) may involve an endothelium-dependent mechanism (Holden & McCall, 1984) and it is thought that vasodilator factors produced and released from vascular endothelium have a major role (Tesfamariam et al, 1987). Hypoxia was shown to have an inhibitory effect on the vascular endothelium-derived relaxing factor in bovine pulmonary artery endothelial cells and in rabbit pulmonary arteries (Warren et al, 1989; Johns et al, 1989). Hypoxia diverts blood from areas where the O_2 tension is low to the better ventilated areas, hence it aids in the regulation of ventilation-perfusion matching (Dawson, 1984). The pulmonary abnormalities that are related to hypoxia, include chronic bronchitis, chronic obstructive pulmonary disease, emphysema and cystic fibrosis, the hypoxia-induced vasoconstriction may add to the pulmonary hypertension (Rubin, 1984) and further aggravate the rise in the blood pressure.

Acute and chronic hypoxia have been shown to influence agonist-induced contractions. This has been attributed to different sites of action in blood vessels, including the endothelium and smooth muscle cells. In the vascular endothelium hypoxia modulates the release of vasoactive substances (Rakugi et al, 1990) and it may have a direct effect upon vascular smooth

muscle cells (Murray et al, 1990; Dempsey et al, 1991; Butler et al, 1991). The underlying molecular mechanisms may be through the hydrolysis of phosphatidylinositol (4,5) bisphosphate [Ptd Ins (4,5) P_2], the mobilisation of both extra- and intracellular Ca^{2+} ions or the activation of protein kinase C (Dempsey et al, 1991; MacLean & Nally, 1992; Jin et al, 1992).

In this study, in the perfused lungs, the pressor responses to NA were smaller when 14 % O_2 (hypoxia) was used than they were when the normal 95 % O_2 was used. This indicates the necessity of enough O_2 to generate sufficient energy (ATP) for vascular responses to vasoactive agents. Propranolol (10 μM) still enhanced the pressor responses to NA when 14 % O_2 was used (Figs-52,53) and suggested that the antagonism of ß-adrenoceptors and their suppressor effect on the NA-induced pressor response were still possible under a controlled level of hypoxia (14 % O_2).

Cyclic nucleotides including cAMP and cGMP are the main second messengers involved in vascular relaxation. Isoprenaline and various other ß-adrenoceptor agonists are generally considered to be endothelium-independent vasodilators, mediating their effects by activation of adenylate cyclase (Kukovetz et al, 1981) and increasing cAMP synthesis in the smooth muscle (Furchgott & Martin, 1985; Furchgott & Vanhoutte, 1989). In this study, increasing concentrations of isoprenaline (10^{-7}-10^{-4} M) perfused to obtain equilibrium responses and superimposed on the ongoing pressor responses to submaximal concentrations of 5-HT, PE and KCl, produced inhibitory effects that were dependent on the mechanism of action of the pressor agents involved (Figs-54,55,56). The inhibitory effect of isoprenaline on the pressor responses to 5-HT and PE, which act through PI-turnover, was powerful enough to cause complete inhibition of these vasoconstrictions. However, in the case of KCl, the inhibitory effect of

isoprenaline was only partial and reduced the peak pressor response to KCl by approximately 10 %. These results indicate that isoprenaline can oppose the rise in the $[Ca^{2+}]_i$ resulting from 5-HT- and PE-induced increases in PI-turnover in densely distributed resistance arteries and so there may be some blood vessels that are involved to a greater extent than others in responses to 5-HT and PE. Isoprenaline may also act on limited parts of the vasculature to reduce the vascular resistance in the AV-shunts (or AV anastomoses) through $ß_2$-adrenoceptor activation and increased synthesis of cAMP. On the other hand, KCl has a widespread depolarising effect and so produces large and well-maintained pressor responses, which are only partially inhibited by isoprenaline.

L-NAME (400 μM) did not affect the inhibitory responses to isoprenaline on the ongoing pressor response of 5-HT (50 μM) in the pulmonary vasculature (Fig-54). These results suggest that, the main inhibitory activity of isoprenaline in the pulmonary circulation does not involve endogenous NO but is exclusively due to activation of ß-adrenoceptors and increased synthesis of cAMP.

SNP was perfused cumulatively to obtain equilibrium inhibitory responses that were superimposed on the ongoing pressor responses to submaximal concentrations of PE, 5-HT and KCl. SNP produced inhibitory effects which were greater when the PI-mechanism was involved (PE,5-HT) than when KCl depolarization caused the contraction (Figs-58,57,59). The inhibitory effects of SNP on both PE and KCl were small and partial. However, SNP produced a more marked inhibitory effect in the presence of L-NAME. This was a surprising observation and suggests that normally, in the absence of L-NAME, NO is produced, so that when the SNP is added to the perfusing solution, the inhibition produced by the NO that is derived from SNP has an

effect that is additive with that of the endogenous NO. In effect, the endogenous NO is probably already exerting an inhibitory effect on the pressor response and so to the extent that this is happening, there is less opportunity for the exogenous NO, derived from SNP to have an inhibitory effect. The administration of L-NAME would inhibit the synthesis of endogenous NO but would not affect the release of NO from SNP and so with the loss of the inhibition derived from endogenous NO, there would be more opportunity for the exogenous NO, released from the SNP to produce an inhibitory effect. The inhibitory action of SNP would apparently be enhanced but this would only be because now the full range of the inhibitory dose-response curve for NO^- was available rather than only part of it, as was the case prior to the administration of L-NAME.

PERFUSED HINDQUARTERS PREPARATION

Blood vessels have the ability to maintain a residual vascular tone which is known as basal vascular tone. This occurs even in the absence of neurogenic, metabolic and hormonal influences. Two major physical forces including the fluid shear stress, resulting from the flow of blood, and the distending transmural pressure influence the vascular basal tone. The fluid-induced shear stress only acts on the vascular endothelial layer whereas an increase in the transmural pressure is balanced by viscoelastic forces of the vascular medial and adventitial layers. An increase in the shear stress may be the underlying reason for the flow-induced endothelium-dependent vasodilatation (Davies, 1989; Griffith et al, 1988) which is needed for adequate blood supply to the tissues.

Flow-induced endothelium-dependent vasodilatation was first recognised in conduit arteries (Smiesko et al, 1985; Pohl et al, 1986b; Melkumyants et al, 1987), and since then has also been observed in resistance arteries (Griffith et al, 1987; Koller & Kaley, 1990a). This endothelium-dependent dilatation is mainly mediated by EDRF (Hutcheson & Griffith, 1991), which is NO (Palmer et al, 1987) and additionally in some vascular regions by PGI_2 (Pohl et al, 1986a; Koller & Kaley, 1990b).

The hindquarters of the rat is a sensitive preparation for investigating the effects of vasoactive agents (Folkow et al, 1970; Ostman, 1975 and Cooper and Wylie, 1979). This preparation is easy to set up and takes only a short time to dissect so there will be less damage due to hypoxia and ischaemia, in the vascular tissue as well as in the other surrounding tissues that are involved. This preparation has all the blood vessels including arterioles, capillaries and venules and is part of the systemic vascular system. The physiological effect of 5-HT and endogenous NO which mediates vascular relaxation (Moncada et al, 1987) and modulates agonist-induced contractile responses of the vasculature (Miller et al, 1988) were also examined in this preparation.

The effects of perfusing increasing cumulative concentrations of 5-HT (0.1-100 uM), PE (0.1-100 uM) and KCl (3-300 mM) were examined and found to produce well-maintained dose-related pressor responses in all three strains of rats hindquarters (Wistar, SHR, WKY). The maximal pressor response to equilibrium-perfused, cumulative concentrations of KCl, was approximately the same as they were for 5-HT and PE in Wistar rats.

In hypertension, the resistance of the peripheral vasculature is increased. The underlying reason may be due to an augmentation of the arterial

contractility. The vascular cellular Ca^{2+} ion uptake and/or content may be increased in essential hypertension. In particular, an abnormality in the Ca^{2+} handling of arterial smooth muscle has been identified in spontaneously hypertensive rats (SHR) (Bohr & Webb, 1988; Postnov & Orlov, 1985: Lau & Eby, 1985). Since the vascular contractile-mechanism is activated by a rise in the free cellular Ca^{2+} ion content, a change in the cellular Ca^{2+} ion uptake and/or content may be a likely cause for some of the abnormal functions associated with hypertension, such as an increased incidence of myogenic tone, hyper-reactivity to vasoconstrictors and decreased relaxation to vasodilators.

Arteries from SHR exhibit a spontaneous active tone (Asano et al, 1986; Winquist & Bohr, 1983; Fitzpatrick & Szentivanyi, 1980; Noon et al, 1978). This spontaneous active tone is independent of regional innervation and circulating hormones and is known as myogenic. The myogenic tone is abolished by the removal of external Ca^{2+} ions (Winquist & Bohr, 1983; Fitzpatrick & Szentivanyi, 1980; Noon et al, 1978) and by Ca^{2+} ion channel blockers (Asano et al, 1986), indicating that the influx of Ca^{2+} ions to the vascular smooth muscle cell is required for myogenic tone.

In this study, there was no significant difference between the pressor responses to 5-HT, PE and KCl that were obtained in SHR and WKY control hindquarters vasculature (Fig-69). However, the peak pressor response produced by the maximal concentration of KCl was larger than either the peak pressor response to 5-HT or to PE in WKY control rats, whereas the peak pressor response to KCl was only larger than the peak response to PE in SHR hindquarters vasculature (Fig-71). Therefore, KCl was still capable of producing its potent vasoconstrictor activity in both SHR and WKY control

rats, although there are some functional and structural differences in the blood vessels of these two different strains of rats.

It is well established that 5-HT can cause endothelium-dependent relaxation in a variety of preparations, by acting on a receptor which resembles a 5-HT_1-like receptor (Angus & Cocks, 1989). Much evidence suggests that endothelium-dependent relaxation to 5-HT is not through a specific subtype of 5-HT receptors but occurs via a heterogenous receptor group. In porcine coronary artery (Schoeffter & Hoyer, 1989; 1990), the endothelial receptor shows characteristics, close to the 5-HT_{1D} receptors in both rabbit saphenous vein (Martin et al, 1991) and guinea pig frontal cortex (Middlemiss et al, 1988). In contrast, endothelial 5-HT receptors in both rabbit jugular vein (Leff et al, 1987) and porcine vena cava (Sumner, 1991) show similar characteristics but do not appear to relate to the subtypes of 5-HT receptor currently recognised.

In this study, increasing cumulative concentrations of 5-HT perfused to equilibrium and superimposed on a submaximal, PE-induced pressor response, showed additive pressor effects (Fig-63). The objective of this experiment was to find out if it was possible to obtain a vasodilatation to 5-HT when the hindquarters vascular tone was already raised with PE. However, there was no evidence that 5-HT could relax the PE-induced pressor response at any dose in the Wistar hindquarters vascular bed. This observation, indicates that vascular 5-HT receptors are predominantly excitatory, causing vasoconstriction in the hindquarters vasculature. The final vasodilator component of the 5-HT-induced response in anaesthetized rats could be partly mediated through inhibitory 5-HT receptors in the CNS and particularly in the peripheral nervous system.

It is known that in vascular endothelial cells, an increase in the $[Ca^{2+}]_i$ produced by agonists such as ATP, bradykinin and 5-HT, causes the production and release of NO and PGI_2 (Newby & Henderson, 1990). This mechanisms involves activation of specific receptors on the vascular endothelial cells, leading to stimulation of the phosphoinositol pathway. It is maintained by an influx of extracellular Ca^{2+} ions, presumably through cell surface non-selective cation channels (Hallam et al, 1989; Olesen et al, 1988).

In this study, 5-HT produced larger pressor responses in the hindquarters than in the pulmonary circulation. L-NAME produced a small, prolonged increase in the basal perfusion pressure in the Krebs-buffer perfused hindquarters, suggesting that a small basal release of endogenous NO occurs in this vascular bed. Neither L-NAME nor indomethacin had any effect on the responses to cumulative concentrations of 5-HT in Krebs-buffer perfused hindquarters vasculature. It is notable that the 5-HT-induced response after administration of L-NAME and indomethacin was apparently enhanced. However, when comparison was made with the corresponding time control, there was no significant difference in the responses, suggesting that endogenous NO and vasodilator prostaglandins were not produced in the hindquarters vasculature or had only a slight effect. This suggests that these endogenous vasodilators are less important in the hindquarters than they are in the lungs, where they may contribute to the low pressure system. This might explain the difference between the high resistance blood vessels in the hindquarters vascular bed and low resistance blood vessels in the pulmonary vascular bed. Unlike NO formation, PGI_2 biosynthesis depends on the release of Ca^{2+} ions from intracellular stores (Luckhoff et al, 1988; White & Martin, 1989). However, shear stress may activate a set of non-selective Ca^{2+} stores (Schwarz et al, 1992). As a

consequence, shear stress may elevate endothelial $[Ca^{2+}]_i$ and hence stimulate NO and PGI_2 biosynthesis.

Increasing concentrations of SNP were perfused to equilibrium and the effects of SNP were superimposed on responses to submaximal concentrations of the pressor agents, PE, 5-HT and KCl. SNP was effective in inhibiting these pressor responses and was particularly effective in opposing the pressor responses to PE and 5-HT, which act on receptors that operate the PI-mechanism. In contrast, SNP was less effective in inhibiting responses to KCl, which acts by depolarising vascular smooth muscle. The difference between the ease with which SNP inhibits PI-mediated constriction, produced by PE or 5-HT, and its poorer ability to inhibit KCl-induced constriction, may reflect the fact that the receptors for PE and 5-HT are not uniformly distributed throughout the vasculature but are located mainly in some blood vessels, such as resistance arteries, whereas KCl exerts its depolarizing action on all blood vessels.

The presence of functional vascular endothelial cells has been demonstrated to suppress the contractile actions mediated through α-adrenoceptor agonists in rat isolated aortic rings (Allan et al, 1983; Egleme et al, 1984; Lues & Schumann, 1984; Carrier & White, 1985); an action attributed to the production and release of endogenous NO and its subsequent opposing effect on the pressor responses evoked by α-adrenoceptor agonists. The role of vascular endothelium in influencing the binding of subtype-specific adrenoceptor radioligands to vascular α_1- and α_2-adrenoceptor sites was described by Carman-Krzan (1985). It was reported that the density of the α_1-adrenoceptors would be increased by removal of the vascular endothelial cells, with little effect on the affinity of the agonist for the receptors.

Carman-Krzan (1985) suggested that the loss of vascular endothelium might have uncovered previously undetectable receptor sites.

This study compared the vasodilator potency of SNP (exogenous NO) on PE-induced increases in the systemic vascular tone in three different strains of rats (ie. Wistar, SHR and WKY). EC_{70} concentrations of PE were perfused in all three of them and SNP was perfused in increasing doses and its effect was superimposed on a PE-induced pressor response. An EC_{70} concentration of PE (10 μM) induced a bigger pressor response in Wistar hindquarters (ie. approximately about 2.5 times bigger) than in SHR and WKY hindquarters. The sizes of PE-induced pressor responses were almost the same in the latter two strains.

In general, the hindquarters vasculature, which is part of the systemic vasculature, has been found to be very useful for studying the effect of vasoactive agents (Folkow et al, 1970; Ostman, 1975 and Cooper and Wylie, 1979) including vasoconstrictors such as 5-HT, PE and KCl and vasodilators such as carbachol and SNP. The responses obtained to these vasoactive agents were much larger than those obtained in the pulmonary vasculature. Some of the reasons why this preparation responds well to the vasopressor substances include: there may be larger amounts of structural smooth muscle in the vasculature, lower production and release of endogenous vasodilators such as NO and PGI_2, fewer collateral blood vessels in the hindquarters and less inactivation of vasopressor agents, including 5-HT, in the hindquarters vasculature.

5-HT may release vasodilator substances such as NO and PGI_2 through an increase in the $[Ca^{2+}]_i$ in the vascular endothelial cells (Newby & Henderson, 1990). In this study, L-NAME apparently potentiated the pressor

responses to 5-HT, but when comparisons was made with appropriate time controls, which had not received L-NAME, there was no enhancement of the pressor responses to 5-HT. Indomethacin, which inhibits cyclooxygenase had no effect on the pressor responses to 5-HT. SNP which is a source of exogenous NO, produced large inhibitions when it was added to the Krebs-buffer, containing submaximal concentrations of either 5-HT, PE or KCl. This may indicate that in the hindquarters vasculature, the production and release of endogenous NO is insufficient to inhibit pressor responses to vasoconstrictor agents. There was also no difference between the inhibitory effects produced by increasing concentrations of SNP when it was perfused on the ongoing pressor response to a submaximal concentration of PE (SHR and WKY rats).

Carbachol inhibited NA-induced vasoconstriction, via a mechanism which is dependent on the release of endogenous NO, but independent of the release of PGs in the mesenteric arterial bed of the rat (Randall & Hiley, 1988). The results obtained with carbachol in this study were complex. When carbachol was added to the Krebs-buffer, which contained a submaximal concentration of PE and was used to perfuse the vasculature of the hindquarters of SHR and WKY rats, it produced an initial pressor response, followed by a depressor response. These results suggest that different cholinoceptors may be activated during administration of increasing concentrations of carbachol. However, it appears that the effects of carbachol are not the same in hindquarters from SHR and from WKY rats.

The results obtained in this study indicate that endogenous vasodilators, such as NO and prostacyclin are produced and released in the hindquarters vasculature but to a lesser extent than in the lungs, in which the abundance

of NO in particular may be important in the maintaining the low resistance to perfusion that is characteristic of this vascular bed.

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