THE EFFECTS OF ADENOSINE AND THE ADENOSINE ANALOGUES ON THE CEREBROVASCULATURE

by

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CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

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SUMMARY

1. Aims of study

The initial aims of this study were to monitor the effects of adenosine, ATP and a variety of adenosine analogues on local cerebral blood flow (LCBF) and local cerebral glucose utilisation (LCGU) using the appropriate quantitative autoradiographic techniques. It was anticipated that these initial studies would show that adenosine and ATP increased LCBF in the rat, as had already been found in this laboratory in studies involving baboons (Forrester et al., 1979), and that following these experiments further studies would be carried out to determine whether there was any coupling of the vascular and metabolic effects, whether any particular areas of groups of areas were affected, and whether or not the effects could be antagonised by specific adenosine receptor antagonists.

When the initial LCBF experiments revealed that two known vasodilators (the adenosine analogues N^6-cyclohexyladenosine (CHA) and 2-chloroadenosine (2-CADO)) produced decreases rather than increases in LCBF the aims of the thesis then changed to trying to identify what receptors were involved in mediating these decreases and whether the decreases in LCBF could be antagonised using classical adenosine receptor antagonists.

2. Methods

A wide variety of techniques were used to monitor the effects of adenosine and the analogues of adenosine. Cerebral vessels were studied both in vitro (using isolated procine basilar arteries) and in vivo (using cat pial vessels). Cerebral blood flow in the rat was measured using both laser-Doppler Flowmetry and [^{14}C]-iodoantipyrine autoraiography. Local cerebral glucose utilisation/..

utilisation in the rat was measured using [^{14}C]-2-deoxyglucose autoradiography. In the local cerebral blood flow studies the agonists were administered via the right internal carotid artery at 50µl/min. for either 2 or 15 minutes. In some LCBF experiments an antagonist (8-phenyltheophylline) was given intraperitoneally 20 minutes before the effects of the agonists were assessed. In the local cerebral glucose utilisation experiments the agonists were administered via the right internal carotid artery at 33µl/min. for 15 minutes.

3. Results

3.1./ Studies involving cerebral vessels both in vitro and in vivo (isolated porcine basilar arteries and cat pial vessels, respectively) confirmed the vasodilatory properties of adenosine and the adenosine analogues (Edvinsson & Fredholm,1983). The order of potency in the in vitro study (i.e. NECA > 2-CADO, adenosine > L-PIA > CHA) showed that the vasodilation was mediated via an A_2 adenosine receptor interaction. In vitro and in vivo studies involving the adenosine analogues and the adenosine receptor antagonist 8-phenyltheophylline, however, suggest that the mechanism mediating the vasodilation is not as straightforward as a simple A_2 receptor interaction.

3.2./ The in vivo and in vitro studies mentioned above show the vasodilatory properties of adenosine and the adenosine analogues, therefore, one would expect them to increase local cerebral blood flow. The effect of adenosine and the adenosine analogues on local cerebral blood flow was found to be dependent not only on the analogue used but also on the length of time that the analogue was infused via the right internal carotid artery. 15 minute infusions of/..

of adenosine, ATP and NECA all showed a tendency to increase local cerebral blood flow, whereas 15 minute infusions of the analogues CHA and 2-CADO produced significant decreases in local cerebral blood flow. A 2 minute infusion of adenosine produced significant increases in local cerebral blood flow and a similar 2 minute infusion of CHA showed a tendency to increase local cerebral blood flow.

3.3./ In animals which had received an intraperitoneal injection of the adenosine receptor antagonist 8-phenyltheophylline (30mg/kg) a 15 minute infusion of the analogue CHA was again found to decrease local cerebral blood flow. It seems likely, therefore, since 8-phenyltheophylline is an A_1 and A_2 receptor antagonist, that the decreases in flow produced by CHA are not mediated via an A_1 or A_2 receptor interaction. The recently identified third (A_3) adenosine receptor is also known to be xanthine-sensitive, therefore it is also unlikely that the decreases in flow produced by CHA are mediated via an action on this receptor. Intraperitoneal injections of 8-phenyltheophylline (30mg/kg) were able to antagonise the increases in local cerebral blood flow produced by a 2 minute infusion of CHA. The A_2 receptor is known to mediate vasodilation (and will, in turn, increase flow) and 8-phenyltheophylline is an A_1/A_2 receptor antagonist, it seems likely, therefore, that the increases in flow produced by a 2 minute infusion of CHA are mediated via an A_2 receptor interaction.

3.4./ The possibility of experimental artefact being responsible for the unexpected decreases in local cerebral blood flow being produced by 15 minute infusions of CHA and 2-CADO is unlikely for the following reasons. Firstly, it is unlikely that the artefact should be evident only in the experiments involving these/..

these two compounds and not in any of the other experimental groups. Secondly, studies were carried out involving FAM fixation and pathological examination and there was no evidence of thrombic or embolic damage to the cerebral tissue. Thirdly, the vascular effects of the compounds are not occurring as a secondary response to metabolic changes produced by the compounds, since studies carried out to measure the local cerebral glucose utilisation using [^{14}C]-2-deoxyglucose autoradiography showed that adenosine and the analogues had no effect on local cerebral glucose utilisation.

4. Conclusions

The vessel studies carried out in this thesis confirm the vasodilatory actions of adenosine and the adenosine analogues, with isolated vessel studies indicating that the dilations are mediated, at least in part, by the A_2 adenosine receptor. The in vivo autoradiographic experiments, however, revealed that there was only a barely evident vasodilation with 15 minute infusions adenosine and ATP, whereas substantial significant dilations occurred with the 2 minute infusion of adenosine. In contrast 15 minute infusions of CHA and 2-CADO produced significant decreases in LCBF. Antagonism studies carried out involving the non-specific adenonsine receptor antagonist theophylline showed that although the increases in LCBF produced by 2 minute infusions of CHA could be blocked by theophylline, the decreases in LCBF produced by 15 minute infusions of CHA could not. It seems likely, therefore, that the increase in LCBF produced by a 2 minute infusion of CHA are mediated via an A_2 receptor interaction, whereas the decreases/..

decreases produced by a 15 minute CHA infusion are not mediated via an interaction with a known adenosine receptor. The exact identity of the receptor involved in mediating the decreases in LCBF produced by a 15 minute infusion of CHA, however, was not able to be identified using the experiments detailed in this thesis. In conclusion, therefore, the in vitro experiments detailed in this thesis confirmed the vasodilatory actions of adenosine and its analogues, however, when these compounds are administered in vitro their effects are dependent not only on the particular compound but also on the length of the administration of that compound.

Declaration

The data was obtained from my own original experiments. This material has not been presented in a thesis in any form.

SECTION I

INTRODUCTION

Interest in the field of adenosine research has grown considerably over the past 50 or more years, ever since Drury and Szent-Gyorgi (1929) first showed the pronounced cardiovascular effects of adenylic acid. They showed that the simple extracts of tissues produced both bradycardia and hypotension and that the primary active agent of those extracts was adenylic acid. They also demonstrated, however, that adenosine had even more potent actions than the adenylic acid itself. Adenosine is a purine nucleoside and the structure of it and the other related compounds used in this study are shown in Figures I.1. and I.2.

1. PHYSIOLOGY AND PHARMACOLOGY OF ADENOSINE

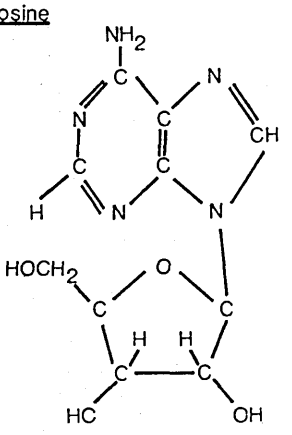
1.1. Pharmacology of purinergic receptors

One of the major advances in our understanding of the actions of adenosine and its analogues has been the identification and characterisation of specific receptors. The concept of multiple receptors for adenosine was developed when it was observed that adenosine had different effects on adenylate cyclase activity in different tissues. It was known from very early on that adenosine was acting at extracellularly located receptors or binding sites, even before the development of specific techniques to look at binding sites using radioactive ligands. One technique which led to the development of this hypothesis showed that adenosine analogues, bound covalently to molecules that were unable to pass through cell membranes, were still as active as adenosine itself (Daly, 1977).

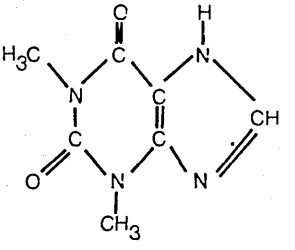
Burnstock first proposed the idea of two groups of receptors in 1978. The two purinoceptors proposed were the P_1 receptor, linked to/..

Figl.1 STRUCTURES OF ADENOSINE AND THE RELATED COMPOUNDS USED IN THE STUDY

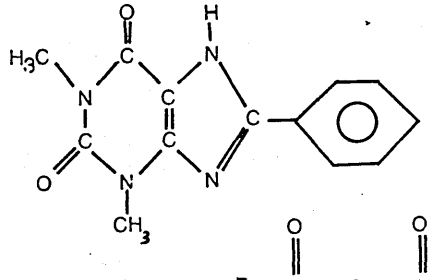
Adenosine



Theophylline



8-Phenyltheophylline



Adenosine Triphosphate (ATP)

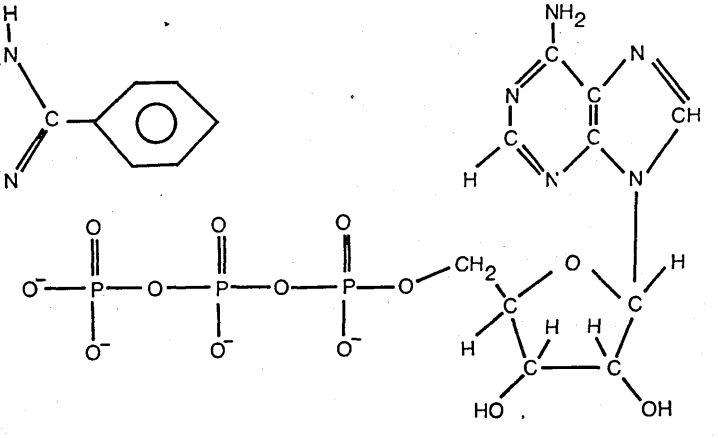
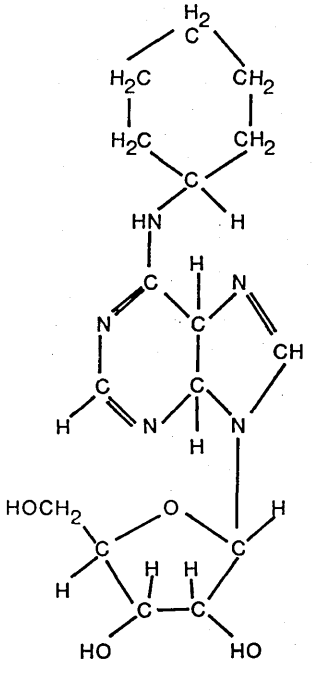
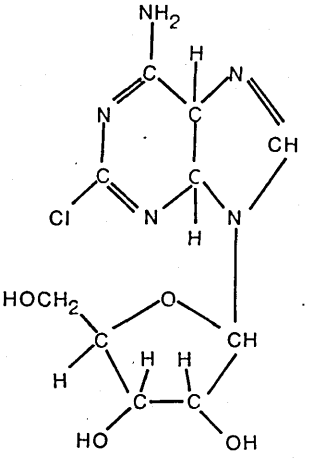


Fig. I.2 STRUCTURES OF ADENOSINE RELATED COMPOUNDS USED IN THE STUDY

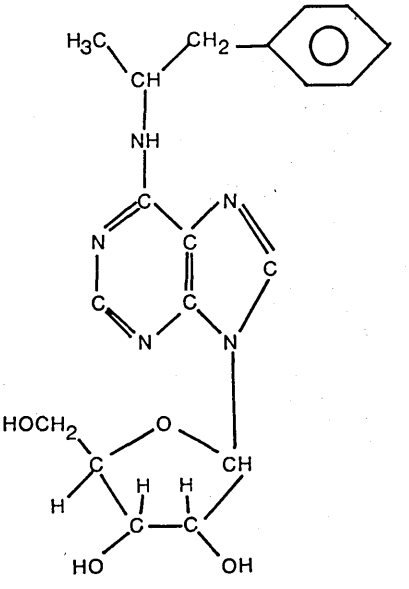
N^6-Cyclohexyladenosine(CHA)



2-Chloroadenosine(2-CADO)



N^6-R(-)-Phenylisopropyladenosine(R-PIA)(L-PIA)



5^1-N-Ethylcarboxamidoadenosine(NECA)

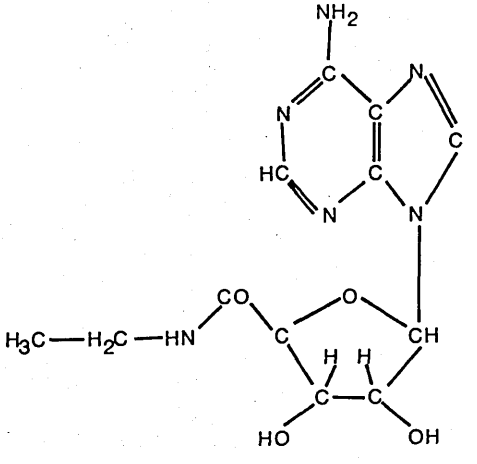
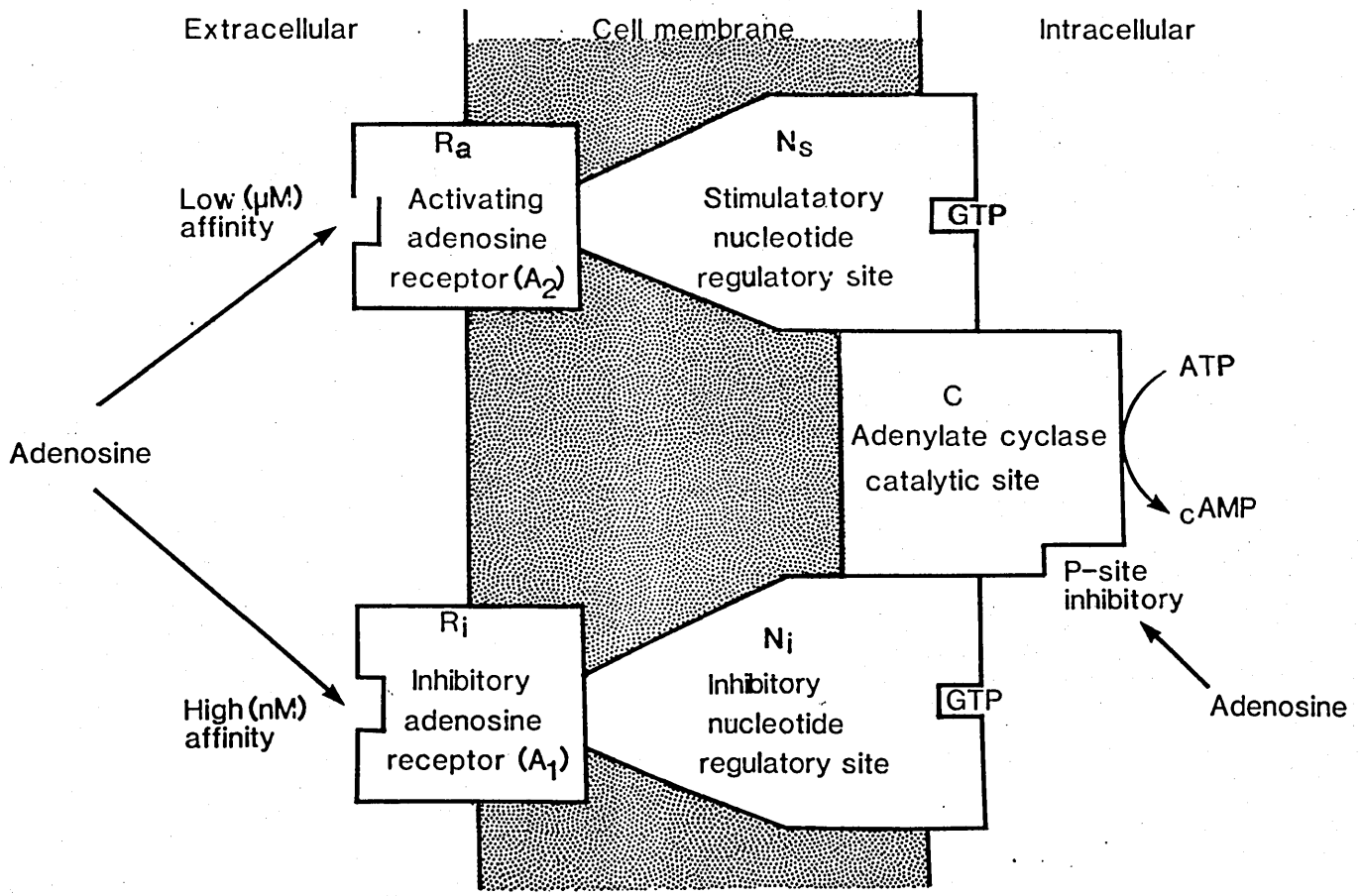


Fig. I.3 Classification of Adenosine Receptor Sub-types



to the adenylate cyclase system, with an agonist potency order of adenosine $\geq$ AMP > ADP $\geq$ ATP, and the P_2 receptor, not linked to adenylate cyclase, with an agonist potency order of ATP $\geq$ ADP > AMP $\geq$ adenosine.

Studies by Burnstock and Kennedy (1985) using structural analogues of ATP have shown that there are, in fact, two sub-types of the P_2-purinoceptor: P_{2x}, with a rank order of potency of α,β-methyleneATP, β,γ-methyleneATP > ATP = 2-methylthioATP; and P_{2y}, with a rank order of potency of 2-methylthioATP >> ATP > α,β-methyleneATP, β,γ-methyleneATP. The P_{2x} receptor mediates contraction of the guinea pig vas deferens, the urinary bladder and the rabbit portal vein, whereas the P_{2y} receptor mediates relaxation of the guinea pig taenia coli and the rabbit portal vein as well as the endothelium dependent vasodilation of the guinea pig aorta and rat femoral artery.

The P_1 (adenosine) receptors can be subdivided into two classes (Figure I.3.): the A_1 (or R_i) receptor, a high affinity receptor which is coupled to adenylate cyclase in an inhibitory manner (van Calker, Muller & Hamprecht, 1979 ; Londos, Cooper & Wolff, 1980); and the A_2 (or R_a) receptor, a low affinity receptor which is coupled to adenylate cyclase in a stimulatory manner (Bruns, 1980). Both these adenosine receptors are linked to separate GTP-regulated N-proteins, the N_i unit for the A_1 receptor and the N_s unit for the A2 receptor. It is these proteins that promote the activation or inhibition of the catalytic unit of the adenylate cyclase. As can be seen in Figure I.3. there is also a third type of adenosine binding site, the purine (P) site. The P-site is intracellularly located and it mediates the inhibitory actions of high concentrations of adenosine on the catalytic site. It was named the P-site because/...

because it was found that adenosine analogues which had an intact purine moiety (e.g. 2',5'-dideoxyadenosine) were particularly potent as direct inhibitors of the adenylate cyclase catalytic site. The actual physiological significance of the P-site is, as yet, unclear.

The A_1 and A_2 receptors show a different affinity for adenosine and its structural analogues, as has been shown by Londos et al. (1978) and Trost and Stock (1977) for the A_1 sub-type, and Bruns (1980) for the A_2 sub-type. The rank orders of potency for the various adenosine analogues are as follows:

A_1 receptor

CHA,L-PIA > 2-CADO > Adenosine > NECA

A_2 receptor

NECA > 2-CADO,Adenosine > L-PIA > CHA

Both classes of the adenosine receptor have similar affinities for methylxanthine compounds such as theophylline and caffeine, and these compounds are competitive antagonists at both sites. Conversely, the P-site is not inhibited by methylxanthines, but rather by 5'-methyl-adenosine.

Although it has been thought that the adenosine receptor classification is a simple A_1/A_2 classification, recent evidence has suggested that it might not be this straightforward. Work summarised by Williams (1984) suggests that there may be multiple A_1 receptor sites, but at present these remain poorly understood. Londos et al. (1980) suggested that there may be a further sub-division in the A_2 classification when they showed that the A_2 receptors in the liver had a higher affinity for NECA than those in the adrenal gland or Leydig cells.

The idea of a third (A_3) adenosine receptor in the brain, not linked to adenylate cyclase but associated with calcium channels has been put forward by both Chin & DeLorenzo (1985) and Ribeiro & Sebastiao (1986). The physiological responses of nerve endings where this A_3 receptor appears to be present are highly calcium dependent, suggesting that the A_3 adenosine receptor is linked to calcium. Although these investigations have been unable to show how adenosine affects the calcium needed for transmitter release they suggest two processes that may be involved, either together or independently. These two processes are that adenosine acts as a calcium channel antagonist to decreases calcium entry, or that there is a reduction in the efficacy of calcium for promoting the physiological response. Two speculative hypotheses have been put forward to explain these findings: the A_3 receptor is a voltage-dependent calcium channel which changes its conformation after binding adenosine; or activation of the A_3 receptor induces a conformational change in the membrane which results in an alteration of the calcium receptor sites (Ribeiro & Sebastiao,1986).

It can be seen, therefore, that the adenosine receptor picture is not as clear as a simple A_1/A_2 receptor classification, and further work is required, particularly with regard to identifying specific A_1, A_2 (and A_3?) receptor antagonists to help clarify the overall picture.

1.2. Synthesis and degradation of adenosine

There is sufficient evidence to suggest that neuronal activity results in adenosine release from neurones in the central nervous system. Studies have shown that electrical stimulation of cerebral slices results in a large outflow of purines (Pull & McIlwain, 1972). This release of adenosine is calcium dependent. Only a small amount of the released compounds have been found to be adenine nucleotides, with the bulk of the material (50%) being adenosine itself (Kuroda & McIlwain, 1974).

ATP is often co-released with a number of other neurotransmitters and thereafter it can be hydrolysed by an ecto-nucleotidase enzyme, found on the outer surface of the neurones, to adenosine. However, it has been suggested that this hydrolysis of the extracellular ATP in the synaptic cleft is not the major source of extracellular adenosine, since stimulation-evoked release of adenine nucleotides in the presence of an inhibitor of the ecto-nucleotidase (e.g. α,β-methylene ADP) has no effect on the proportion of adenosine release (Pull & McIlwain, 1977).

The adenosine which is released from the neurone into the synaptic cleft is obtained from the metabolic degradation of ATP (see Figs. I. 4, 5, 6). It is thought that the release of the adenosine is calcium-dependent (Kuroda & McIlwain, 1974), but by a mechanism different from that observed with other neurotransmitters. (Stone, 1981).

It can be seen, therefore, that the levels of synaptic cleft adenosine are determined by both the release of adenosine from nerve endings and by hydrolysis of released ATP by ectoenzymes to form adenosine (1, 2 and 3 in Fig.I.7). Once in the synaptic cleft the adenosine can be either taken back up into the presynaptic

Fig. I.4 SYNTHESIS AND DEGRADATION OF ADENOSINE

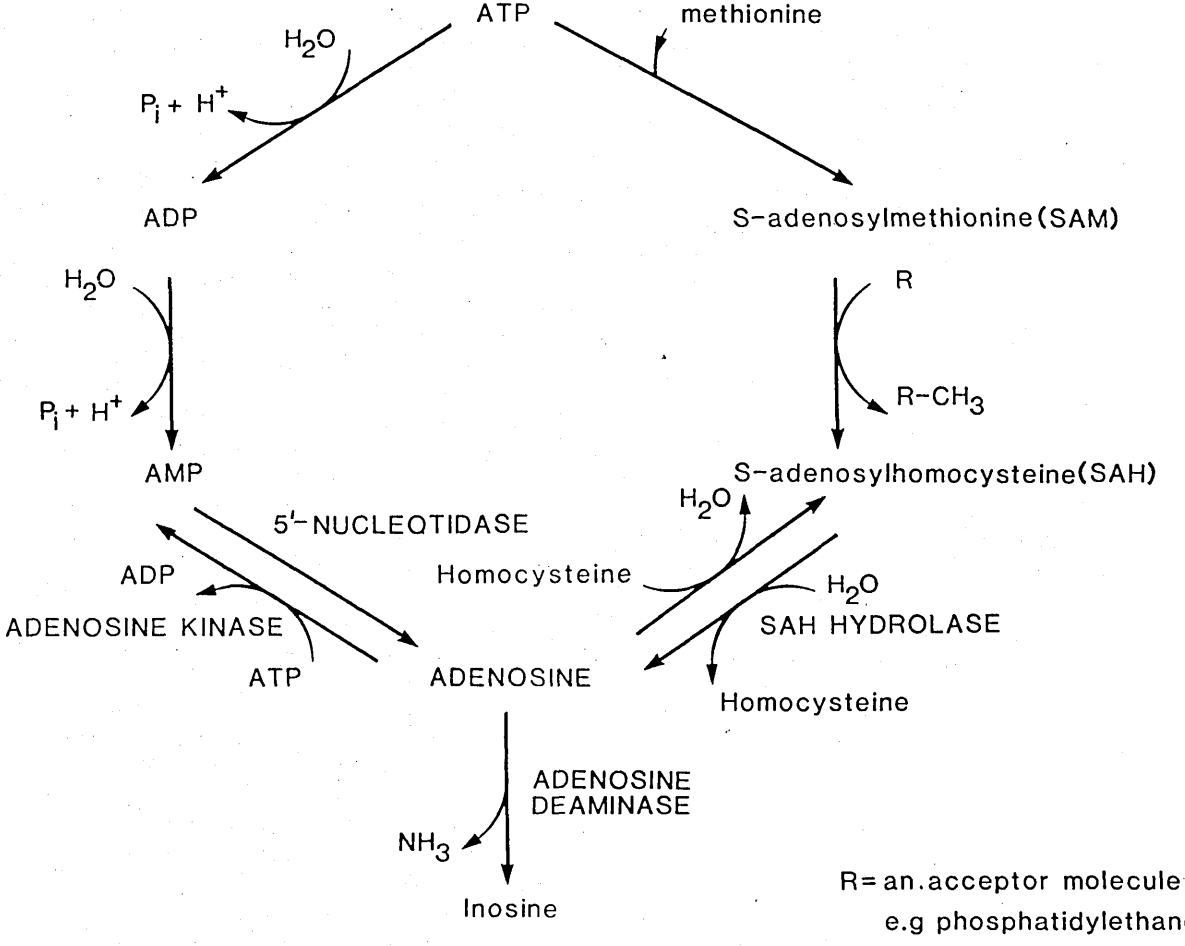
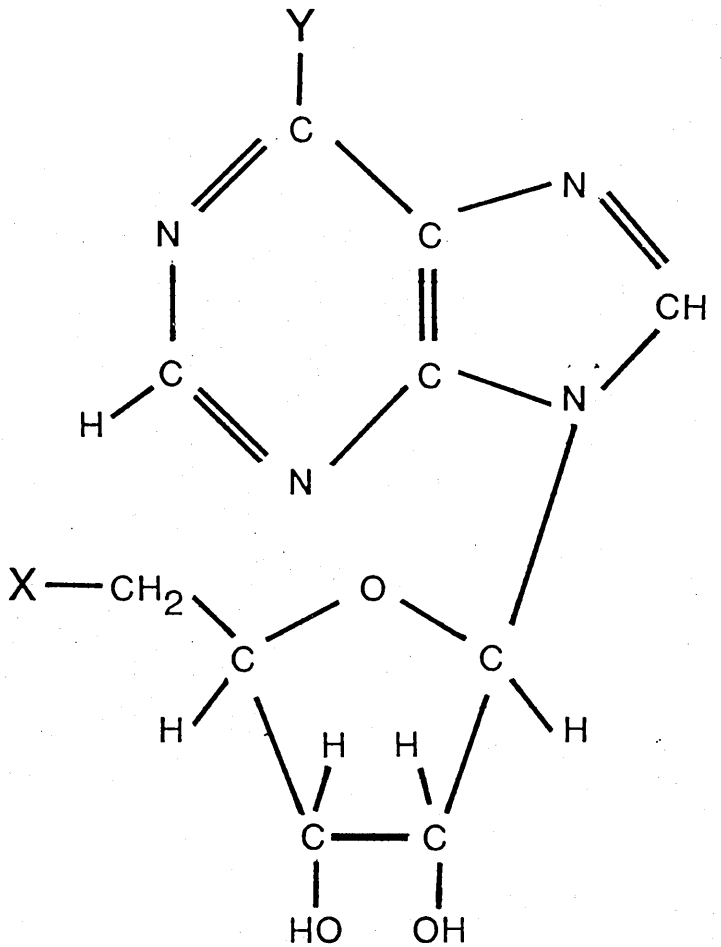
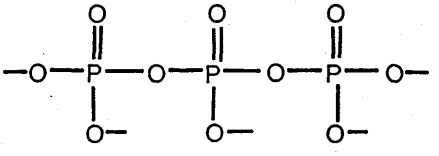
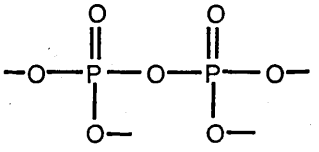
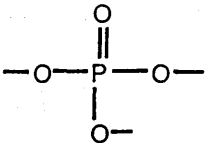
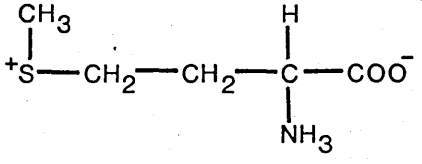
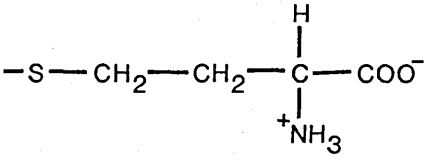


Fig. I .5 STRUCTURE OF THE PARENT MOLECULE IN SYNTHETIC PATHWAY



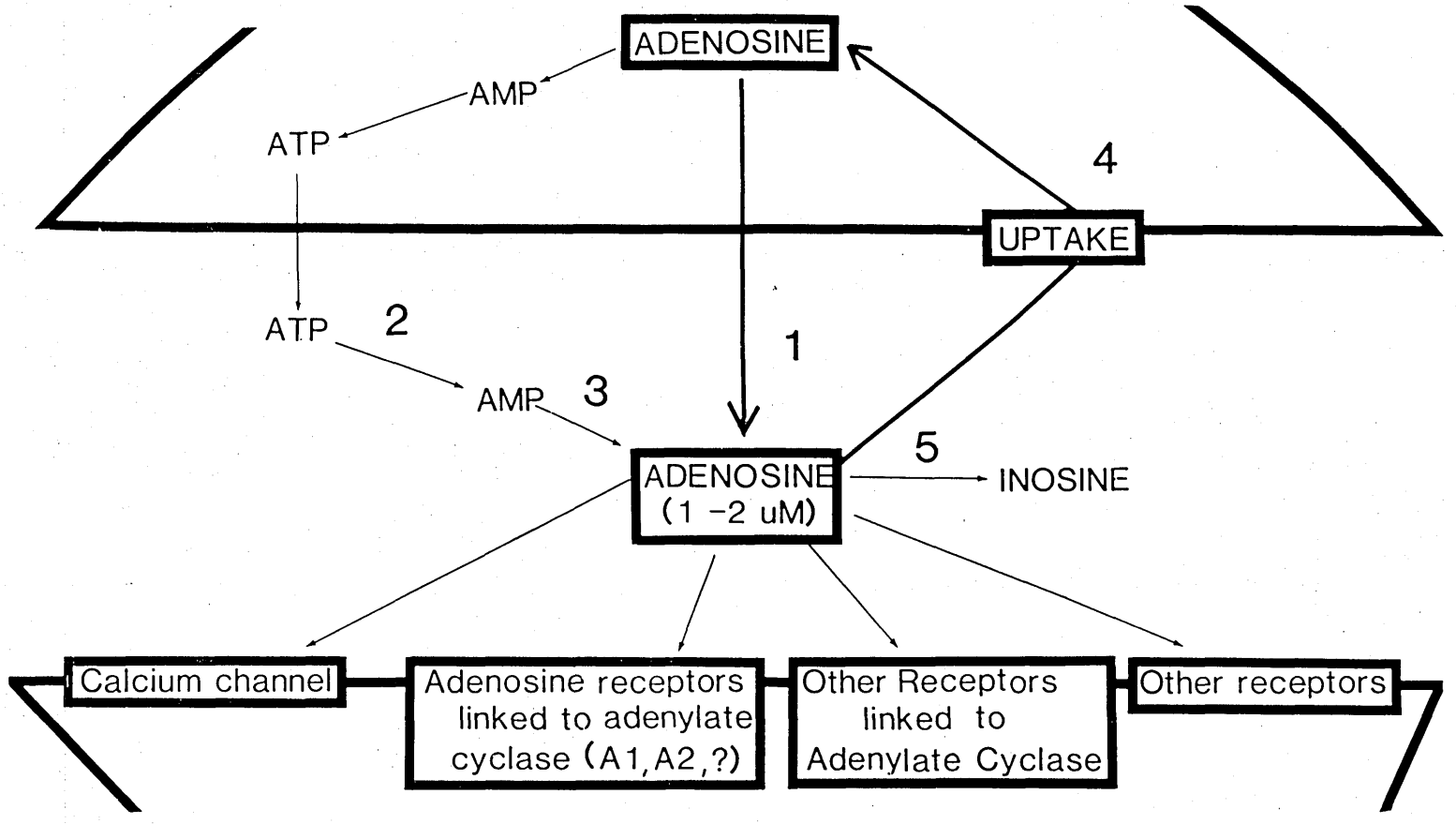
'Parent' molecule

Fig. I.6 INDEX OF STRUCTURES USED IN SYNTHETIC PATHWAY

Compound	X	Y
ADENOSINE	-OH	$-NH_2$
ATP		$-NH_2$
ADP		$-NH_2$
AMP		$-NH_2$
SAM		$-NH_2$
SAH		$-NH_2$
Inosine	-OH	=O

nerve ending via a specific nucleoside transport system (4 in Fig.I.7) or deaminated by adenosine deaminase to form inosine (5 in Fig.I.7). The sites of action of the remaining adenosine are adenosine receptors linked to adenylate cyclase (A_1,A_2), other receptors linked to adenylate cyclase (e.g. P-site), calcium channels and other receptors. The adenosine metabolite, inosine, may also act on other receptor systems. Although Fig. I.7 shows these sites of action as being post-synaptic they may also be located pre-synaptically.

Fig. I.7 **METABOLISM AND ACTION OF ADENOSINE AT THE SYNAPTIC JUNCTION**



1.3. Physiological actions of adenosine

Adenosine has effects on a wide variety of physiological systems. Adenosine has been shown to be a potent vasodilator in a number of vascular beds and it is thought that this may mediate postischaemic hyperaemia of coronary blood vessels (Berne,1980), since after it was conclusively demonstrated that an inadequate oxygen supply led to the production and release of adenosine by the heart, it was then shown that an increased metabolic activity in the heart produced an enhanced production and release of adenosine. These studies were carried out in a variety of preparations including cultured myocytes and unanaesthetised dogs and the results obtained suggested that the release of the adenosine was coupled to the balance of the myocardial oxygen supply and need, and that the alteration in the coronary circulation necessary to keep this balance was mediated in some way by adenosine (Berne,1985). Adenosine is also a potent, fast-acting bronchoconstrictor (Cushley, Tattersfield & Holgate, 1983), and it is thought that blockade of adenosine receptors by methylxanthines may explain their anti-asthmatic effects.

Adenosine inhibits platelet aggregation (Born,1964) and it is thought that the build up of adenosine after cardiac ischaemia could therefore influence blood clotting. The effect of the inhibition of platelet aggregation can be associated with an increase in cyclic AMP and is mediated via A_2 adenosine receptors (Haslam &Cusack,1981). Adenosine has been shown to inhibit noradrenaline-stimulated cyclic AMP accumulation in isolated rat adipocytes (Fain,1973). Lipolysis is partially inhibited by adenosine, only if submaximal concentrations of noradrenaline are used (Schwabe, Ebert & Erbler,1973).

The best characterised physiological action of adenosine is that of inhibition of transmitter release. The inhibition is actually a/..

a heterologous regulation of release, unlike the α_2-autoinhibition of noradrenaline or the D_2-inhibition of dopamine release, in which the neurotransmitter inhibits its own release via a negative feedback mechanism.

Adenosine, in fact, has been shown to inhibit neurotransmitter release and therefore synaptic transmission at a variety of synapses where adenosine is not the transmitter. Although the best examples of adenosine inhibition of neurotransmitter release are to be found in the peripheral nervous system, it has also been shown in the central nervous system, where evidence suggests that the release or turnover rate of many neurotransmitters is reduced by adenosine. Included in this list of neurotransmitters are acetylcholine (Pedata et al,1983), serotonin (Fredholm, Jonzon & Lindgren,1983), dopamine (Harms, Wardeh & Mulder,1979), γ-aminobutyric acid Hollins & Stone,1980) and glutamate (Dolphin & Archer,1983).

In the central nervous system adenosine has inhibitory actions on neuronal firing (Phillis & Wu,1981). Although some direct postsynaptic actions can be seen, the major effects reflect a presynaptic blockade of excitatory transmitter release. These effects of adenosine are once again blocked by xanthine compounds.

Administration of adenosine and some of its analogues illicit a wide variety of behavioural effects including sedation, analgesia (Crawley, Patel & Marangos, 1981), hypnosis (Marley & Nestico,1972) and an anticonvulsant action (Maitre et al.,1974). Since adenosine has such marked effects on blood pressure it is difficult to establish whether the effects of peripherally administered adenosine are mediated by the central nervous system or as a consequence of its action on the cardiovascular system. Recent work by Patel, Marangos & Boulenger (1984), however, suggests that at least the sedative effects of adenosine are centrally mediated. They found/..

found that when they administered the adenosine receptor antagonist 8-p-sulfophenyltheophylline (which does not cross the blood-brain barrier) intravenously along with N^6-cyclohexyladenosine (CHA), it was able to block most of the cardiovascular actions of CHA. Under these conditions, however, the sedative action of CHA was unaffected.

2. PROPOSED ROLE FOR ADENOSINE IN THE REGULATION OF CEREBRAL BLOOD FLOW

Several factors have been proposed as possible mediators involved in the mechanism by which the brain regulates its own blood flow. Included in this list of factors are hydrogen ions (Kontos, Raper & Patterson,1977), carbon dioxide (Severinghaus & Lassen,1967), oxygen (Courtice,1941), lactate (Siesjo & Zwetnow,1970), potassium ions (Kuschinsky et al.,1972) and a more recent addition, adenosine, which was proposed as a coronary blood flow regulator by Berne (1963).

In order for a substance to be considered as a mediator in cerebral blood flow regulation it must meet certain criteria (Winn, Rubio & Berne,1981): (i)it must be a potent dilator of cerebral resistance vessels; (ii)the brain must be capable of producing the mediator during periods of altered cerebral blood flow and metabolism; (iii) the perivascular concentrations must be sufficient to produce the vasodilation observed with the metabolic stimulation; (iv)the time course and magnitude of the increase in substance must parallel the metabolically induced rise in cerebral blood flow; and (v)potentiators of the substance should increase cerebral blood flow while inhibitors should cause decreases in cerebral blood flow.

Adenosine is a likely candidate for the regulation of cerebral/..

cerebral blood flow for a variety of reasons. Adenosine has been shown to dilate pial vessels both in the cat (Wahl & Kuschinsky,1976) and the dog (Berne, Rubio & Curnish,1974). It can be seen, therefore, that topical application of adenosine causes a significant increase in pial vessel calibre, however, intracarotid infusions of adenosine in the dog have no effect on CBF (Berne et al.,1974). Following intravenous or intracarotid infusions of radioactively labelled adenosine little redioactivity is found in the brain tissue or the cerebrospinal fluid (CSF)(Rubio, Berne & Winn,1978) and similarly, following convexity-cisternal infusions of [^{14}C]-adenosine very little radioactivity is found in the venous blood (Winn et al.,1980c). It appears, therefore, that a relative blood-brain barrier and CSF-blood barrier exists for adenosine (Berne et al.,1974; Cornford & Oldendorf, 1975) and unlike blood, CSF does not contain the enzymes which metabolise adenosine and its degradative products (Winn et al.,1980c). As a consequence of these findings the changes in brain reflect cerebral rather than systemic events and, therefore, adenosine is a local metabolite and an ideal candidate for the regulation of cerebral blood flow.

Winn et al. (1979) demonstrated that adenosine levels were rapidly increased 2.5-fold during profound ischaemia. The adenosine levels were measured five seconds after decreasing the mean arterial blood pressure from normal levels of more than 100mmHg down to 0mmHg. Kontos et al. (1978) also showed that during similar periods of short-lasting ischaemia and hypotension pial vessel dilation occurred. It can be see, therefore, that the time course of these increases in adenosine levels in response to ischaemia occur with sufficient rapidity to account for the vascular changes observed. During sustained hypotension in rats brain adenosine levels were increased even if the blood/..

blood pressure remained within the normal autoregulatory range (Winn et al.,1981). With sustained hypoxia and the use of a rapid brain sampling technique Rubio et al.(1975) showed increased levels of adenosine in the brain. Work carried out by Rehncrona et al. (1978), however, using a slow-freezing method, failed to support this relationship between adenosine levels and PO_2.

With the onset of hypoxia CBF was increased 2-fold within 30 seconds (Astrup et al.,1978) and during similar periods of hypoxia adenosine levels increased 6-fold (Winn, Rubio & Berne,1981). It appears, therefore, that adenosine is a relatively sensitive index of cerebral hypoxia and the changes in cerebrovascular resistance and brain adenosine levels are temporally related during hypoxia.

Previous work has shown that bicuculline-induced seizures in rats increased adenosine concentrations in the brain 6-fold (Winn et al., 1980b) and cerebral blood flow 8- to 9-fold (Meldrum & Nilsson,1976) and that these responses occur in parallel.

On examination of the data it can be seen that the concentration of adenosine in the brain is increased in conditions of both increased oxygen demand (seizure) and decreased oxygen delivery (hypotension and ischaemia) thus suggesting that adenosine may well be involved in cerebral blood flow regulation. Further direct support for the adenosine hypothesis has been obtained using both adenosine potentiators (Heistad & Marcus, 1980) and inhibitors (Kontos & Wei, 1981). Both adenosine and ATP have previously been shown to produce increases in cerebral blood flow in baboons (Forrester et al.,1979). Adenosine and its analogues have also been shown to dilate feline cerebral vessels in vitro (Edvinsson & Fredholm,1983).

It can be seen, therefore, from these data that adenosine meets many of the criteria of a metabolic regulator of cerebral blood flow:/..

flow: it is a potent dilator of cerebral blood vessels, particularly the smaller ones which play a role in cerebrovascular resistance (CVR); the brain produces adenosine under conditions where CVR is decreased; the increases in cerebral adenosine levels are temporally related to the increases in CBF and cerebral metabolism; the concentrations in brain tissue by rapid-freezing techniques are apparently within the vasoactive range. All of the data are consistent, therefore, with the hypothesis that adenosine is an important metabolic factor in the regulation of cerebral blood flow.

3. REASONS FOR STUDY

The studies detailed in this thesis were carried out to monitor the effects of adenosine and a variety of adenosine analogues on the cerebrovasculature using a variety of in vitro and in vivo techniques. Adenosine and the adenosine analogues had previously been shown to dilate feline cerebral arteries in vitro via an A_2 adenosine receptor interaction (Edvinsson & Fredholm,1983), and the isolated vessel experiments detailed in this study were carried out to confirm these results in another species, namely the pig, and try to determine whether the effects could be blocked using known A_1/A_2 receptor blockers. The pial vessel experiments were designed to monitor the effects of adenosine and the adenosine analogues on vessel calibre in situ, where the innervation, absent in vitro, is present, to determine whether the effects are in any way different.

The effects of adenosine on cerebral blood flow have been well documented. When adenosine was administered via the internal carotid artery of dogs at 10^{-8} and 10^{-7}moles/min. there were significant increases of 15% and 40%, respectively, in CBF of the whole brain/..

brain as measured by xenon clearance (Kozniewska, Trzebski & Zielinski,1975). Similarly, intracarotid infusions of adenosine at 10^{-7}moles/min. and 10^{-6}moles/min. in baboons have been seen to produce significant increases of 20% and 80%, respectively, in CBF of the whole brain as measured using xenon clearance (Forrester et al., 1979). Intracarotid infusions of adenosine have also been shown to produce significant increases in CBF in the cerebrum, brain stem and cerebellum of dogs, as measured using microspheres (Heistad et al., 1981). These studies confirmed the expected effects of known vasodilators on CBF i.e. they increase CBF. Other studies, however, have shown that adenosine does not always produce the expected increase in CBF. When adenosine was injected into the hypothalamus of rabbits at concentrations of 10^{-4}M and 10^{-3}M there were the expected increases in blood flow as measured by Xenon clearance (Livemore & Mitchell, 1983), however, when adenosine was injected at 10^{-6}M in the same study there was a significant 25% decrease in hypothalamic blood flow. This observed vasoconstriction was unaffceted by adrenergic blockade with phenoxybenzamine and propranolol, or by the inhibition of neuronal activity using barbiturate, which suggests that the vasoconstriction is not dependent on adrenergic mechanisms or on reduced neuronal metabolism, but is a result of a direct action of adenosine on adenosine receptors on blood vessels. No changes were found in CBF in young (6 month) rats following infusions of adenosine (Hoffman, Albrecht & Miletich,1984). Similarly no changes in CBF were found in dogs following intravertebral infusions of adenosine (Boarini et al.,1984). In both of these studies CBF was measured using radioactive microspheres. It was because of these discrepancies in the results from species to species that it was decided to test the effects of adenosine and a variety of adenosine/..

adenosine analogues on local cerebral blood flow in the rat as measured by [^{14}C]-iodoantipyrine autoradiography. When these initial autoradiographic experiments showed that two of the adenosine analogues produced decreases in LCBF further studies were carried out to determine whether these effects could be blocked by known adenosine receptor antagonists, thus uncovering the receptor mechanisms mediating the effects.

SECTION II

MATERIALS AND METHODS

1. IN VITRO STUDIES USING PORCINE BASILAR ARTERIES

1.1. Preparation of porcine basilar arteries for in vitro studies

Specimens of basilar artery were obtained from adult pigs immediately after slaughter and stored in the bathing medium (see 'Compounds under examination and solutions used') at 4° until use. The vessels were stored for up to four days before use and the responsiveness of the vessels was not altered within this time span. However, on average, the vessels were used within one or two days of obtaining them. The vessels were trimmed to a length of 4mm. and the segments were suspended between two L-shaped metal hooks in an organ bath containing 20ml. of the bathing medium, which was being continuously gassed with 5% carbon dioxide (CO_2) in oxygen (O_2) in both the stock solution and the bath, to produce a resulting pH of between 7.35 and 7.45. The temperature of the bathing medium was kept constant at 37°C.

1.2. Experimental procedure

Isometric contractions of the circular smooth muscle were measured. The optimal passive load on the vessels of 37.28 milliNewtons (mN) was chosen as the tension which produced the largest contractile response to $3x10^{-6}$M $PGF_{2\alpha}$. After application of this load the vessels were allowed to stabilise for one hour. The vessels were sensitised with 30mM KCL for ten minutes which produced a contractile response. The vessels were washed for one minute (three changes of the bathing medium) and left for thirty minutes with further one minute washes at fifteen and thirty minutes. At this point the $PGF_{2\alpha}$ ($3x10^{-6}$M) was added to the bath to elicit a contriction of the vessels. The optimal $PGF_{2\alpha}$ concentration of $3x10^{-6}$M was determined by means of a concentration-response curve from which the concentration producing/...

producing the maximum constriction of the vessels was determined. Once the $PGF_{2\alpha}$ effect had stabilised concentration-response curves were obtained for each of the compounds being studied by their cumulative administration to the bath. In the experiments carried out to study the antagonistic effects of 8-phenyltheophylline, the 8-phenyltheophylline was administered when the $PGF_{2\alpha}$ effect was at its peak. The cumulative concentration-response curves were begun twenty minutes after the administration of the 8-phenyltheophylline. The concentrations of 8-phenyltheophylline chosen (10^{-8}M and 10^{-7}M) were those which were found to have minimal effects on relaxed and $PGF_{2\alpha}$-constricted vessels (see Figures III.1 and III.2).

2. ANIMAL PREPARATION

2.1. Preparation of animals for autoradiographic experiments

The experiments were carried out using male Sprague-Dawley rats weighing between 250g and 500g. Anaesthesia was induced using a mixture of 70% nitrous oxide (N_2O) and 30% oxygen (O_2), containing 5% halothane. Anaesthesia was maintained by means of a face mask, using 2% halothane in the same N_2O:O_2 mixture. A tracheostomy was performed and anaesthesia with 0.5% halothane in the same N_2O:O_2 mixture was delivered by mechanical ventilation. Small incisions, approximately 1.5cm long, were made in the groin on both sides of the animal to expose the femoral vessels. Polythene catheters (15cm long, external diameter 0.96mm) filled with heparinised saline (10 IU/ml) were inserted into both femoral arteries and veins, thus allowing the continuous measurement of arterial blood pressure, the withdrawal of arterial blood samples and the administration of drugs and radioactive tracers. Prior to commencement of the neck surgery required to expose the external carotid artery, the halothane/...

halothane level was raised from 0.5% to 1.0% in order to ensure a sufficient depth of anaesthesia. An incision was made in the neck adjacent to the tracheal cannula to expose both the right common and external carotid arteries. A heparinised catheter (35cm long, external diameter 0.63mm) was inserted into the external carotid artery and advanced until it lay at the bifurcation with the internal carotid artery, thus allowing any substances injected into the catheter to mix with the blood flowing up the common carotid artery and pass into the internal carotid artery. On completion of the neck surgery the halothane level was gradually decreased from 1.0% to 0.5% over a period of approximately thirty minutes, and it was under this level of anaesthesia that the autoradiographic experiments were carried out. Arterial blood pressure, Pco_2, Po_2, pH, bicarbonate (HCO_3-) concentration, base excess and core body temperature were monitored throughout the course of the experiments.

2.2.Preparation of animals for the laser-Doppler experiments

The animals were prepared in the same way as for the autoradiographic experiments until the external carotid artery had been cannulated. At this point, with the halothane level in the N_2O:O_2 anaesthetic mixture remaining at 1.0%, the animal was placed face down and an incision made in the scalp. A 5mm diameter hole was drilled in the skull and the dura removed to expose the cortical surface of the brain. During the actual experiments the laser beam was positioned over the hole to allow the appropriate measurements to be made. After the hole had been drilled the halothane level was gradually decreased from 1.0% to 0.5% over the next thirty minutes and it was at this level of anaesthesia that the experiments were carried out. The same blood parameters were/...

were monitored in these experiments as in the autoradiographic experients (see 2.1. above).

2.3. Preparation of animals for the pial vessel study

The study was carried out using male and female cats weighing 2.5kg to 4.0kg. Anaesthesia was induced using saffan (18ml/kg) administered via one of the forepaw veins. Saffan is a short-lasting steroid anaesthetic, 1ml of which contains 9mg of alphaxalone and 3mg of alphadalone acetate. After induction of anaesthesia the trachea was intubated using a cuffed rubber endotracheal tube, the cuff of which was inflated to give maximum contact between the tube and the tracheal wall. The cats were then mechanically ventilated using a gaseous mixture of 70% nitrous oxide (N_2O) and 30% oxygen (O_2). The rate and volume of the mechanical ventilator were constantly adjusted to maintain the arterial Pco_2 around 30mmHg. Two femoral arteries and one femoral vein were cannulated to allow the continuous measurement of arterial blood pressure, the removal of arterial blood samples for analysis and the intravenous administration of anaesthetics. After these cannulations the anaesthesia was maintained using intravenous doses of 1% α-chloralose which was prepared by dissolving 1g of powder in 100ml of deionised water heated to 60°C and filtering off any undissolved powder. The α-chloralose was kept in a water bath at 50°C throughout the experiment to prevent precipitation and was given to the animal when necessary to control the depth of anaesthesia. The cat received α-chloralose when either the blood pressure or respiratory trace suggested that the animal was not fully anaesthetised, or when a twitch reflex was found on stimulation of the animal's eye. Having completed the initial surgery the animals were ventilated/....

ventilated with oxygen enriched air.

The head of the animal was then placed in a stereotactic frame and a longitudinal incision was made in the centre of the scalp. The edges of the incision were sewn on to a metal ring (6cm in diameter) to create a circular pool over a skull. The left temporal muscle was retracted and a craniotomy, measuring approximately 2cm by 1.5cm, was carried out over the left parietal region using a dental drill, the tip of which was cooled by a jet of saline. Any bleeding from the vessels in the bone was arrested by the application of bone wax to the area, taking care not to put any on to the brain surface, and the exposed brain surface was covered by surgical patties soaked in saline. The pool created in the scalp by the metal ring was filled with paraffin oil, the temperature of which was kept at around 37°C by passing heated water from the water bath through a jacket surrounding the oil inlet tube. The oil was continuously renewed by having a system whereby fresh oil was passed in via the inlet tube while the overflow was collected by suction via the outlet tube. When the pool was filled with oil the patties were removed and the area was illuminated with a cold light source (Schott, Mainz,F.R.G.) With the aid of a stereomicroscope (Bausch & Lamb) the dura was cut and reflected to reveal the cortical surface. The preparation was left undisturbed for approximately thirty minutes before any measurement of pial vessel diameter was made.

Arterial blood pressure was monitored continuously throughout the course of the experiments by a pressure transducer connected to one of the femoral artery cannulae. Airway carbon dioxide (CO_2) percentage was also measured throughout the experiments using a capnograph (Godart). The end tidal percentage $CO_2$was displayed on a chart recorder (Devices). The animal's temperature was monitored/...

monitored via a rectal probe (Palmer) and maintained at 37°C by means of a feedback mechanism which connected the rectal probe to a heating blanket. Arterial blood smaples (1 - 2ml) were taken at regular intervals and used to measure the following: Pco_2, Po_2, pH, base excess and bicarbonate (HCO_3-) concentration. If a highly negative base excess was found (i.e. HCO_3- concentration too low) it was corrected by giving the cat an intravenous injection of 8.4% bicarbonate solution. The required volume of HCO_3- was calculated using the following equation:-

$$\text{Volume of } HCO_3^- \text{ (ml)} = \frac{0.3 \times \text{animal weight (kg)} \times \text{base excess}}{2}$$

$$= 0.15 \times \text{animal weight (kg)} \times \text{base excess}$$

where 0.3 is the empirical correction factor.

3. DETERMINATION OF LOCAL CEREBRAL BLOOD FLOW (LCBF) USING [^{14}C] - IODOANTIPYRINE (IAP) AUTORADIOGRAPHY

3.1. Theory

The radioactive tracer used in the determination of LCBF is [^{14}C] - iodoantipyrine (IAP), which is freely diffusible across the blood-brain barrier in the wall of the cerebral vessels. The blood-brain partition coefficient is 0.8. Since the tracer is a freely diffusible molecule the equation devised by Kety (1960) which relates the accumulation of radioactive tracers in the tissue to blood flow can be applied. The equation is as follows:-

$$C_i(T) = \lambda K \int_0^T C_A \cdot e^{-K(T-t)} \, dT$$

where Ci = tracer concentration in the tissue at time T, and λ = the blood brain partition co-efficient (0.8). The integral is the area under the curve described by the function arterial tracer concentration (C_A) against time from onset of tracer/...

tracer circulation. The constant K incorporates the tissue blood flow per unit of weight and another constant m , which indicates the extent of blood - tissue tracer equilibrium attained in a single passage of the tracer through the circulation:-

$$K = m \frac{F}{W} \lambda$$

Since IAP is a freely diffusible tracer , m is taken as being unity, therefore the equation becomes:-

$$K = \frac{F}{W} \lambda$$

which indicates that K is equal to the flow per unit weight times the partition co-efficient. It can be seen from these equations that since we can calculate the tissue tracer concentration at time T(Ci), can monitor the arterial tracer concentrations through the course of the experiment ($\int_0^T C_A$) and know the blood-brain partition co-efficient (λ), we can derive the value of the only unknown quantity, the flow (F) per unit weight e.g. ml of blood per 100g of brain tissue.

In order to be able to resolve differences in regional blood flow the arterial tracer concentrations must not be allowed to reach constant levels, since under these conditions the accumulation of radioisotope in the various brain regions is dependent not on the flow through the region, but only on the blood -brain partition co-efficient.

If the tracer is infused at a constant rate for a long enough period and equilibrium is attained, one merely gets a picture of the partition co-efficient. This problem has been overcome by administering the radioisotope at a logarithmically increased rate over a period of thirty seconds.

3.2/....

3.2.Determination of local cerebral blood flow (LCBF)

LCBF was measured using the quantitative autoradiographic technique with [^{14}C]-iodoantipyrine (IAP) as the radioactive tracer (Sakurada et al.,1978). The IAP (50μCi in 1.5ml of saline) was infused intravenously at a logarithmically increased rate over thirty seconds using a Harvard infusion pump. During this time arterial blood was allowed to drip freely from a catheter in one of the femoral arteries and eighteen samples of blood were collected on pre-weighed filter paper discs. The discs were weighed after the experiment, thus allowing the calculation of the weight of each sample. The course of the experiment was recorded on an audio cassette recorder, thus allowing the time of each sample and the decapitation by guillotine of the animal at approximately thirty seconds to be measured precisely by a deci-minute timer. Following the decapitation the brain was dissected out and frozen in isopentane at -42^{o}C within about two to three minutes. The filter paper discs were placed in scintillation vials and 0.4ml of hydrogen peroxide and 0.1ml of water were added to bleach the blood and extract the radioisotope. 10ml of liquid scintillant was then added, the vials tightly capped and liquid scintillation analysis carried out.

Brain sections (20μm thick) were cut on a cryostat at -22^{o}C and three out of every thirteen sections were mounted on glass coverslips and dried on a hot plate. Autoradiograms were prepared from these sections together with a set of calibrated plastic standards (44-1175 nCi/g.) by placing them in a light tight cassette in contact with X-ray film (Kodak GRS) for ten to fourteen days. The resulting images on the X-ray films were analysed using a computer based densitometer (Quantimet 720, Cambridge Instruments, U.K.) with reference to the precalibrated standards. Five optical/..

optical density readings were measured for each of the thirty seven regions of the brain being studied and the mean optical density obtained. Using this data and making comparisons with the optical density of the precalibrated standards and the arterial ^{14}C levels, the cerebral blood flow of each region was calculated using the equation derived by Kety (1960) and developed for this technique by Sakurada et al (1978).

4. MEASUREMENT OF CEREBRAL BLOOD FLOW USING LASER-DOPPLER FLOWMETRY

4.1. Theory

The flowmeter used in the experiments (Periflux Pf2, Perimed KB, Stockholm, Sweden) measures total microvascular blood cell flow through the measured volume i.e., the region of the brain surface that the laser beam covers. The measurement comprises all the vessels crossing this area, irrespective of the direction of blood cell movement. The flowmeter reacts only to the movement of discrete bodies, such as blood cells, and not to the flow of homogenous liquids like blood plasma or lymph. It responds to all movement relative to the probe head, therefore the probe head was held in position by means of a clamp.

The flowmeter emits a beam of low power laser light which is led by an optical fibre to the probe head. When the probe head is positioned over the brain surface the light enters the tissue and there it becomes repeatedly reflected, refracted and gradually absorbed. This multiple scattering of the beam produces a volume of almost isotopic illumination in front of the probe head. All blood vessels crossing through this volume are struck by light, partly reflecting it, whereupon the light undergoes a Doppler shift. The light in this volume is therefore a mixture of unshifted/...

unshifted and Doppler shifted light, the magnitude and frequency distribution of the latter being related to the number of red blood cells passing through this volume, and to their velocity.

Part of the light is back scattered from the brain surface and detected by efferent optical fibres which convey the back scattered fraction to photodetectors which convert it into electrical signals. Therefore, the greater the blood flow the greater the electrical signal there will be. These electrial signals from the flowmeter are transmitted to and displayed on a chart recorder (Linseis). The recording is a measure of blood cell flux i.e., the blood cell flow through the microvasculature from the arterial to the venous side. The measurements made are only relative and no numerical value can be put on them in terms of ml/100g/minute.

4.2.Experimental procedure

The probe head was positioned over the hole in the skull and lowered until a stable baseline flowrate was achieved on the chart recorder. The drug under study was then infused at a rate of 50µl/minute via the internal carotid artery for the fifteen and a half minutes of the experiment to give an infusion rate of 10^{-10}moles/minute. In the control animals 0.9% saline was infused at the same rate, 50µl/minutes, for the same length of time, fifteen and a half minutes. The changes in the flow rate were recorded on the chart recorder and for both saline control and CHA-treated animals the percentage changes relative to the baseline were calculated at five, ten and fifteen and fifteen and a half minutes. Each animal acted as its own control i.e., they received the saline infusion first and after a recovery period of approximately twenty minutes/...

minutes, during which time the baseline returned to its pre-saline level, they received the drug infusion. Mean arterial blood pressure, Pco_2, Po_2, pH, core body temperature, bicarbonate (HCO_3-) concentration and base excess were monitored throughout the experiments.

5. MEASUREMENT OF LOCAL CEREBRAL GLUCOSE UTILISATION (LCGU) USING [^{14}C] -2- DEOXYGLUCOSE (2-DG)

5.1. Theory

Many problems arise when it comes to measuring the functional activity of the central nervous system, since the various sub-units which integrate to produce the functional networks of the brain are not only anatomically discrete, but also have diverse physiological roles. Another problem is the fact that the brain is continuously reacting to any input which it receives.

It is known that there is close relationship between brain activity and its consumption of energy substrate. Under non-pathological conditions the brain receives all its energy requirements from the oxidative catabolism of glucose (Sokoloff, 1960). For this reason one might assume that either the rate of oxygen or glucose utilisation might be a useful method for measuring the functional activity of the brain. However, one encounters many problems when trying to measure such parameters directly. Oxygen electrodes can be used to measure the levels of oxygen or the extent of oxygen turnover, but with this method one encounters the problems of the insult to the brain caused by the electrodes. Another possible method would be to measure the turnover of oxygen radioisotopes. However, since oxygen radioisotopes have both a/...

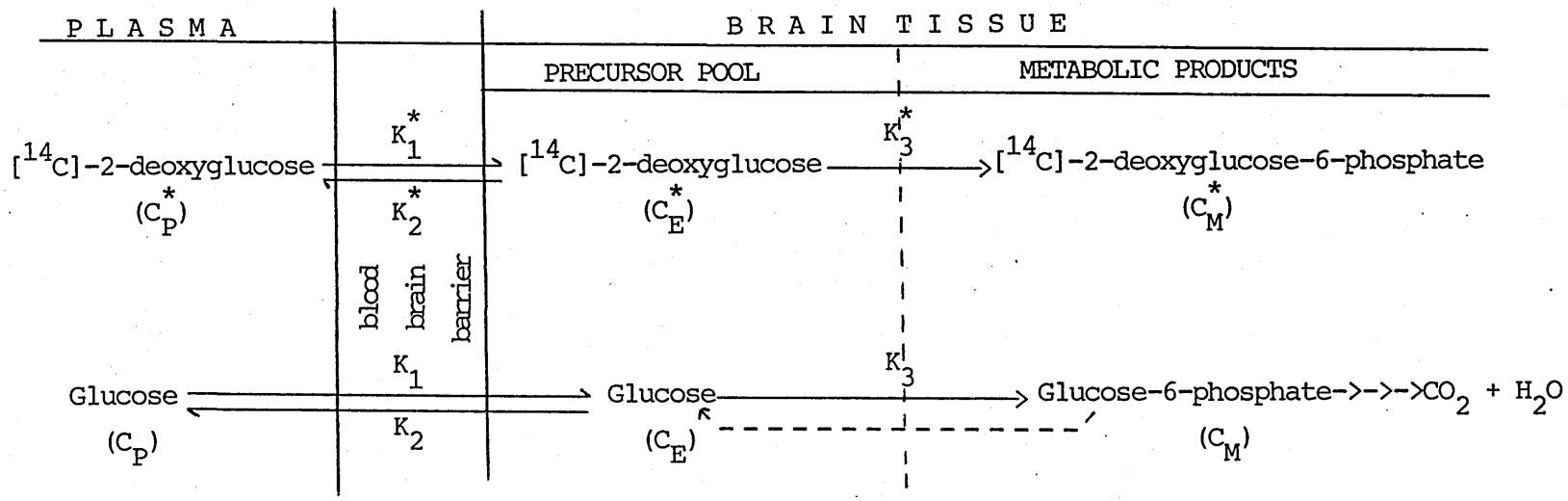
a short half-life and are also rapidly cleared from cerebral tissue, this leads to further problems. Similar problems are also encountered when trying to use the glucose radioisotope, [^{14}C]-glucose. However, these problems can be overcome by using the radioactive glucose analogue, [^{14}C]-2-deoxyglucose (2DG).

The theoretical model for the [^{14}C]-2-deoxyglucose method of functional activity is shown in Figure II.3. The model shows that both 2-DG and glucose enter a common precursor pool in the brain via a common carrier in the blood-brain barrier. Once in this pool they both compete for either the carrier, which would transport them back to the plasma, or for the enzyme which would convert them to their respective hexose-6-phosphates. This phosphorylation is far from equilibrium and since, unlike the glucose-6-phosphate, the [^{14}C]-2-deoxyglucose-6-phosphate takes no part in any of the known metabolic pathways it is trapped within the cell.

The operational equation used to measure glucose utilisation is shown in Figure II.4. The equation shows that in order to calculate the amount of glucose utilised by a specific brain region the amount of radioactively labelled products formed during the experimental period must be calculated. The amount of labelled product which is in the form of [^{14}C]-2-2deoxyglucose is subtracted from the total amount of labelled products and the remainder is divided by the lumped constant. The lumped constant is the value which expresses the differences in kinetics and distribution of deoxyglucose and glucose multiplied by the plasma integral for [^{14}C]-2-deoxyglucose and glucose during the experimental period, which is corrected for the lag time of equilibration.

The rate constants and lumped constant used in the operational equation were obtained from Sokoloff et al.(1977) and for the albino/...

Fig.II.3. THEORETICAL MODEL OF THE 2-DEOXYGLUCOSE TECHNIQUE



Total $[^{14}C]$ tissue concentration $(C^*_i) = C^*_E + C^*_M$.

C^*_E and C_E are the precursor pool concentrations of $[^{14}C]$-2-deoxyglucose and glucose respecticely.

C^*_M is the tissue concentration of $[^{14}C]$-2-deoxyglucose-6-phosphate.

K^*_1, K^*_2 and K^*_3 are the rate constants for $[^{14}C]$-2-deoxyglucose transport from plasma to brain, brain to plasma and its use by hexokinase, respectively.

K_1, K_2 and K_3 are the respective rate constants for glucose.

Fig.III.4. OPERATIONAL EQUATION OF THE 2-DEOXYGLUCOSE TECHNIQUE

$$\text{Rate of reaction} = \frac{\text{labelled product formed in interval of time, 0 to T}}{\text{isotope effect correction factor} \times \text{integrated specific activity of precursor}}$$

LABELLED PRODUCT FORMED IN INTERVAL OF TIME, 0 to T.

total ^{14}C in tissue at any time T

^{14}C in precursor remaining in tissue at time, T

$$R_i = \frac{C_i(T) - k_1^* \cdot e^{-(k_2^* + k_3^*)T} \int_0^T C_p^* \cdot e^{(k_2^* + k_3^*)t}\, dT}{\left[\frac{\lambda \cdot V_m^* \cdot K_m}{\Phi \cdot V_m \cdot K_m^*}\right]\left[\int_0^T \frac{C_p^*}{C_p^*}\, dt - e^{-(k_2^* + k_3^*)T} \int_0^T \frac{C_p^*}{C_p} \cdot e^{(k_2^* + k_3^*)t}\, dT\right]}$$

isotope effect correction factor

integrated plasma specific activity

correction for lag in tissue equilibration with plasma

INTEGRATED SPECIFIC ACTIVITY OF PRECURSOR IN TISSUE

C_i^* - total ^{14}C concentration in tissue.

T - time at termination of experiment.

C_p^* and C_p - plasma [^{14}C]-2-deoxyglucose and glucose concentrations, respectively.

K_1^*, K_2^* and K_3^* - rate constants for [^{14}C]-2-deoxyglucose transport from plasma to brain, brain to plasma and its use by hexokinase, respectively.

K_1, K_2 and K_3 - respective rate constants for glucose.

λ - the ratio of distribution space of [^{14}C]-2-deoxyglucose in the tissue to that of glucose.

Φ - fraction of glucose that, once phosphorylated, continues down glycolytic pathway.

K_m^* and V_m^* - Michaelis-Menten kinetic constants for [^{14}C]-2-deoxyglucose.

K_m and V_m - Michaelis-Menten kinetic constants for glucose.

K_m^*, V_m^*, K_m and V_m together make up the 'lumped' constant.

albino rat, they are:-

$K_1^* = 0.189\ min^{-1}$ $K_2^* = 0.245\ min^{-1}$

$K_3^* = 0.052\ min^{-1}$ Lumped constant = 0.481.

5.2. Determination of local cerebral glucose utilisation (LCGU)

LCGU was measured using the quantitative autoradiographic technique with [^{14}C]-2-deoxyglucose (2-DG) as the radioactive tracer (Sokoloff et al.(1977).

The measurement was initiated by the intravenous injection of a bolus of [^{14}C]-2-DG (50μCi in 0.7ml of saline). During the following forty five minutes fourteen samples of arterial blood were obtained at pre-determined times (0, 15, 30, 45 seconds, 1, 2, 3, 5, 7.5, 10, 15, 25, 35, and 45 minutes). These arterial samples were then centrifuged and approximately 23μl of plasma were taken for the determination of [^{14}C] concentration in the plasma, with another 10μl being used to measure the plasma glucose levels by means of a semi-automated glucose oxidase assay (Beckman, USA). Forty five minutes after the administration of the [^{14}C]-2-DG the rat was killed by decapitation. Following the decapitation the brain was dissected out and frozen in isopentane at -42°C. within two to three minutes. The frozen brain was coated in an embedding matrix and coronal sections 20μm thick were cut on a cryostat at -22°C. Three sections out of every thirteen were mounted on glass coverslips and rapidly dried on a hot-plate.

Autoradiograms were prepared from these sections, together with a set of calibrated standards (44 - 1175 nCi/g) by placing them in a light-tight cassette in contact with X-ray film (Kodak GRS) for/...

for up to thirty days. The length of exposure time was determined by the value of the plasma integral, as obtained from the raw blood data. The resulting images on the X-ray films were analysed using a computer based densitometer (Quantimet 720, Cambridge Instruments, U.K) with reference to the pre-calibrated standards. Five optical density readings were measured in both hemispheres for each of the thirty seven brain regions under examination, and the mean optical density for both hemispheres obtained. Using this data (i.e.,the [^{14}C] concentration in each brain region), the history of [^{14}C] and the glucose levels in the arterial plasma during the experimental period, the appropriate rate constants for the rat and the operational equation devised by Sokoloff et al. (1977) (see 5.1. above), the rate of glucose utilisation in each brain region was calculated.

6. MEASUREMENT OF CHANGES IN CAT PIAL ARTERIOLAR DIAMETER BY THE IMAGE SPLITTING TECHNIQUE

6.1. Equipment

Changes in pial arteriolar calibre were measured by the television image splitting technique of Baez (1966) as modified by Wahl et al. (1973). Individual pial vessels on the surface of the brain were viewed through a stereomicroscope (Bausch & Lomb) at either x40 or x70 magnification. A video camera (Sony) was incorporated into one eyepiece of the stereomicroscope, and the magnified image transmitted on to a video monitor (Shibaben).

The image-splitting component of the set up consisted of a prism within the same eyepiece of the stereomicroscope as the video camera. This prism was able to be rotated by means of a screw adjacent to the eyepiece,and the more the screw was turned, the more the prism/...

prism would rotate and, therefore, the greater the resulting split of the image.

The diameter of a vessel was measured in the following way. The prism was rotated so that the image of the vessel was split on the video screen to give two images of the vessel lying parallel to each other with their edges touching. After application of the test compound, if the images are found to have overlapped, the vessels will have dilated and, conversely, if the images are found to have moved apart the vessels will have constricted.

The screw which rotates the prism was connected to a potentiometer which was in turn linked to a Servoscribe pen recorder. At the start of each experiment the Servoscribe recorder had been calibrated with monofilament fibres of known diameter. The diameter of the pial vessel observed on the video screen could then be calculated directly from the servoscribe recorder. Pial arterioles in the range 25μm - 250μm were studied.

6.2.Application of solutions

Glass micropipettes were pulled, sharpened to produce a tip size of approximately 10μm and filled with the various test solutions on the morning of each experiment. The pipettes were filled by capillarity and sealed with mineral oil to reduce any CO_2diffusion which might have occurred.

The micropipettes were moved into position over the vessel to be studied by means of a micromanipulator (Leitz). The manipulator consisted of a moveable arm mounted on a stable, weighted base and connected by a length of clear tubing to a glass syringe. The glass syringe, the tubing and the arm were all filled with mineral oil. The micropipette, filled with a test solution, was attached to the arm of the micromanipulator and by turning a knob/...

knob on the edge of the glass syringe the movement of the mineral oil through the system injects a small quantity of the test solution (approximate 5µl).

The tip of the micropipette was carefully placed in the vicinity of the arteriole being measured and the calibre of that vessel was then calculated. The test solution was then injected and the calibre of the vessel was re-measured. The effect of the drug was calculated as a percentage change in the vessel calibre from the pre-injection value.

All the test solutions were dissolved in mock cerebrospinal fluid (CSF) and before any of these solutions were injected the effects of both the mock CSF and a 10mM K^+ solution were tested. The CSF was tested to ensure that the vehicle in which the compounds were dissolved had no effect of its own, and the 10mM K^+ was administered to test the reactivity of the vessels.

7. PATHOLOGICAL EXAMINATION OF THE RAT BRAIN FOR THE PRESENCE OF AIR EMBOLI AND THROMBI

The rats were prepared in the same way as for the autoradiographic experiments (see 2.1.) On completion of the surgery each animal received a fifteen and a half minute infusion of 0.9% saline. This was carried out to parallel those experimental conditions used in the autoradiographic experiments for the determination of local cerebral blood flow (LCBF). After the saline infusion, the animals were left for four hours before fixation was carried out using a mixture of formaldehyde/acetic acid/methanol (FAM). The FAM fixation was carried out in the following way. The rat's chest was opened to expose the heart/...

heart. A small incision was made in the left ventricle and a catheter was placed into the heart via this incision. 0.9% saline was infused into the heart through this catheter and when a swelling of the right atrium was observed a small incision was made in it to allow the blood to be washed out by the infused saline. When all the blood had been washed out the saline infusion was stopped and an infusion of the FAM mixture begun. The FAM infusion was stopped when the animal had gone completely rigid, indicating the fixation of all the tissues. The animal was then decapitated and the brain was sent for pathological examination to look for any damage which may be apparent.

8. COMPOUNDS UNDER EXAMINATION AND SOLUTIONS USED

The following compounds were used in the course of the studies: Theophylline, 5'-(N-ethyl)carboxamidoadenosine (NECA), 2-chloroadenosine (2-CADO), adenosine, adenosine triphosphate (ATP) and prostaglandin $F_{2\alpha}$ (PG $F_{2\alpha}$) (all Sigma, St.Louis, USA), N^6-cyclohexyladenosine (CHA) (Calbiochem, California, USA), L-phenylisopropyladenosine (L-PIA) (Boehringer-Mannheim, Ingelheim, F.R.G.) and 8-phenyltheophylline (a gift from Dr. Thorwart, Hoechst AG Werk Albert, Wiesbaden, F.R.G.).

In all the rat in vivo experiments the compounds were dissolved in 0.9% saline and infused via the internal carotid artery cannula at either 50µl/minute (LCBF studies) or 33µl/minute (LCGU studies) at the concentration necessary to produce the infusion rates of moles/minute shown in the text. In the autoradiographic experiments the solutions were infused for 15 minutes prior to commencement of the experiment and for the 30 seconds (LCBF) or 45 minutes (LCGU) of the experiment. In the Laser-Doppler experiments the solutions were/...

were infused for 15 mins and 30 seconds, thus corresponding with the time course of the autoradiographic determination of cerebral blood flow.

In the antagonist studies either saline (5ml/kg) or theophylline (30mg/kg) was administered intra-peritoneally 20 minutes prior to the start of the internal carotid infusion.

In all the cat in vivo experiments the compounds were dissolved in mock cerebrospinal fluid (CSF) the constituents of which were:-

NaCl, 143.9mM; KCl, 3mM; $NaHCO_3$, 12.1mM; and $CaCl_2$, 2.5mM.

In the isolated vessel work the compounds were dissolved in distilled water. The bathing medium for the vessels consisted of the following:-

NaCl, 148mM; KCl, 5.4mM; $CaCl_2$,2.2mM; $NaHCO_3$, 12mM; and glucose, 12mM.

9. ANALYSIS OF DATA

9.1. Analysis of isolated vessel data

From the dose response curves the relative potencies of the various agonists used can be calculated. The order of potency is determined by the $_pD_2$ value i.e. the greater the $_pD_2$ value, the more potent the agonist is. The $_pD_2$ values can be calculated by both analysis of the dose-response curves and analysis of the data according to Hill (Bowman & Rand, 1982).

From the dose-response curves we can determine the concentration of agonist producing half maximal response (EC_{50}), and the $_pD_2$ value is equal to negative the log of the EC_{50} value.

The Hill analysis involves the use of the following equation/....

equation.

$$r = \frac{Ka\ [D]^n}{1 + Ka[D]^n} \qquad \text{---- equation (1)}$$

where $r = \frac{E}{Emax}$; E = response produced by agonist concentration [D] ; Emax = maximum response produced by agonist ; Ka = a constant ; [D] = agonist concentration ; and n = molecular ratio of drug per adsorptive site (receptor).

From equation (1) we devise:-

$$\frac{r}{1-r} = Ka[D]^n \qquad \text{---- equation (2)}$$

Equation (2) in the logarithmic form gives:-

$$\log\left(\frac{r}{1-r}\right) = n.\log[D] + \log Ka \qquad \text{---- equation (3)}$$

If we then plot $\log\left(\frac{r}{1-r}\right)$ against log [D] (i.e. Hill plot) we get a straight line with gradient ,n. This gradient, n, is a direct measure for the Hill co-efficient.

As stated above:-

$$r = \frac{E}{Emax} \qquad \text{---- equation (1)}$$

$$\text{and } \frac{E}{Emax} = \frac{[D]}{[D] + K_D} \qquad \text{---- equation (4)}$$

Therefore $$r = \frac{[D]}{[D] + K_D} \qquad \text{---- equation (5)}$$

$$\Rightarrow \quad r\ [D] + r.K_D = [D] \qquad \text{---- equation (6)}$$

$$\Rightarrow \quad r.K_D = [D] - r[D] \qquad \text{---- equation (7)}$$

$$\Rightarrow \quad K_D = \frac{[D](1-r)}{r} \qquad \text{---- equation (8)}$$

Using equation (8) the K_D values for a particular drug can be calculated at each concentration of agonist used ([D]), and the corresponding pD_2 values were also calculated[2].

$$pD_2 = -\log K_D \qquad \text{---- equation (9)}$$

The pD_2 values are calculated as mean pD_2 value ± standard error of mean for each agonist used.

The antagonist results were analysed using the Schild plot (Arunlakshana & Schild, 1959) to calculate the pA_2 value. The pA_2 value is calculated in the following way. The log of (dose ratio -1) is plotted against the log of the antagonist concentration. The dose ratio is the concentration ratio of agonist giving equal responses in the presence of a particular concentration of antagonist and in the absence of the antagonist. This line intercepts the X-axis at $(-pA_2, 0)$, therefore the pA_2 is calculated by taking the negative of the X-intercept.

The linear portion of each concentration-response curve in the absence and presence of 8-phenyltheophylline is analysed using linear regression analysis and tested to see if there is a significant parallel shift in the linear part of the curve in the presence of 8-phenyltheophylline.

Student's t-tests are carried out to compare the response obtained in the absence of antagonist with that in the presence of antagonist.

9.2./....

9.2.Analysis of pial vessel data

In this study the control values are those obtained with the mock CSF, and each drug response is compared to the control by means of a Student's t-test, incorporating the Bonferroni correction factor where appropriate, and significance is looked for at the 5% level.

The Bonferroni correction takes into account the fact that multiple comparisons are being made e.g. if we are comparing four drug treated groups to control (i.e. four comparisons) and looking for significance at the 5% level (i.e. $P < 0.05$), we must look for significance at the 5/4% (1.25%) level for a result to be considered significant i.e. $P<0.0125$.

In the antagonist studies involving the pial vessels the effect of the drug alone and the effect of the drug in the presence of the antagonist are compared by means of a Student's t-test, incorporating the Bonferroni correction factor where appropriate.

9.3.Agonist autoradiographic studies

In these autoradiographic studies the drug treated groups are compared to control by means of a Student's t-test, incorporating the Bonferroni correction factor.

9.4.Antagonist autoradiographic studies

In the antagonist studies the data are analysed by means of a one way analysis of variance with Newman-Keuls multiple range test.

SECTION III

RESULTS

1. ISOLATED PORCINE VESSELS

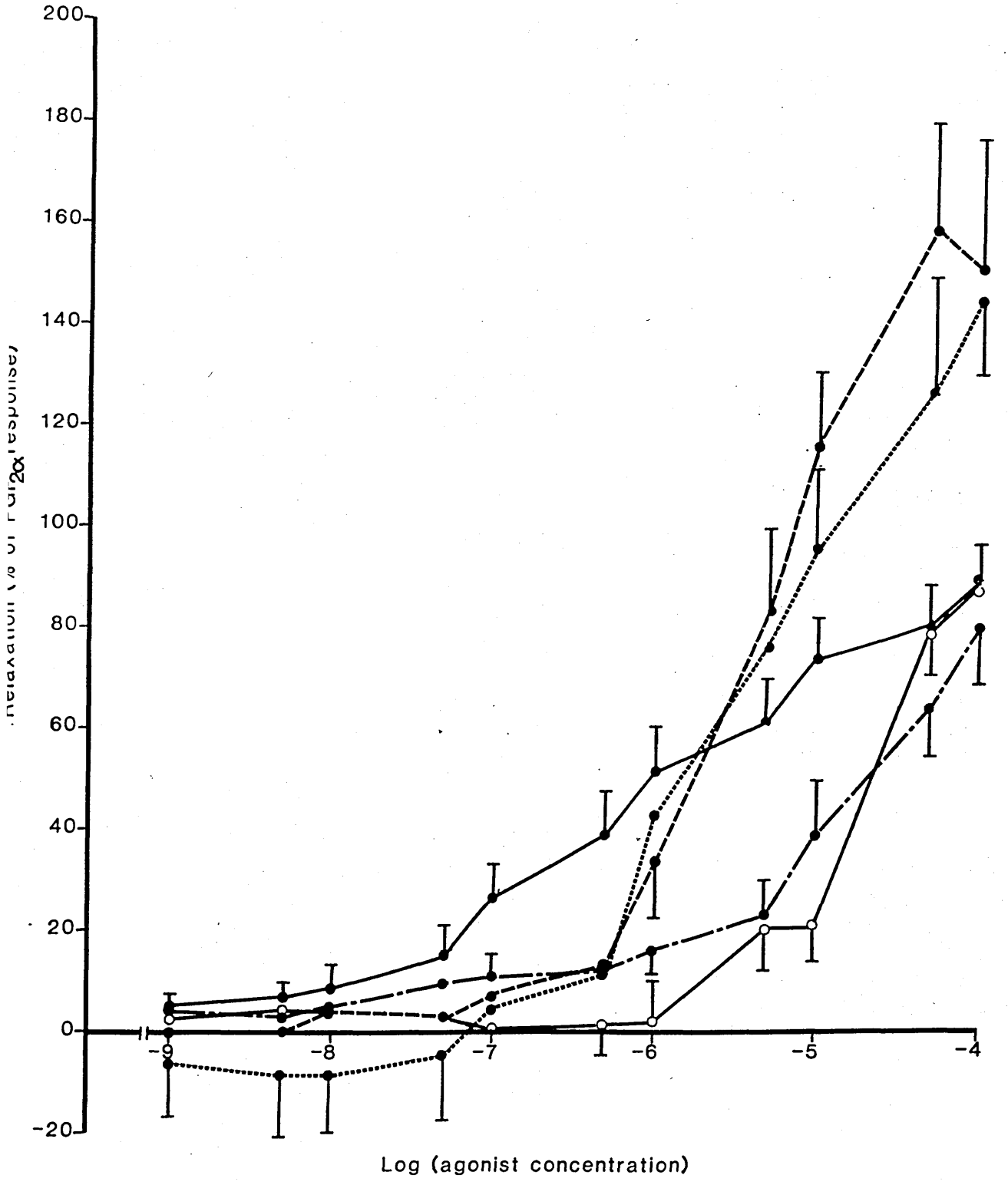
1.1. Effects of adenosine, some adenosine analogues and 8-phenyltheophylline on isolated porcine basilar arteries

Pig basilar arteries were relaxed in a concentration-dependent manner by adenosine, 2-chloroadenosine (2-CADO), N^6-cyclohexyl-adenosine (CHA), L-phenylisopropyladenosine (L-PIA) and 5'-(N-ethyl) carboxamidoadenosine (NECA) (Fig.III.1). The relative potencies of adenosine and these adenosine analogues were calculated in two ways. Firstly, the $_pD_2$ values were calculated by analysis of each dose response curve (Table III.1) and secondly by analysis of the data according to Hill (Table III.2) (see Section II. Materials and Methods) The rank order of potency of the compounds was:

NECA > 2 -CADO, Adenosine > L-PIA > CHA

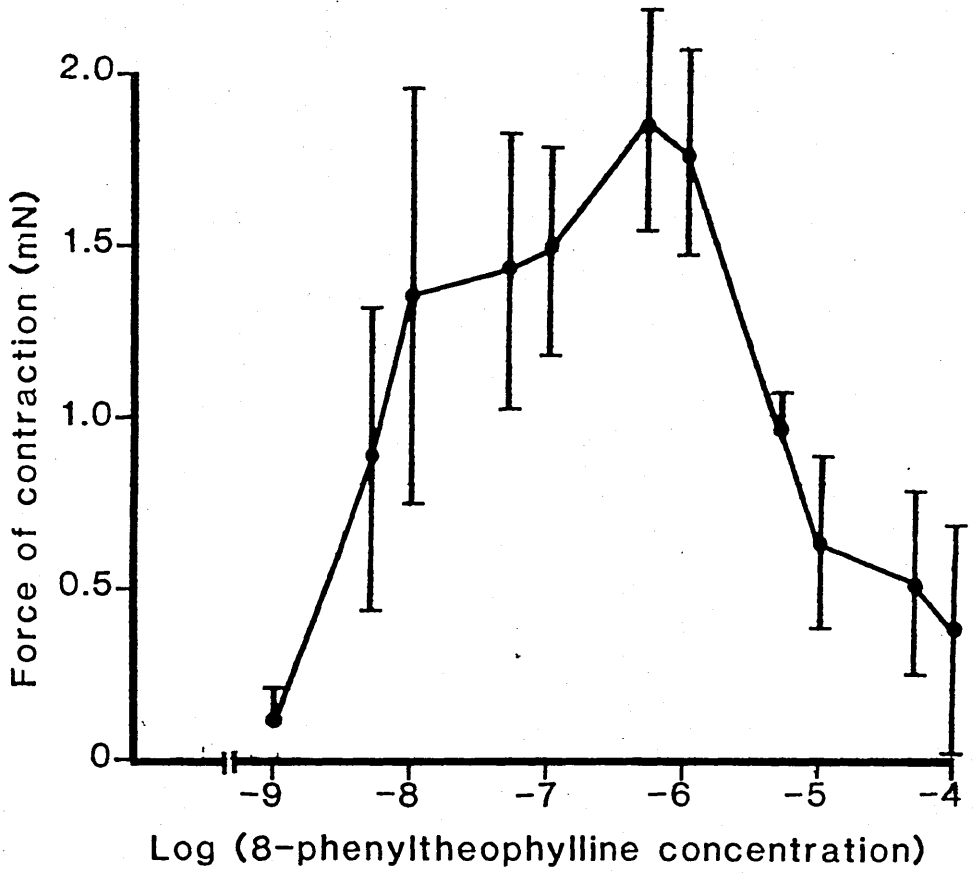
The effects of 8-phenyltheophylline were tested in both relaxed and $PGF_{2\alpha}$-constricted vessels and, as shown in Figs.III.2 and III.3 it can be seen that the 8-phenyltheophylline constricts the resting vessels and dilates the pre-constricted vessels. The concentrations of 8-phenyltheophylline chosen for use in further experiments were those which had minimal effects, namely 10^{-8} and 10^{-7}M. At these concentrations the 8-phenyltheophylline had no significant effect on the concentration response curves to adenosine, 2-CADO, CHA and L-PIA. However, 10^{-7}M 8-phenyltheophylline did produce a significant parallel shift to the right of the NECA concentration response curve (Fig.III.4) indicating a competitive antagonism. Construction of a Schild plot (see Section II Materials and Methods) for NECA in the presence of 10^{-8}M and 10^{-7}M 8-phenyltheophylline gave a graphically calculated/...

Fig III 1. CONCENTRATION RESPONSE CURVES TO ADENOSINE AND SOME ADENOSINE ANALOGUES.



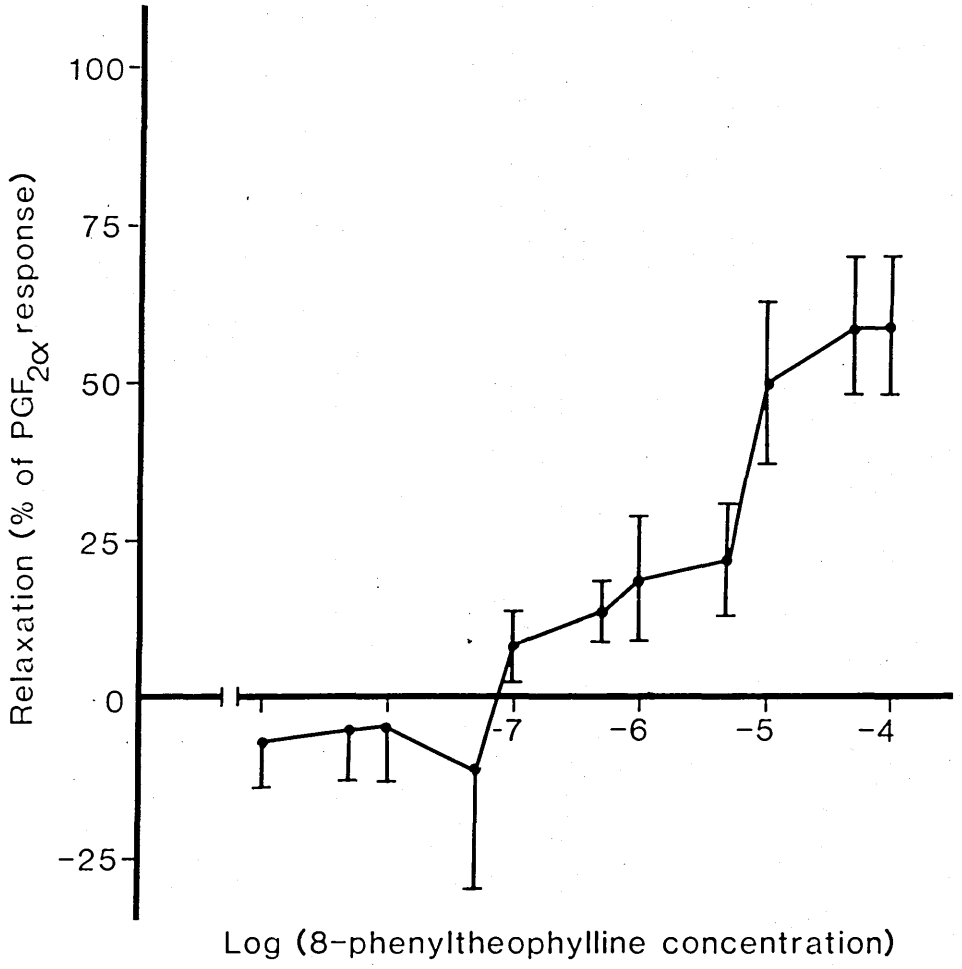
This figure shows the effects of adenosine(·····●·····)(n=8),NECA(——●——)(n=13), CHA(——○——)(n=8),2-CADO(— ● —)(n=9)and L-PIA(·· ● ··)(n=7). All values are expressed as mean ± standard error of mean.

Fig.III.2 EFFECTS OF 8-PHENYLTHEOPHYLLINE ON RELAXED PORCINE BASILAR ARTERIES



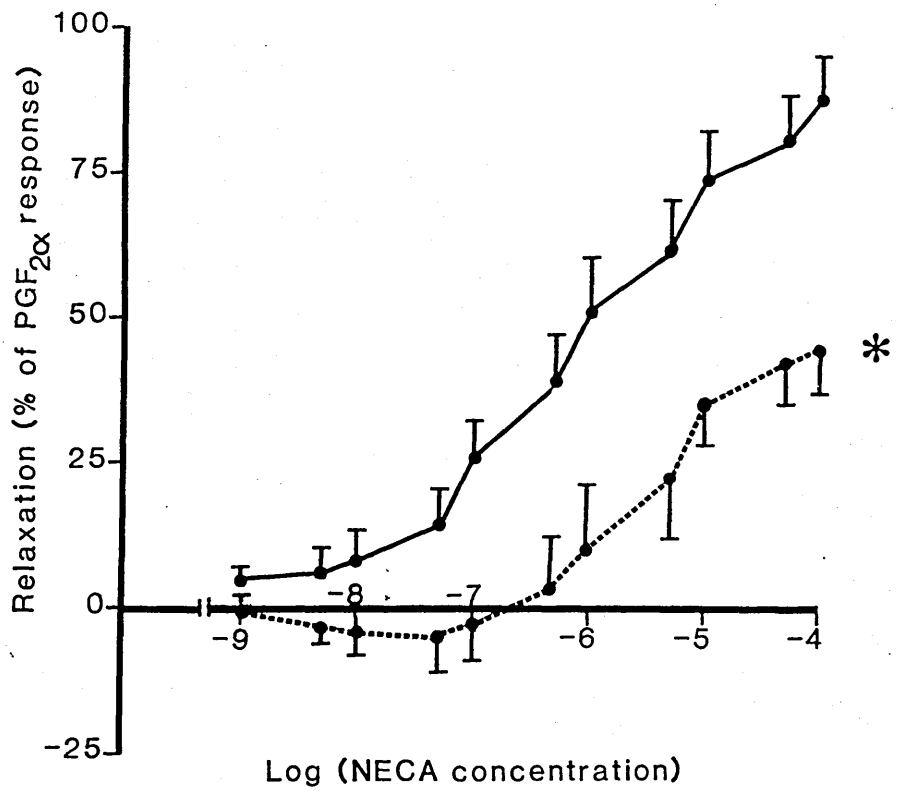
This figure shows the effects of various concentrations of 8-phenyltheophylline on isolated porcine basilar arteries (n=9) All values are expressed as mean ± standard error of mean.

Fig.III.3 EFFECTS OF 8-PHENYLTHEOPHYLLINE ON PORCINE BASILAR ARTERIES PRE-CONSTRICTED WITH $PGF_{2\alpha}$



This figure shows the effects of various concentrations of 8-phenyltheophylline on pre-constricted porcine basilar arteries (n=9). All values are expressed as mean ± standard error of mean

Fig. III .4. EFFECTS OF NECA ALONE AND IN THE PRESENCE OF 8-PHENYLTHEOPHYLLINE ON PORCINE BASILAR ARTERIES PRE-CONSTRICTED WITH $PGF_{2\alpha}$



This figure shows the effects of NECA (●——●) (n = 13) and NECA + 10^{-7}M 8-phenyltheophylline (●·····●) (n=5) on pre-constricted porcine basilar arteries.

* indicates a significant parallel shift in the curve in the presence of 8-phenyltheophylline ($P<0.05$) as determined by linear regression analysis. All values are expressed as mean standard error of mean.

calculated $_pA_2$ value for the antagonist of 7.7 (Fig.III.5).

With 10^{-8}M 8-phenyltheophylline for adenosine and 10^{-7}M 8-phenyltheophylline for 2-CADO, CHA and NECA, the vessels constricted rather than dilated, in the agonist concentration range of 10^{-9}M to 10^{-6}M (Figs III.6, III.7, III.8 and III.4). This effect was absent with L-PIA in the presence of either concentration of antagonist.

1.2.Discussion

The study confirms the vasodilatory properties of adenosine (Berne et al., 1974) and its analogues (Hardebo & Edvinsson, 1979) In comparison to adenosine, NECA is more potent, 2-CADO almost equipotent, while L-PIA and CHA are less potent. The relative order of potency is in keeping with that of the A_2 adenosine receptor sub-type.

When a group of compounds are all acting on a single receptor to produce the same effects the concentration response curves are normally in parallel. This is, in fact, the case for adenosine, 2-CADO, L-PIA and CHA. However, the concentration-response curve for NECA is not in parallel with those of the other four compounds and although NECA is the most potent analogue of adenosine (i.e. highest $_pD_2$ value) it has one of the lowest relative Emax values. These observations, coupled with the fact that the Hill co-efficients for NECA and L-PIA are less than one (Table III.2), suggest that the dilatatory effect of these compounds are possible being produced by more than a simple A_2 receptor interaction.

The affinity of 8-phenyltheophylline to A_2 receptors (Ki$\sim$100$_\mu$M) is about two to three orders of magnitude lower than that of NECA/...

Fig.III.5 SCHILD PLOT FOR NECA IN THE PRESENCE OF 10^{-7} M AND 10^{-8} M 8-PHENYLTHEOPHYLLINE

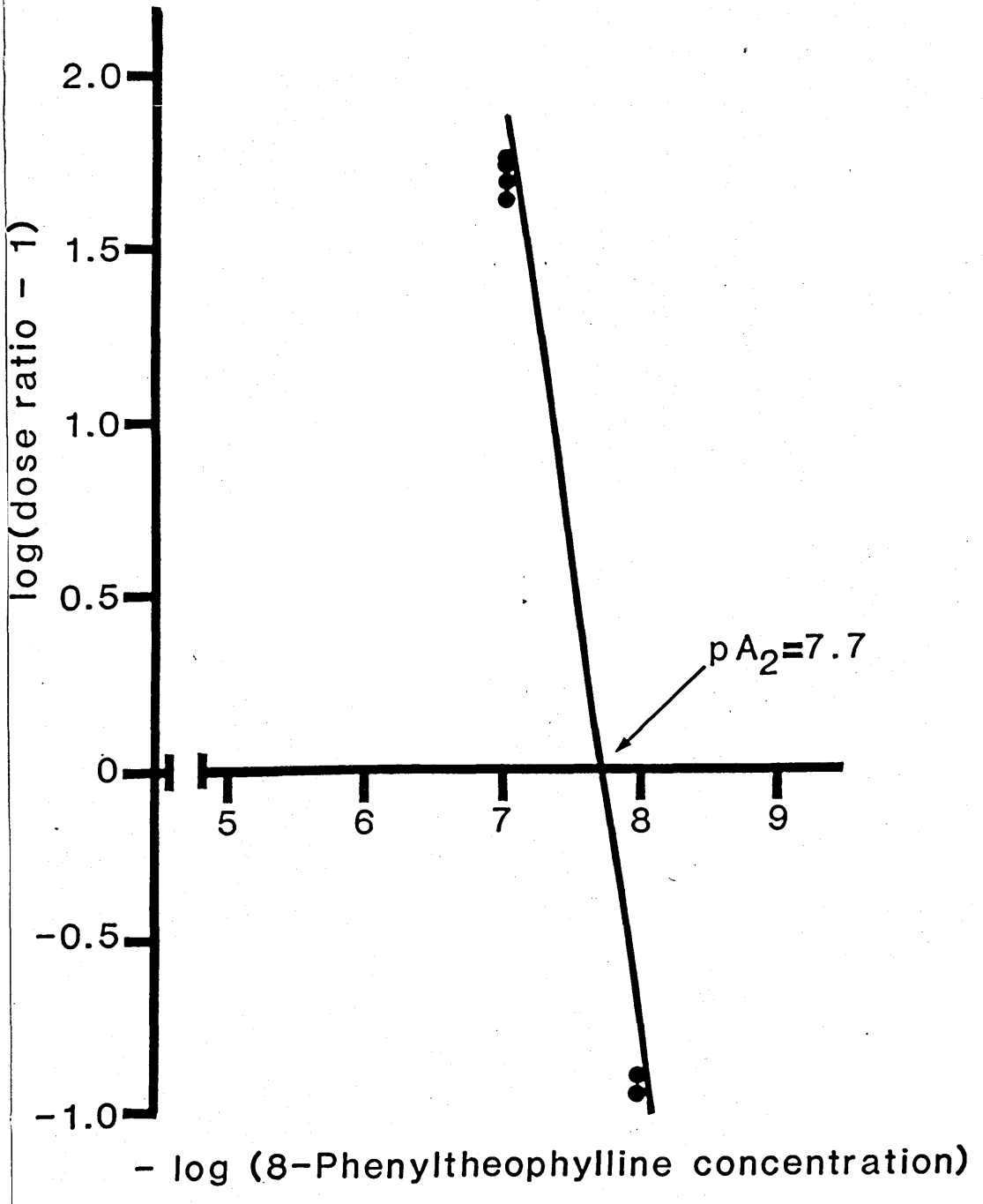
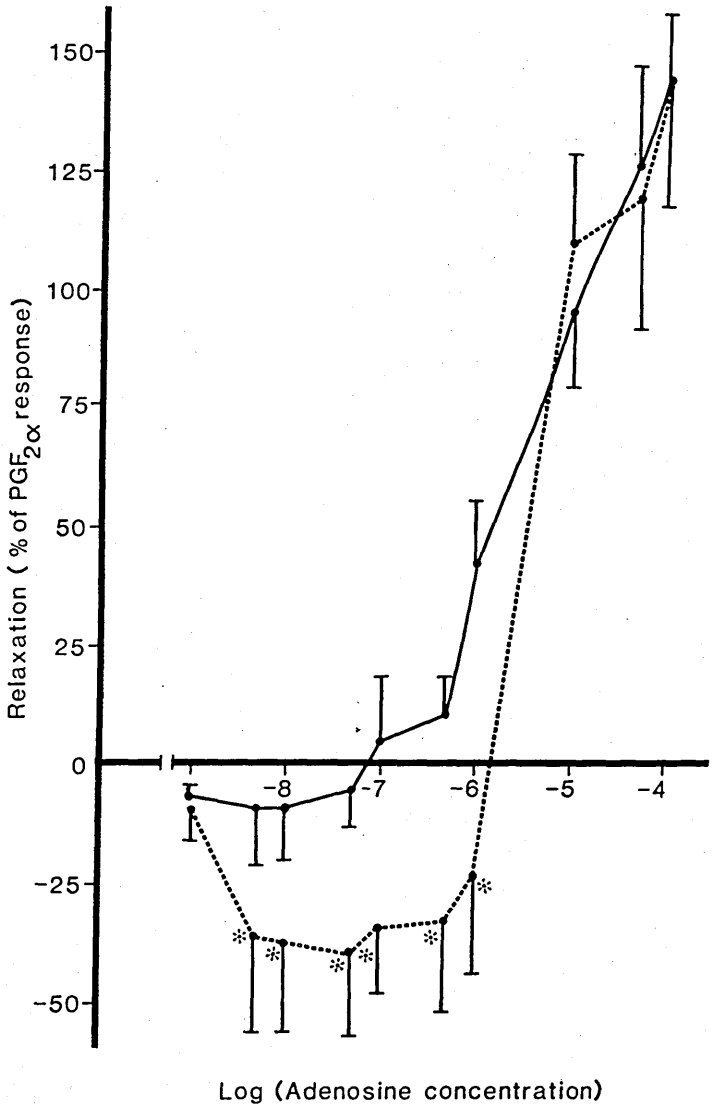


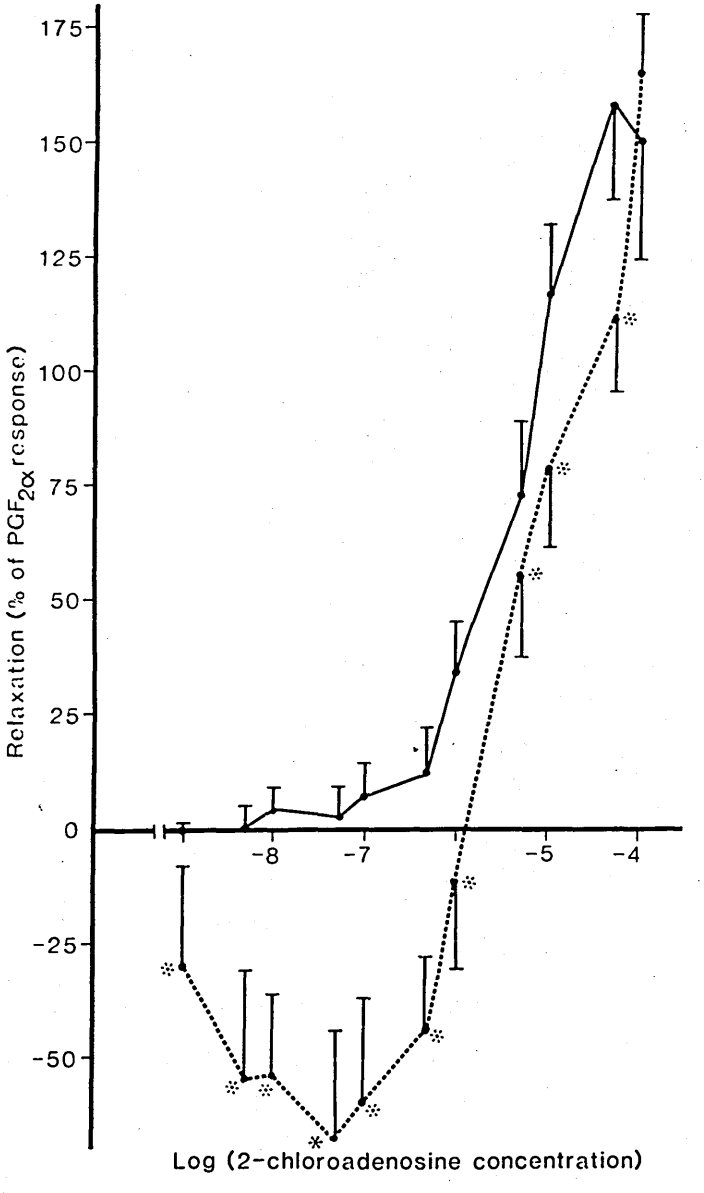
Fig. III 6 EFFECTS OF ADENOSINE ALONE AND IN THE PRESENCE OF 8-PHENYLTHEOPHYLLINE ON PORCINE BASILAR ARTERIES PRE-CONSTRICTED WITH $PGF_{2\alpha}$



This figure shows the effects of adenosine (●—●)(n=8) and adenosine + 10^{-8}M 8-phenyltheophylline (●·····●)(n = 8) on pre-constricted porcine basilar arteries.

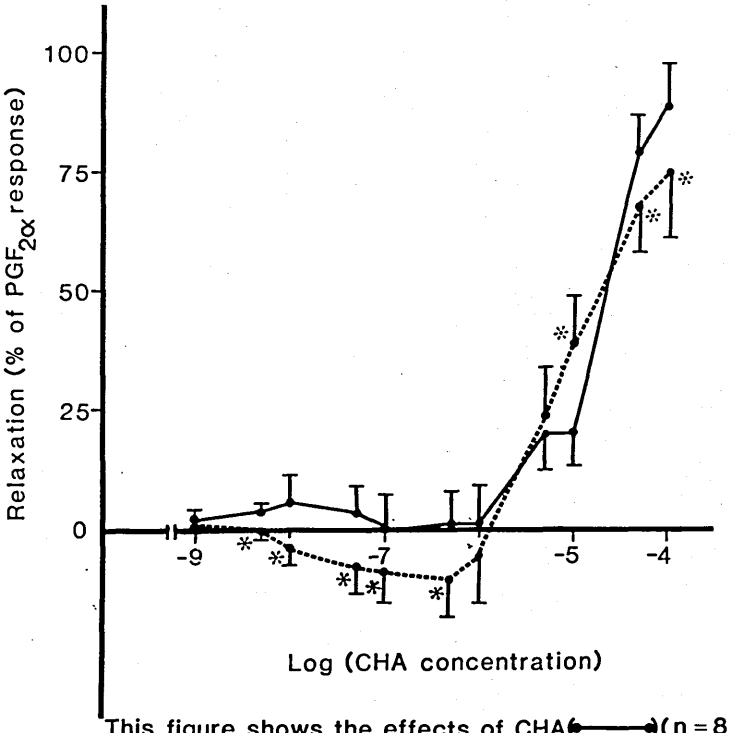
* indicates a significant difference between the response to agonist alone and the response to agonist + 8-phenyltheophylline at the same concentration ($P < 0.05$) as determined by a Student's t-test. All values are expressed as mean ± standard error of mean.

Fig.III.7. EFFECTS OF 2-CADO ALONE AND IN THE PRESENCE OF 8-PHENYLTHEOPHYLLINE ON PORCINE BASILAR ARTERIES PRE-CONSTRICTED WITH $PGF_{2\alpha}$



This figure shows the effects of 2-CADO (——•)(n 9) and 2-CADO + 10^{-7} M 8-phenyltheophylline (•·····•) (n 7) on pre-constricted porcine basilar arteries
* indicates a significant difference between the response to agonist alone and the response to agonist · 8-phenyltheophylline at the same concentration ($P < 0.05$) as determined by a Students t-test
All values are expressed as mean · standard error of mean

Fig.III.8 EFFECTS OF CHA ALONE AND IN THE PRESENCE OF 8-PHENYLTHEOPHYLLINE ON PORCINE BASILAR ARTERIES PRE-CONSTRICTED WITH $PGF_{2\alpha}$



This figure shows the effects of CHA(●——●)(n = 8) and CHA +10^{-7}M 8-phenyltheophylline(●·····●)(n=7) on pre-constricted porcine basilar arteries. ✻ indicates a significant difference between the response to agonist alone and the response to agonist + 10^{-7}M 8-phenyltheophylline at the same concentration($P < 0.05$) as determined by a Students t-test. All values are expressed as mean ± standard error of mean

NECA (Schwabe, Ukena & Lohse, 1985). This low affinity has also been demonstrated in studies with isolated arteries (Edvinsson & Fredholm, 1983) showing a $_pD_2$ value of approximately 6.5. On the basis of this information it would perhaps be thought that 8-phenyltheophylline at the concentrations used (10^{-8}M to 10^{-7}M), would not be able to block the NECA response. However, since 10^{-7}M 8-phenyltheophylline did antagonise the NECA response, this can be seen as adding further support to the idea that the action of NECA is not completely due to an A_2 receptor interaction, but may involve another receptor which 8-phenyltheophylline can block (e.g. the A_1 adenosine receptor). The concentrations of 8-phenyltheophylline used (10^{-8}M and 10^{-7}M) were not able to antagonise the actions of the other adenosine agonists, but higher antagonist concentrations could not be tested since as stated earlier, they possessed their own intrinsic action.

Other facets of the results suggest that there may be more than one adenosine receptor site involved. If one examines the concentration-response curves to the various agonists in the presence of 8-phenyl-theophylline there appears to be a trend in the ability of the agonists to produce further constriction at concentrations between 10^{-9}M to 10^{-6}M. Of the four agonist curves which are parallel adenosine and 2-CADO show a large further constriction, whereas CHA and L-PIA show little or no further constriction. The magnitude of these effects parallels the relative potency of the four agonists. The NECA curve exhibits a very slight further constriction (5%) within the concentration of 10^{-9}M to 10^{-6}M NECA. These results may suggest that although 8-phenyltheophylline acts on both A_1 and A_2 receptor sub-types it could have a greater affinity at the A_2 receptor thus uncovering the vasoconstrictive A_1 effects, or secondly, that the/...

the 8-phenyltheophylline is blocking the A_1 and A_2 receptors and, in the low dose range, the agonists are acting on another receptor to produce a vasoconstriction.

Another explanation for the complexity of the picture could be that there are further subdivisions in the A_1/A_2 receptor classification, since it has already been shown by Londos et al. (1980) that the A_2 receptors in the liver had a very high affinity for NECA, an affinity much higher than those in the adrenal or Leydig cells.

On examination of the data the results for NECA seem to be apart from those of the other compounds: (i) 8-phenyltheophylline, at the concentrations studied, antagonised the action of NECA; (ii) the vasoconstrictive effect apparent in the presence of 8-phenyltheophylline is markedly less for NECA than for the other agonists; (iii) the NECA concentration-response curve has a different gradient than those of the other agonists; and (iv) the slope of the Hill plot was different from that of the other agonists (with the exception of L-PIA). This may support the idea of an atypical adenosine receptor at which NECA has a greater affinity than the other adenosine agonists. A possible third adenosine receptor has been identified in rat fat cells (Garcia, Sainz & Torner, 1985) and also centrally (Chin & DeLorenzo, 1985, 1986). Recent work by Lee & Reddington (1986) also speculates on the existence of a previously undescribed non-A_1 receptor site.

The agonist data in this study suggests that adenosine and its analogues produce their dilatatory effects via an action on the A_2 receptor, as has previously been described (Edvinsson & Fredholm, 1983). However the results with 8-phenyltheophylline antagonism, along with|...

with the different gradient of the NECA concentration-response curve suggest that the mechanism of action of adenosine and its analogues on pig cerebral arteries is not via a single sub-type of adenosine receptor, but a more complicated picture, with the possible involvement of other receptor sub-types.

1.3. Summary of isolated vessel results

Compounds (in order of potency)	Vasodilatation (D) or Constriction (C)	Antagonised by 8-phenyltheophylline (8-PT)	Constriction produced by 10^{-9}M - 10^{-6}M agonist in the presence of 8-PT
NECA	D	Yes	Yes
2-CADO	D	No	Yes
Adenosine	D	No	Yes
L-PIA	D	No	No
CHA	D	No	Yes

2. IN VIVO FELINE VESSELS

2.1. Effects of adenosine and some adenosine analogues and antagonists on cat pial vessels in vivo

Since all of the compounds tested in this study were dissolved in mock cerebrospinal fluid (CSF), the response obtained to the mock CSF alone (1.5% dilatation) was taken as the control response and the effects of all the compounds were compared to this value.

Injections of a 10mM K^+ solution were administered to test the responsiveness of the vessels and were found to produce a mean dilatation of 36% (vessel calibre range 46-194μm; mean vessel calibre ± standard deviation = 98 ± 40μm). This response was significantly different from control (see Table III.3 and Fig.III.9).

Two adenosine antagonists, theophylline and 8-phenyltheophylline, at concentrations of 10^{-9}M and 10^{-7}M, were tested to determine their effects on vessel calibre. Neither of the compounds produced any significant changes in vessel calibre (see TableIII.3 and Fig.III.9) 10^{-9}M 8-phenyltheophylline was chosen as the antagonist concentration to be used in further antagonism studies.

Adenosine, at concentrations of 10^{-9}M, 10^{-7}M and 10^{-5}M produced a 7% constriction (vessel calibre range 58-123μm; mean vessel calibre ± standard deviation = 92 ± 26μm), a 13% dilatation (vessel calibre range 34-161μm; mean vessel calibre ± standard deviation = 88 ± 40μm) and a 22% dilatation (vessel calibre range 40-223μm; mean vessel calibre ± standard deviation = 123 ± 56μm), respectively. The responses obtained to both 10^{-7}M and 10^{-5}M adenosine were significantly different from the mock CSF control and both, in turn, were significantly antagonised by 10^{-9}M 8-phenyltheophylline (see Table III.4 and Fig.III.10).

N6/...

g.III.9 EFFECTS OF MOCK CEREBROSPINAL FLUID (CSF) 10mM K^+ THEOPHYLLINE (Theo) AND 8-PHENYLTHEOPHYLLINE (8-PT) ON CAT PIAL VESSEL DIAMETER

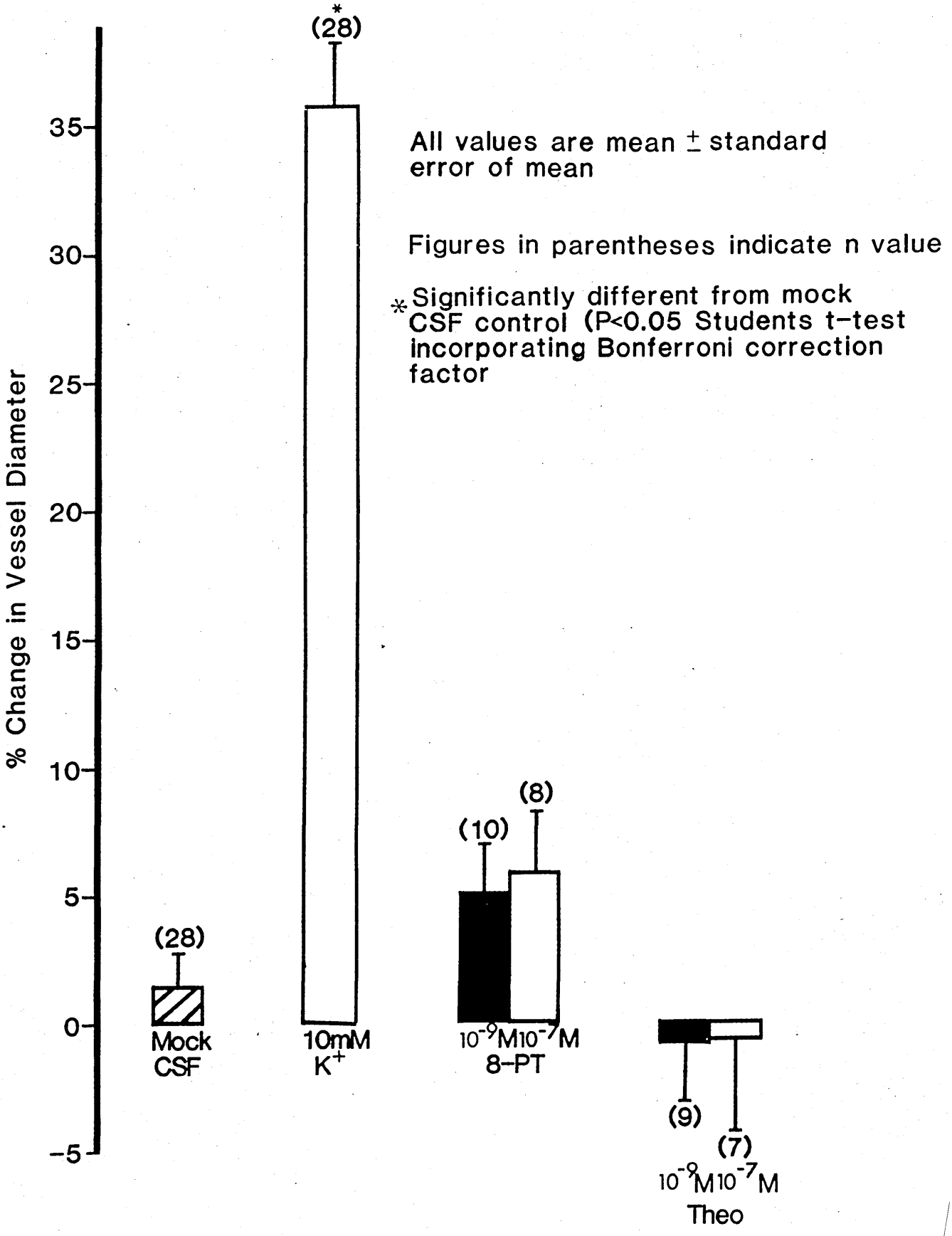
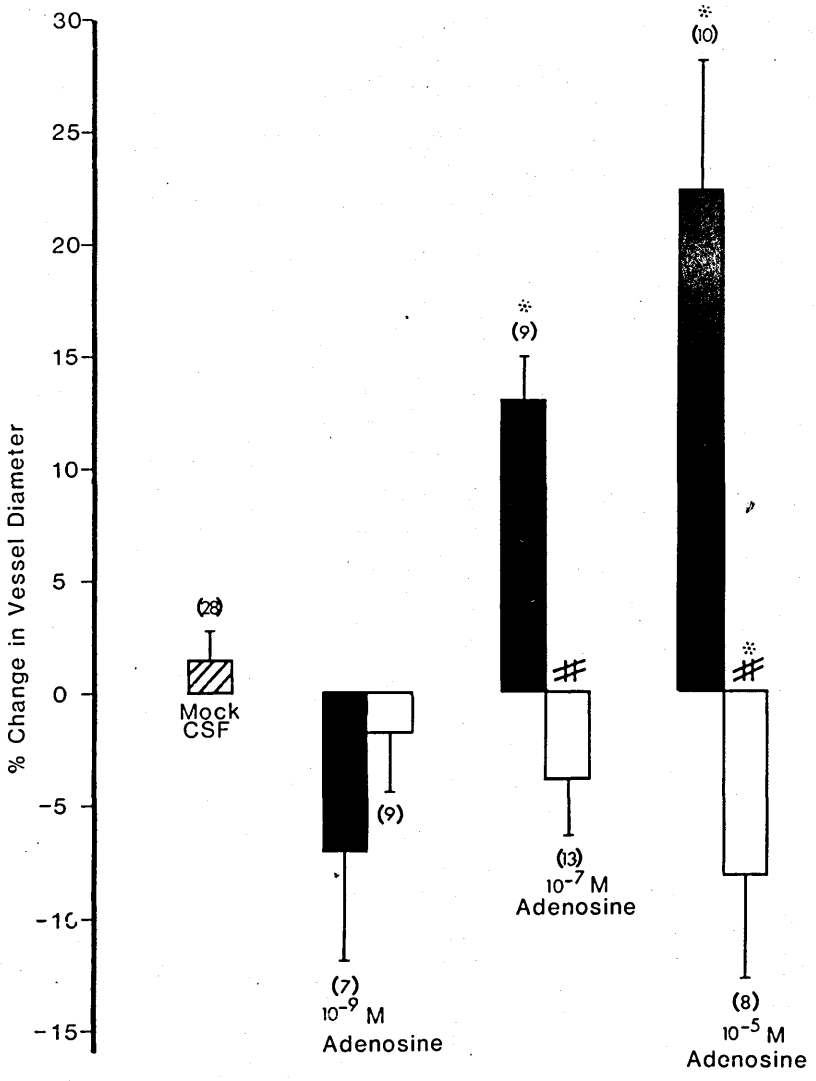


Fig.III.10 EFFECTS OF ADENOSINE ON CAT PIAL VESSEL DIAMETER IN THE ABSENCE AND PRESENCE OF 8-PHENYLTHEOPHYLLINE



Agonist alone

Agonist plus 10^{-9}M 8-phenyltheophylline

All values are mean ± standard error of mean.

Figures in parentheses indicate n value.

* Significantly different from mock CSF control ($P<0.05$: Students t-test incorporating Bonferroni correction factor)

\# Significantly different from agonist only response ($P<0.05$: Student,s t-test incorporating Bonferroni correction factor)

N^6-cyclohexyladenosine (CHA), at concentrations of 10^{-9}M, 10^{-7}M and 10^{-5}M produced dilations of 10% (vessel calibre range 66-248μm; mean vessel calibre ± standard deviation = 139 ± 67μm), 8% (vessel calibre range 46-251μm; mean vessel calibre ± standard deviation = 110 ± 63μm) and 21% (vessel calibre range 68-189μm; mean vessel calibre ± standard deviation = 127 ± 42μm), respectively. The responses obtained to both 10^{-7}M and 10^{-5}M CHA were significantly different from that of the mock CSF control and both, in turn, were significantly antagonised by 10^{-9}M 8-phenyltheophylline (see Table III.5 and Fig III.11).

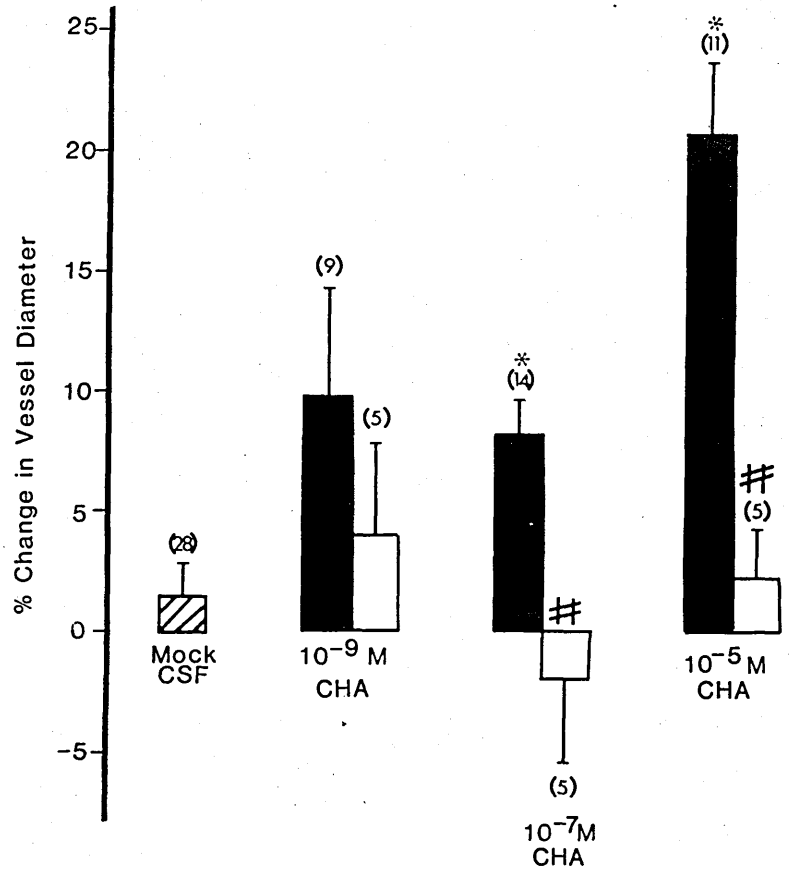
5'-(N-ethyl)carboxamidoadenosine (NECA), also at concentrations of 10^{-9}M, 10^{-7}M and 10^{-5}M produced dilatations of 9% (vessel calibre range 48-182μm; mean vessel calibre ± standard deviation = 111 ± 42μm), 15% (vessel calibre range 63-179μm; mean vessel calibre ± standard deviation = 119 ± 43μm) and 18% (vessel calibre range 57 -192μm; mean vessel calibre ± standard deviation = 114 ± 51μm), respectively. The responses obtained to all three concentrations of NECA were significantly different from that of the mock CSF control and both the 10^{-7}M and 10^{-5}M, in turn, were significantly antagonised by 10^{-9}M 8-phenyltheophylline (see Table III.6 and Fig.III.12).

The response obtained to each compound was not dependent on either the resting vessel calibre or the mean arterial blood pressure of the cat at the time of application of compound.

2.2.Discussion

The results obtained in this study show that in pial vessels which are responsive (as seen by the action of 10mM K^+) adenosine, CHA and NECA all produce increases in vessel calibre, with the exception of 10^{-9}M adenosine which produces a 7% constriction. The/..

Fig. III.11 EFFECTS OF N^6-CYCLOHEXYLADENOSINE (CHA) ON CAT PIAL VESSEL DIAMETER IN THE ABSENCE AND PRESENCE OF 8-PHENYLTHEOPHYLLINE



Agonist alone

Agonist plus 10^{-9}M 8-phenyltheophylline

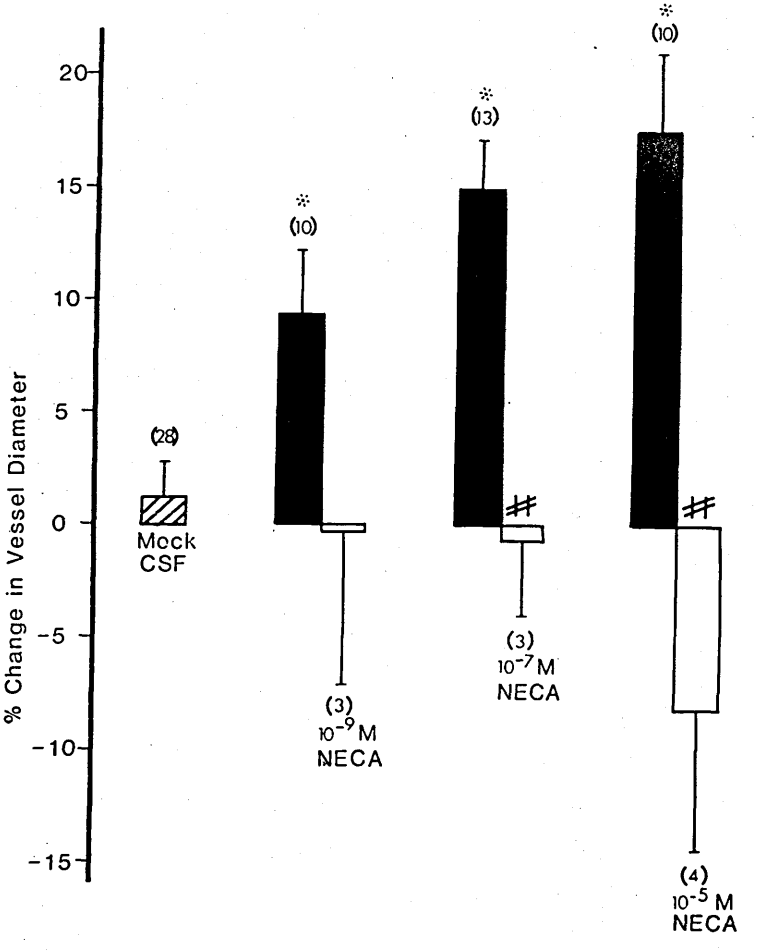
All values are mean ± standard error of mean.

Figures in parentheses indicate n value.

* Significantly different from mock CSF control
(P<0.05: Students t-test incorporating Bonferroni correction factor)

\# Significantly different from agonist only response
(P<0.05: Student,s t-test incorporating Bonferroni correction factor)

Fig.Ⅲ.12 EFFECTS OF 5'-(N-ETHYL)CARBOXAMIDOADENOSINE (NECA) ON CAT PIAL VESSEL DIAMETER IN THE ABSENCE AND PRESENCE OF 8-PHENYLTHEOPHYLLINE



Agonist alone

Agonist plus 10^{-9}M 8-phenyltheophylline

All values are mean ± standard error of mean.

Figures in parentheses indicate n value.

* Significantly different from mock CSF control
($P<0.05$: Students t-test incorporating Bonferroni correction factor)

\# Significantly different from agonist only response
($P<0.05$: Student,s t-test incorporating Bonferroni correction factor)

The change in vessel calibre produced by 10^{-5}M adenosine (i.e. a 22% dilatation at a P_{CO_2} of approximately 30mmHg) compares favourably with values obtained in previous studies using cat pial vessels: a 14% dilatation at a P_{CO_2} of 25mmHg and a 29% dilatation at a P_{CO_2} of 34mmHg (Gregory, Boisvert & Harper, 1980) and a 24% dilatation at a P_{CO_2} of approximately 31mmHg)Wahl & Kuschinsky, 1976).

The results also confirm the vasodilatory properties of adenosine and its analogues which have been shown in isolated cerebral vessel preparations (Edvinsson & Fredholm 1983). Since the vasodilatory effects of adenosine and both the adenosine analogues CHA and NECA can be blocked by the A_1/A_2 adenosine receptor antagonist, 8-phenyltheophylline, and also since the A_2 receptor mediates vasodilatation (Edvinsson & Fredholm, 1983), it seems likely that the effects of all three compounds are mediated via an A_2 receptor interaction. However, as was found in the isolated vessel study (Results Section 1), adenosine and NECA are seen to constrict the vessels in the presence of 10^{-9}M 8-phenyltheophylline)see Tables III.4 and III.6) and this may suggest the involvement of other mechanisms as well.

2.3. Summary of cat pial vessel results

Compound	Vasodilatation (D) or Constriction (C)	Antagonised by 8-phenyltheophylline (8-PT)	Effect in presence of 8-PT:D or C
10^{-9}M Adenosine	C	No	C
10^{-7}M Adenosine	D	Yes	C
10^{-5}M Adenosine	D	Yes	C
10^{-9}M CHA	D	No	D
10^{-7}M CHA	D	Yes	C
10^{-5}M CHA	D	Yes	D
10^{-9}M NECA	D	No	C
10^{-7}M NECA	D	Yes	C
10^{-5}M NECA	D	Yes	C

3.LASER-DOPPLER FLOWMETRY

3.1.Effect of the adenosine analogue N^6-cyclohexyladenosine (CHA) on cerebral blood flow (CBF) as measured in the rat by laser-Doppler flowmetry

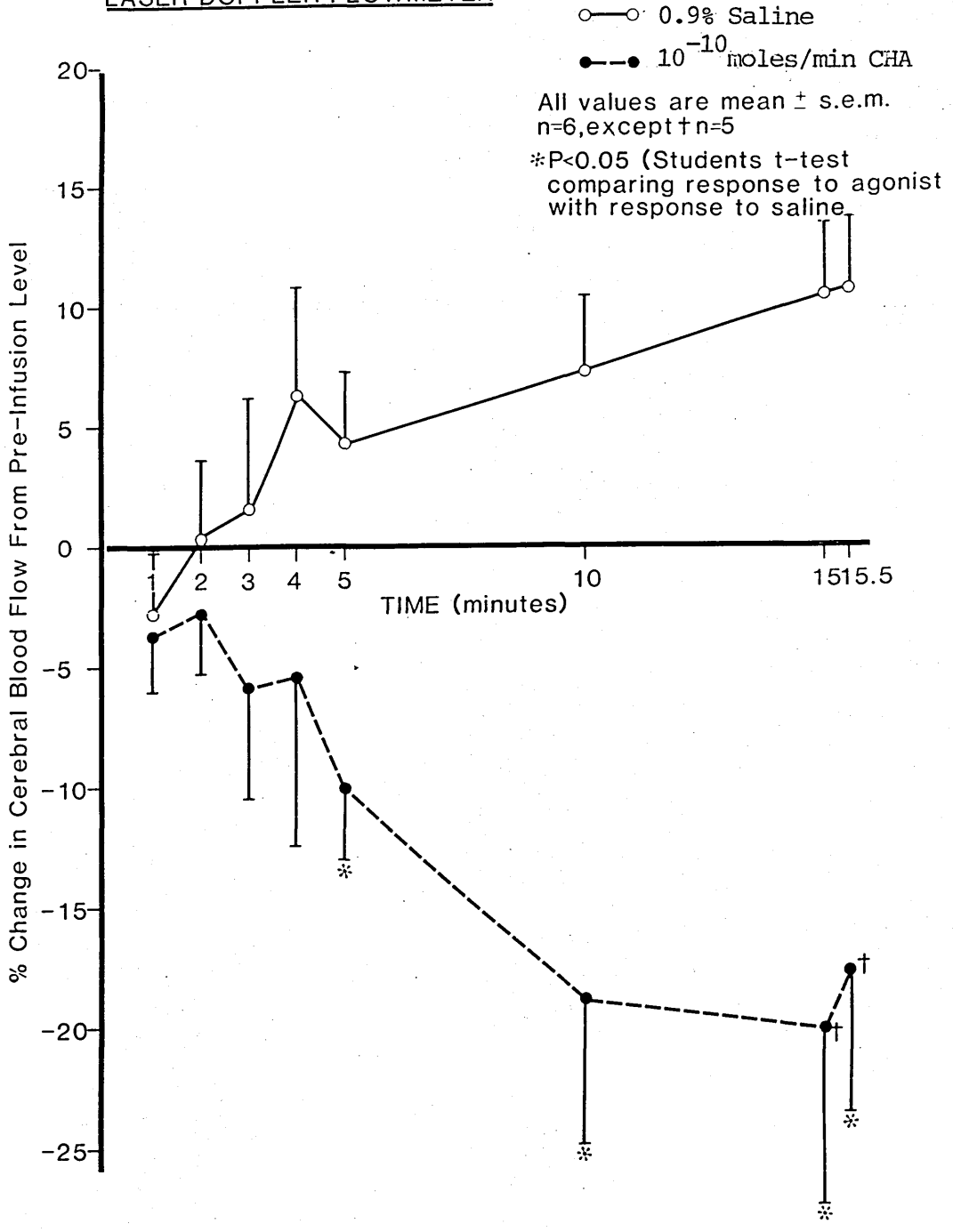
The percentage changes in CBF produced by infusions of saline were taken as the control values and the changes produced by CHA infusion were compared to those controls at each time point (see Table III.7 and Fig.III.13). Within 5 minutes CHA had decreased CBF by 10% (saline, 4% increase); after 10 minutes CHA had decreased CBF by 19% (saline, 7% increase); after 15 minutes CHA had decreased CBF by 20% (saline, 11% increase); and after 15.5 minutes CHA had decreased CBF by 18% (saline 11% increase). The changes produced by CHA were significant at 5, 10, 15 and 15.5 minutes.

Various physiological parameters were monitored before both the saline and CHA infusions (see Table III.8) but there was found to be no difference between the two groups for each of the parameters studied.

3.2.Discussion

The results show that the adenosine analogue, CHA, produces a decrease in CBF and that this effect is monophasic i.e. at no time is there any apparent increase in CBF. One must now explain, however, how CHA, which has already been shown to be a vasodilatator in earlier studies, is decreasing flow. One possible explanation could be that at the low concentration of CHA being used (10^{-10}moles/min.) in order to prevent any hypotensive effect, the CHA is acting either on a different adenosine receptor or on a/..

Fig.III.13 EFFECTS OF INFUSION OF SALINE AND CHA ON CEREBRAL BLOOD FLOW IN ANAESTHETISED RATS AS MEASURED BY LASER DOPPLER FLOWMETER



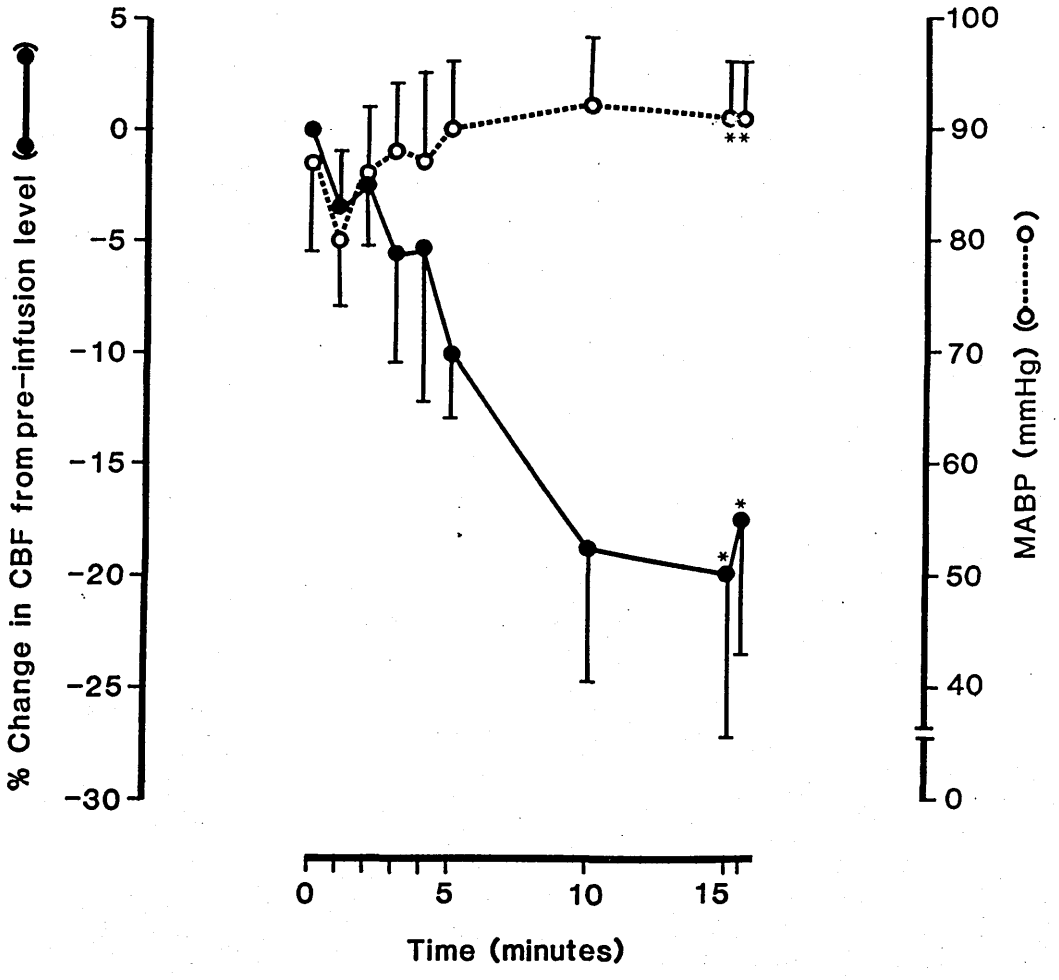
a completely different receptor type, rather than the A_2 receptor which is known to mediate vasodilatation. The results obtained in this study, however, do not allow us to identify the mechanism mediating this vasoconstrictive action of CHA.

It is more likely that the laser-Doppler technique is measuring red cell velocity, however, a decrease in velocity is probably due to an increase in resistance to the flow i.e. a vasoconstriction, which would in turn decrease flow. The rises in flow caused by saline may be due to a haemodilution effect which the flowmeter picks up as an increase in flow.

One factor which can not be used to explain the decreases in CBF produced by CHA is any change in the mean arterial blood pressure (MABP) since as Fig.III.14 shows there is no significant alteration in the MABP during the CHA infusion.

Since the maximum effect of the CHA is seen after 15 minutes of the infusion it was decided to monitor the effects of this compound and other adenosine related compounds using [^{14}C]-iodo-antipyrine autoradiography to measure local cerebral blood flow (LCBF) after a 15 minute infusion of the compounds. The purpose of this is to determine whether the effects of the compounds on LCBF in 35 discrete brain regions are the same as the effect of CHA on cortical blood velocity/flow as measured in this study.

Fig. III.14 CHANGES IN MEAN ARTERIAL BLOOD PRESSURE (MABP) WITH RESPECT TO CHANGES IN CEREBRAL BLOOD FLOW (CBF) DURING INFUSION OF CHA



All bars indicate mean ± standard error of mean.
n=6 in all cases, except * where n=5

4. [^{14}C]-IODOANTIPYRINE AUTORADIOGRAPHY

4.1. Effects of a 15 minute infusion of adenosine, ATP and some adenosine analogues on local cerebral blood flow (LCBF) in the rat as measured by [^{14}C]-iodoantipyrine (IAP) autoradiography

The following physiological parameters were monitored throughout the course of all the experiments: PCO_2; PO_2; mean arterial blood pressure; core body temperature; bicarbonate (HCO_3^-) concentration; base excess and pH. No significant changes in any of these parameters were found in any of these experiments reported in this section (see Tables III.15, III.16 and III.17).

Adenosine and ATP, at concentrations of 10^{-7} moles/min and $3x10^{-7}$ moles/min, did not produce any significant change in LCBF either ipsilateral or contralateral to the side of infusion (see Tables III.9 and III.10). Both adenosine and ATP, however, showed a tendency to increase LCBF in a number of the 34 brain regions studied e.g. the visual and parietal cortices, the lateral geniculate and the globus pallidus.

5'-(N-ethyl)carboxamidoadenosine (NECA) at a concentration of 10^{-11} moles/min, did not produce any significant change in LCBF either ipsilateral or contralateral to the side of infusion (see Tables III.11 and III.12). As was the case for adenosine and ATP, however, NECA also showed a tendency to increase LCBF in a number of the 32 brain regions studied e.g. the visual, auditory and anterior cingulate cortices and the genu.

2-chloroadenosine (2-CADO), at a concentration of 10^{-9} moles/min, produced significant decreases in LCBF in 3 of the 32 ipsilateral brain regions studied: globus pallidus, red nucleus and cerebellum nucleus (see Table III.11 and Figs.III.21, III.24 and III.25)./..

III.25). 2-CADO also produced significant decreases in 3 of the 33 contralateral brain regions studied: sensory-motor cortex, cochlear nucleus and cerebellum nucleus (see Table III.12). 2-CADO also showed a tendency to decrease LCBF in the other brain regions where significant changes were not evident.

N^6-cyclohexyladenosine (CHA), at a concentration of 10^{-10} moles/min, produced significant decreases in 11 of the 32 ipsilateral brain regions studied: sensory-motor cortex, frontal cortex, medial geniculate, lateral geniculate, lateral habenula, caudate nucleus, globus pallidus, superior olive, dentate gyrus, cerebellum nucleus and cerebellum hemisphere (see Table III.11 and Figs.III.15 - III.23, III.25 and III.26). CHA also produced significant decreases in LCBF in 6 of the 33 contralateral brain regions studied: sensory-motor cortex, globus pallidus, vestibular nucleus, red nucleus, cerebellum nucleus and cerebellum hemisphere (see Table III.12). The effect of CHA on LCBF can be seen to be apparent at a variety of concentrations (see Tables III.13 and III.14). The effect of CHA on LCBF can be seen to be apparent with particular reference to the ipsilateral cortical structures (see Fig.III.27).

4.2.Discussion

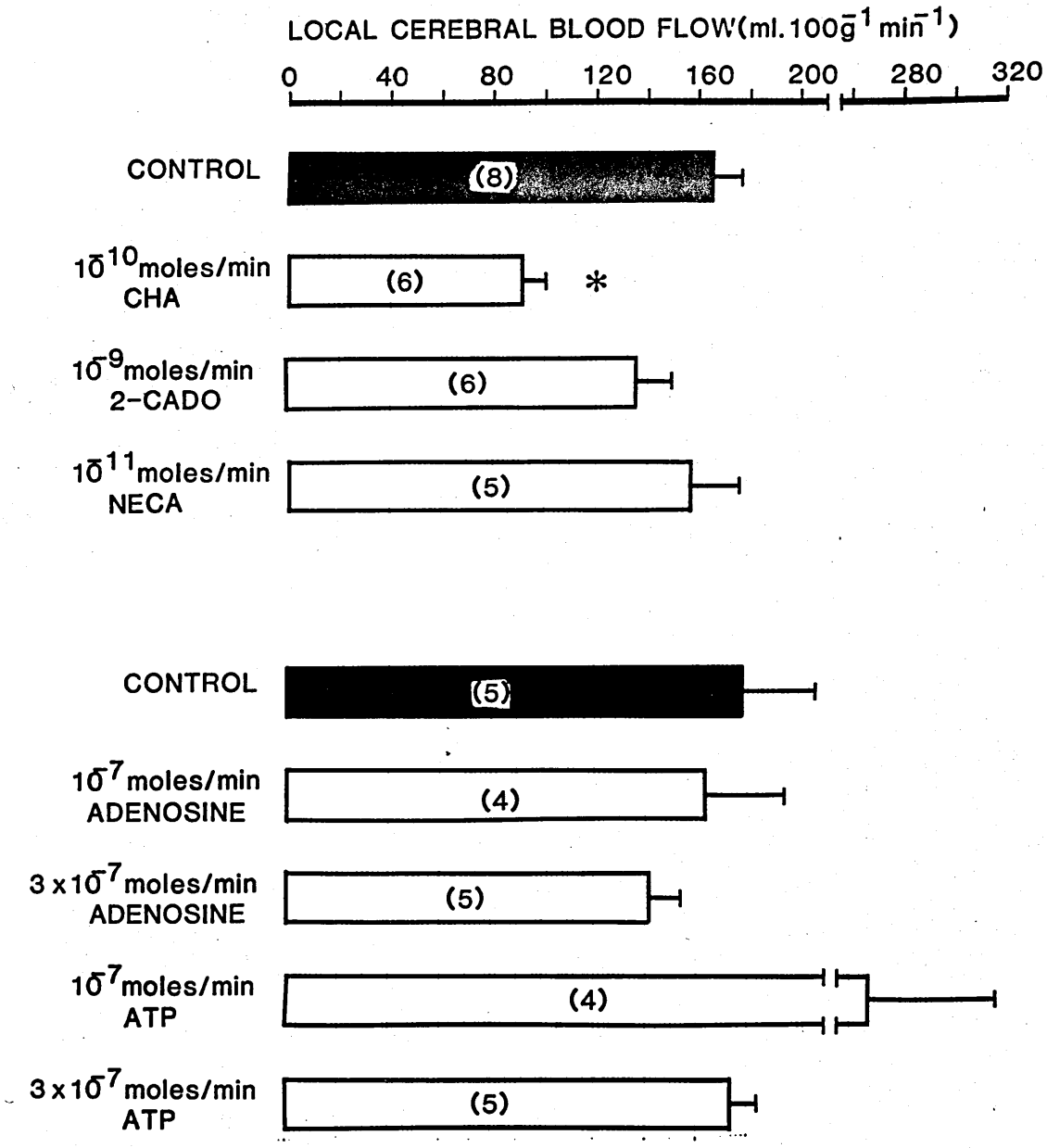
Since adenosine and the adenosine analogues have previously been shown to be vasodilatators both in vitro and in vivo (Results Section 1 and 2) one would expect them to produce increases in LCBF. This is, in fact, the case with adenosine, ATP and NECA which all show a tendency to increase LCBF. These increases in LCBF, however, are not significant unlike the changes produced by adenosine and ATP in other species. Adenosine and ATP, infused via the carotid artery in/..

in baboons, have been shown to produce significant increases in global CBF (Forrester et al.,1979). Adenosine, injected via the carotid artery at a variety of concentrations, has been shown to increase blood flow in the cerebrum, brain stem and cerebellum of the dog (Heistad et al.,1981). The lack of a significant effect with 15 minute infusions of adenosine or ATP could be due to degradation of the compounds by adenosine deaminase, thus limiting the concentration of the compounds reaching the brain.

An explanation still has to be found for the decreases in flow produced by 2-CADO and CHA. 2-CADO, along with adenosine and NECA, has been shown to increase local cerebral blood flow in the rat when infused locally into the brain tissue (van Wylen et al.,1987). The results also show that decreases in local cerebral blood flow were apparent in areas other than those supplied by the internal carotid artery and also in the hemisphere contralateral to the side of infusion. Since both CHA and 2-CADO are stable analogues of adenosine and therefore not susceptible to degradation by the enzyme adenosine deaminase, these changes probably occur as a consequence of sufficient concentrations of CHA and 2-CADO circulating round the animal and passing back into the brain via the contralateral internal carotid artery, as well as via the other arteries (i.e. the basilar artery) supplying both the contra- and ipsilateral hemispheres of the brain.

One possible explanation for the observed decreases in flow could be that the CHA and 2-CADO are acting on another receptor to constrict the vessels rather than the A_2 receptor known to mediate vasodilatation. One other possible explanation for the observed changes in blood flow could be that any vascular actions of CHA and 2-CADO could be secondary to any metabolic changes that they may/..

Fig. III .15 LCBF IN THE IPSILATERAL SENSORY MOTOR CORTEX

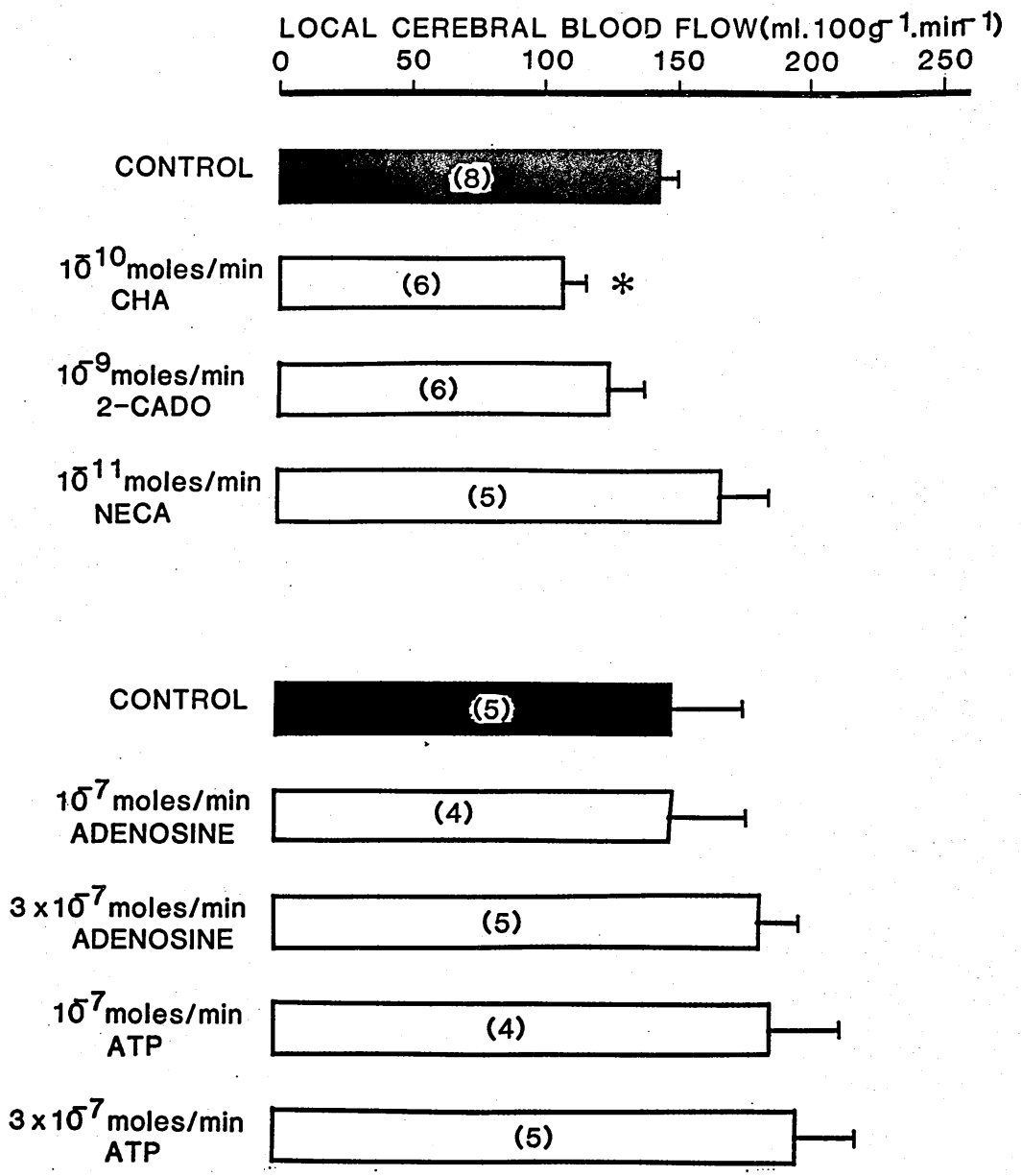


All bars indicate mean ± standard error of mean.

Figures in parentheses indicate n value

* $P < 0.05$ (Students t-test with Bonferroni correction factor)

Fig. III.16 LCBF IN THE IPSILATERAL FRONTAL CORTEX

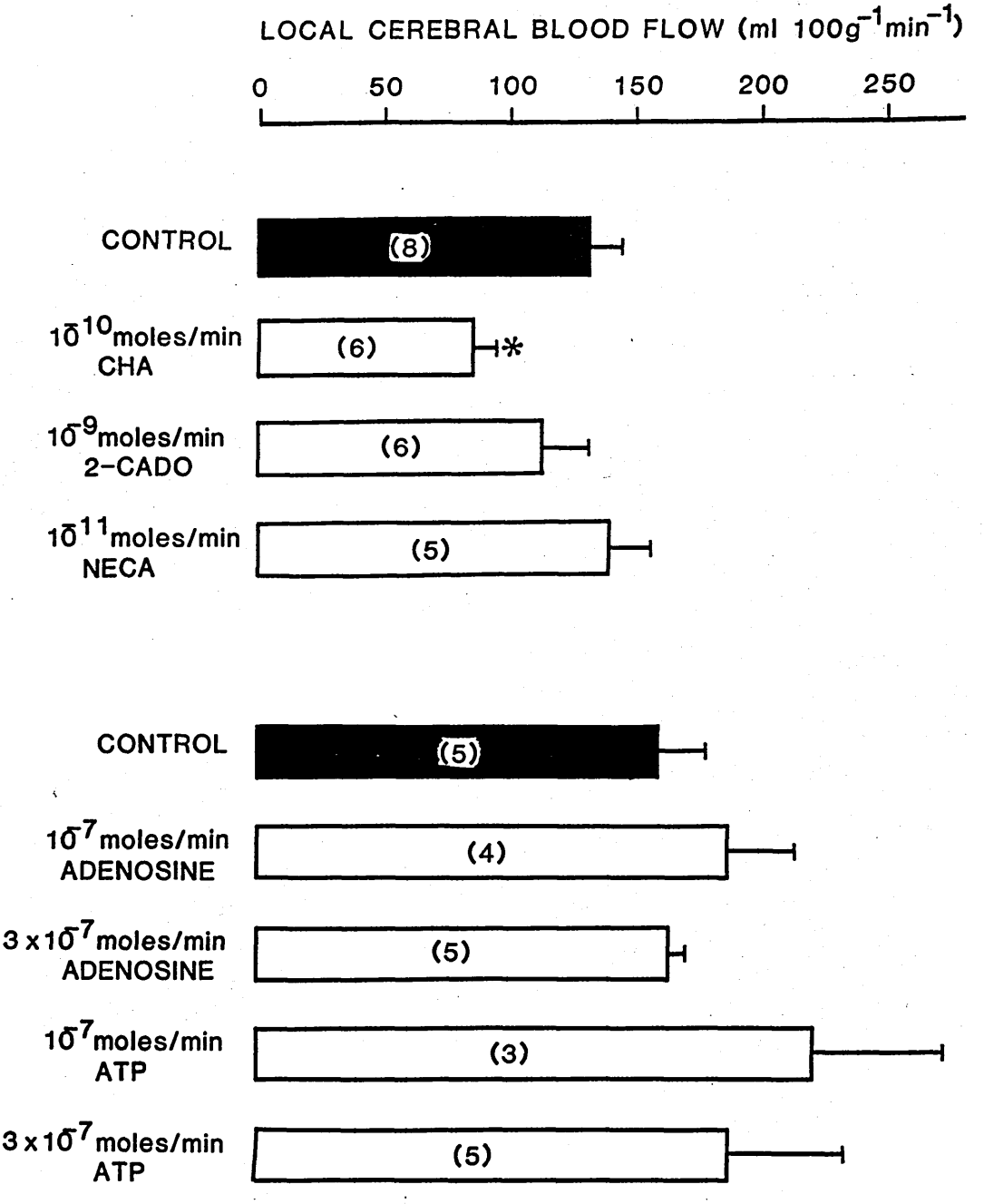


All bars indicate mean ± standard error of mean .

Figures in parentheses indicate n value

* $P < 0.05$(Students t-test with Bonferroni correction factor)

Fig.III.17 LCBF IN THE IPSILATERAL MEDIAL GENICULATE

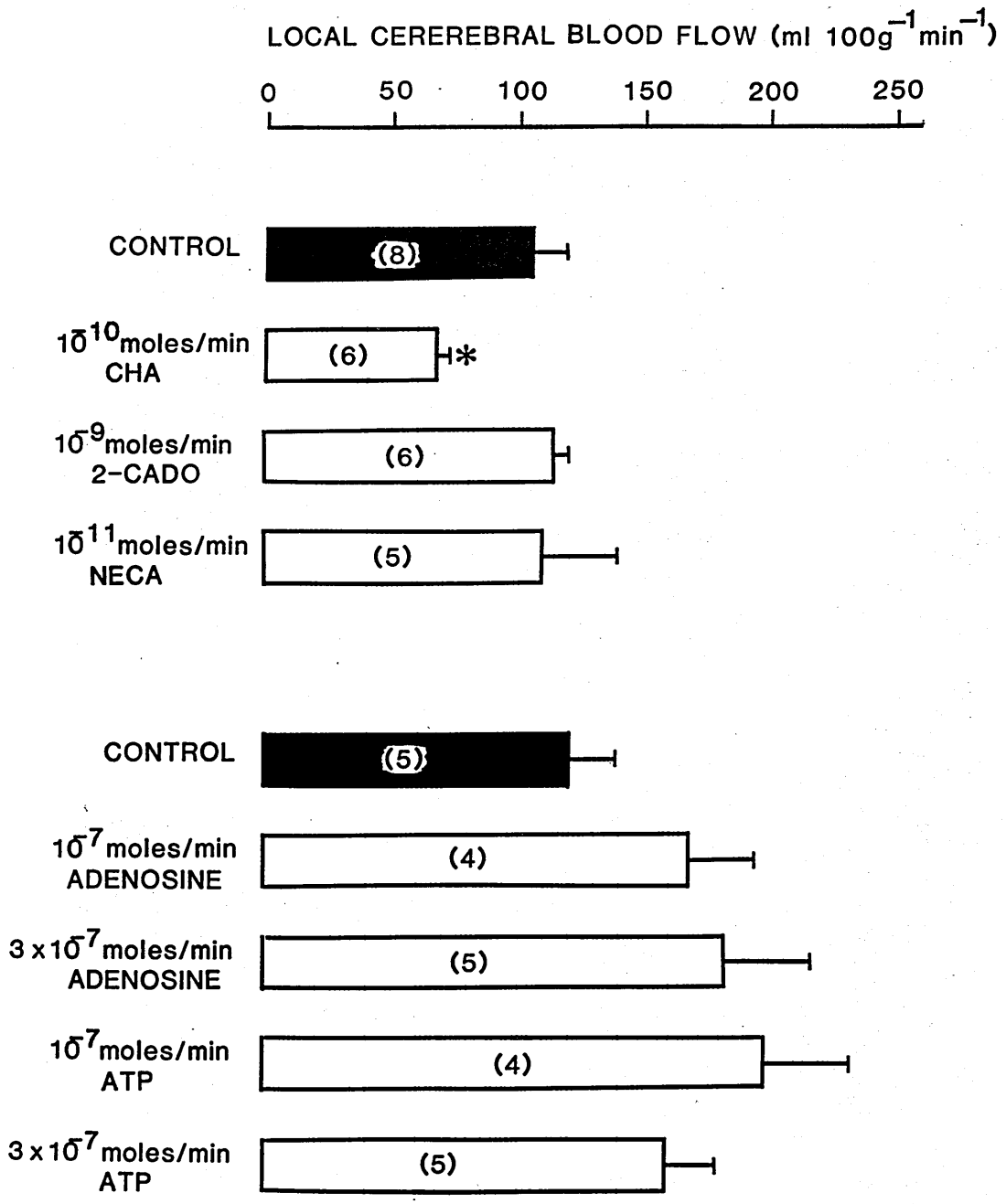


All bars indicate mean ± standard error of mean .

Figures in parentheses indicate n value

* $P < 0.05$(Students t-test with Bonferroni correction factor)

Fig.III 18 LCBF IN THE IPSILATERAL LATERAL GENICULATE

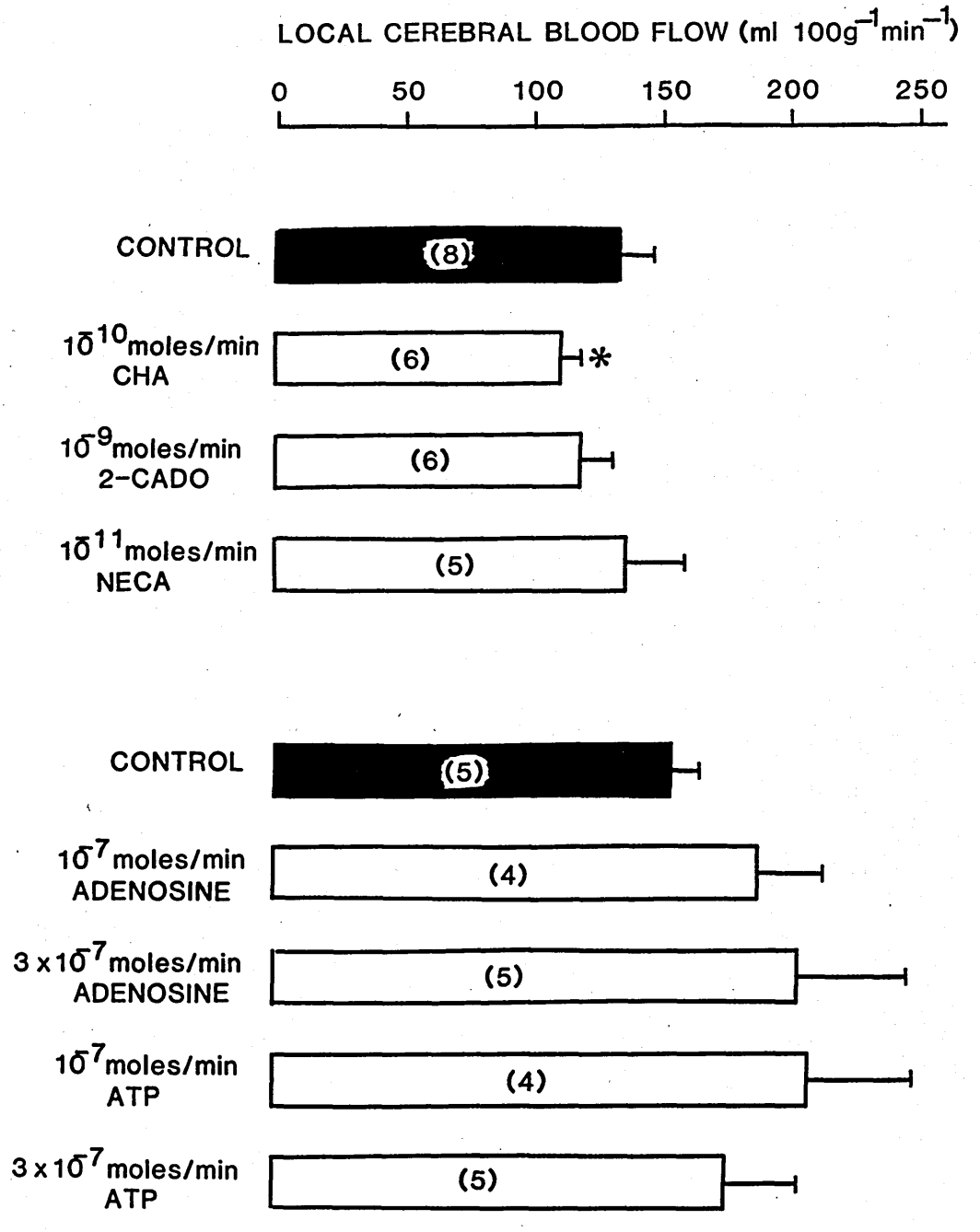


All bars indicate mean ± standard error of mean .

Figures in parentheses indicate n value

* $P < 0.05$ (Students t-test with Bonferroni correction factor)

Fig.III19 LCBF IN THE IPSILATERAL LATERAL HABENULA

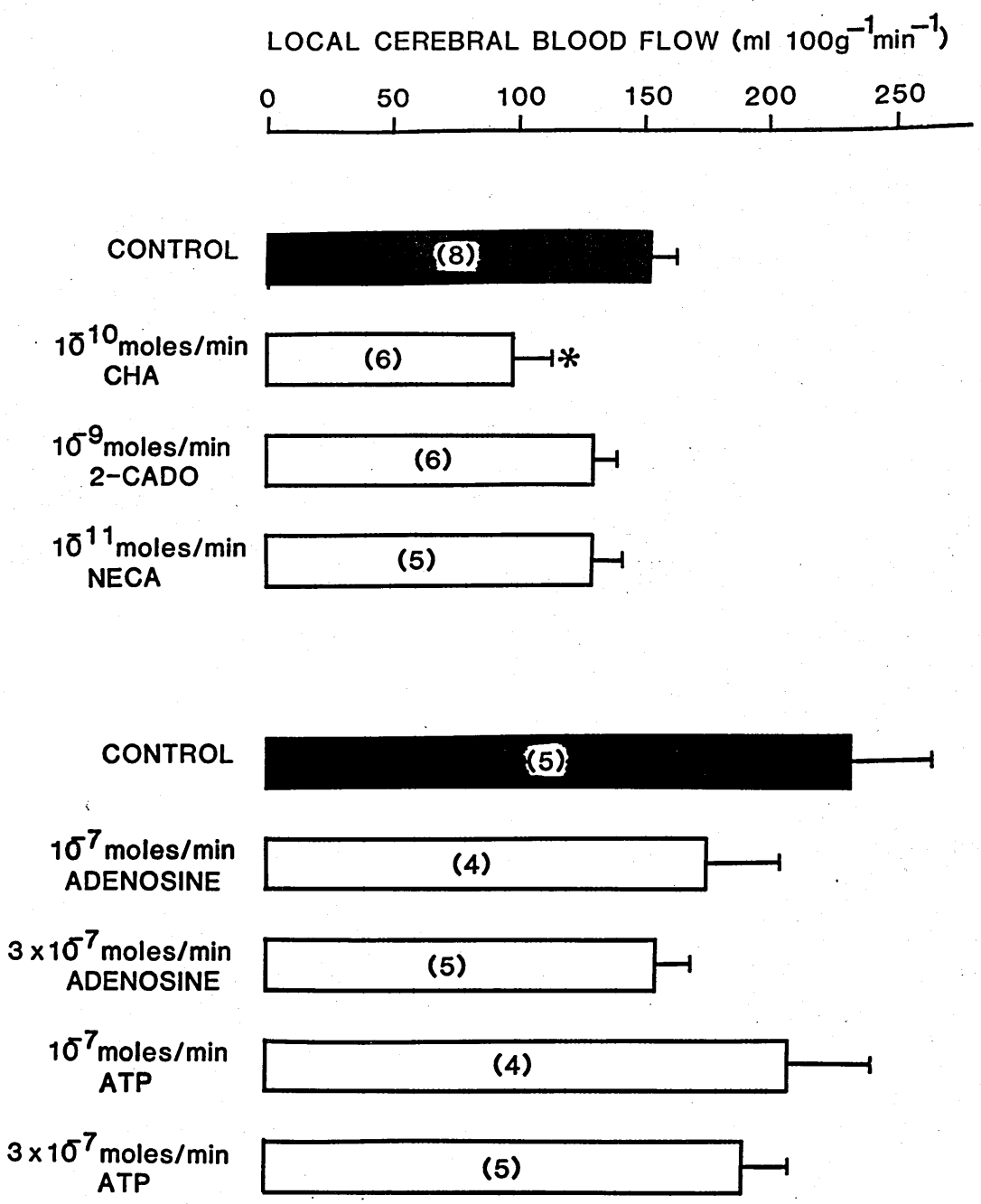


All bars indicate mean ± standard error of mean .

Figures in parentheses indicate n value

* $P < 0.05$(Students t-test with Bonferroni correction factor)

Fig.III 20 LCBF IN THE IPSILATERAL CAUDATE NUCLEUS

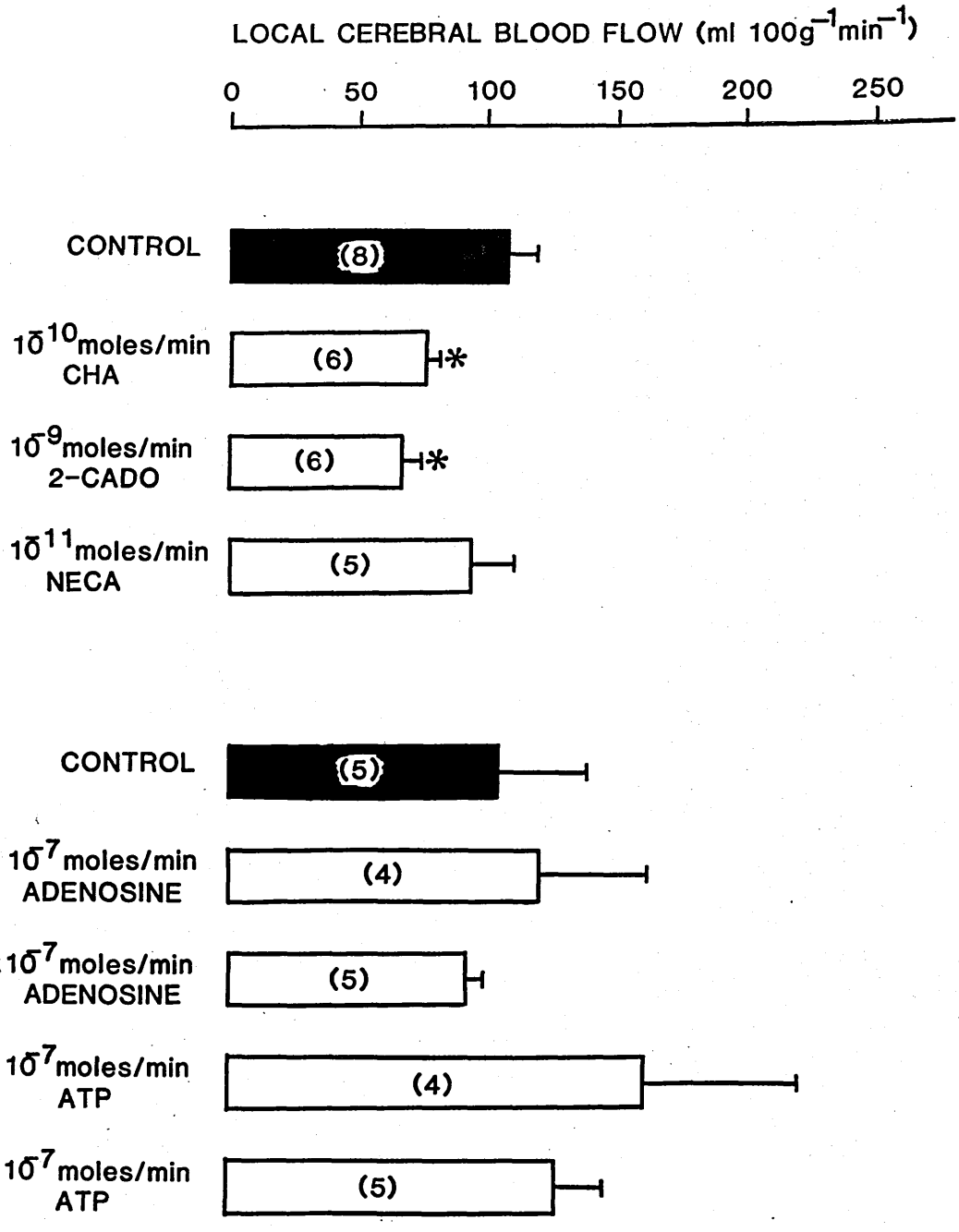


All bars indicate mean ± standard error of mean .

Figures in parentheses indicate n value

* $P < 0.05$(Students t-test with Bonferroni correction factor)

Fig.III 21 LCBF IN THE IPSILATERAL GLOBUS PALLIDUS

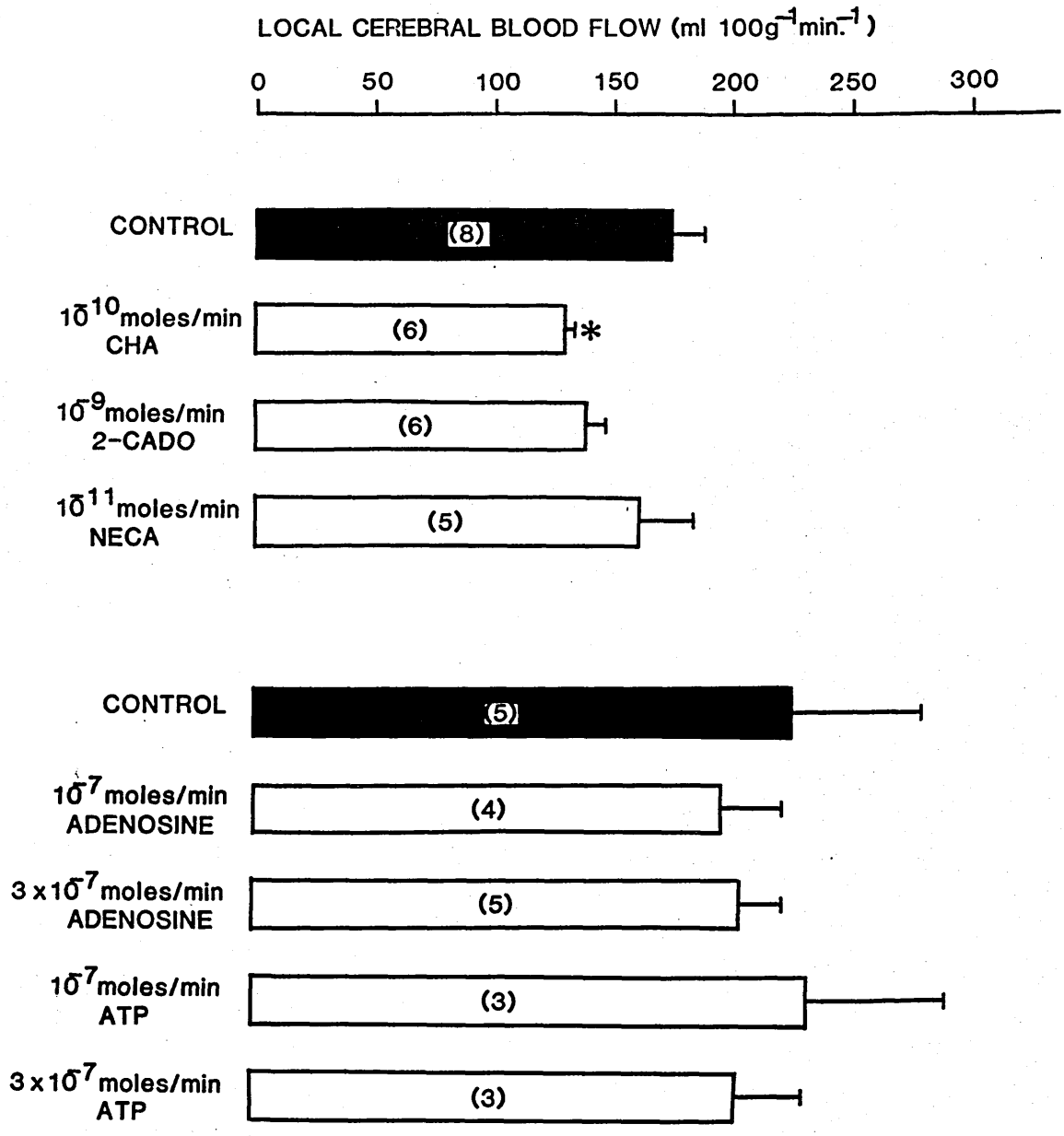


All bars indicate mean ± standard error of mean .

Figures in parentheses indicate n value

* $P < 0.05$(Students t-test with Bonferroni correction factor)

Fig.III 22 LCBF IN THE IPSILATERAL SUPERIOR OLIVE

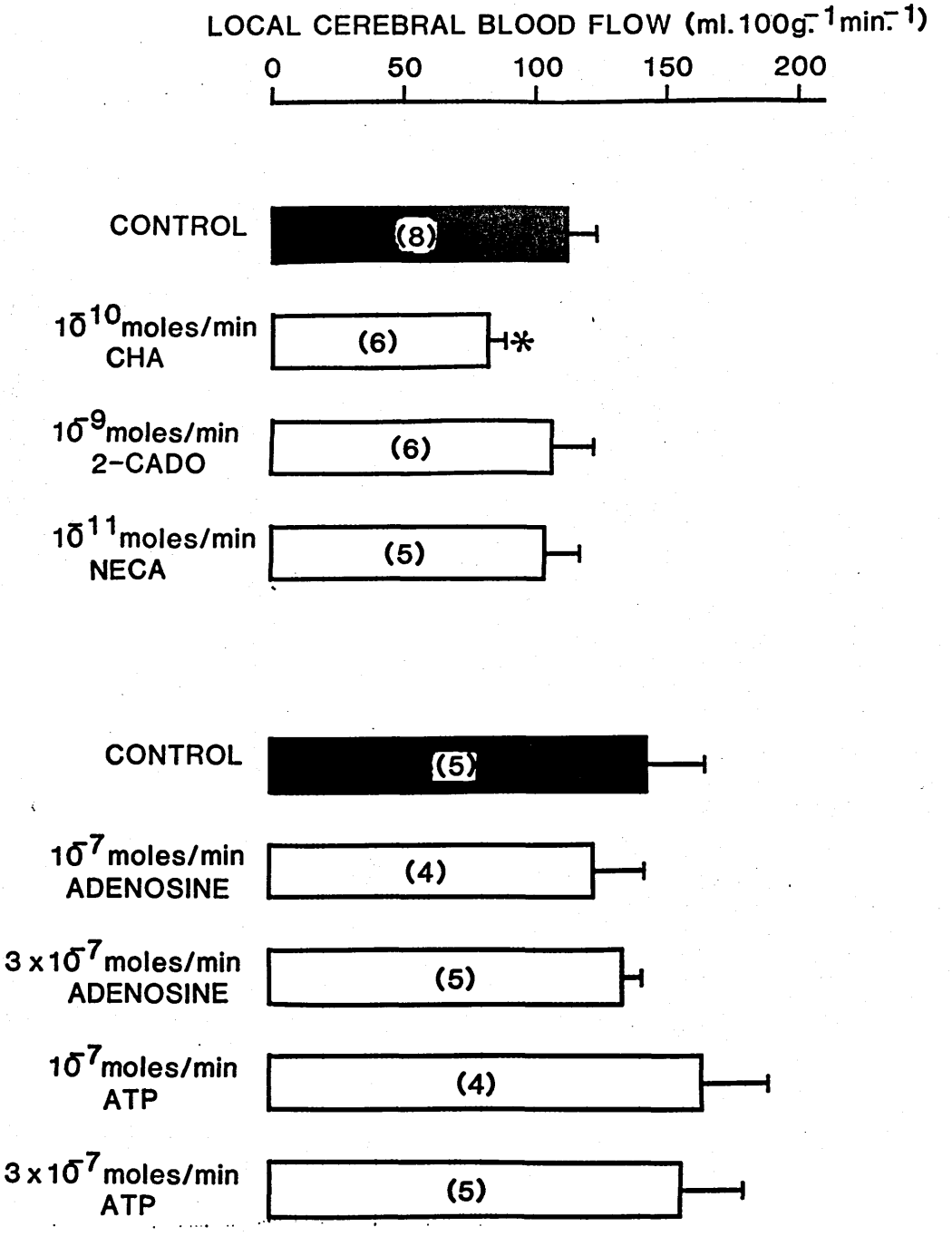


All bars indicate mean ± standard error of mean .

Figures in parentheses indicate n value

* $P < 0.05$(Students t-test with Bonferroni correction factor)

Fig. Ⅲ.23 LCBF IN THE IPSILATERAL DENTATE GYRUS

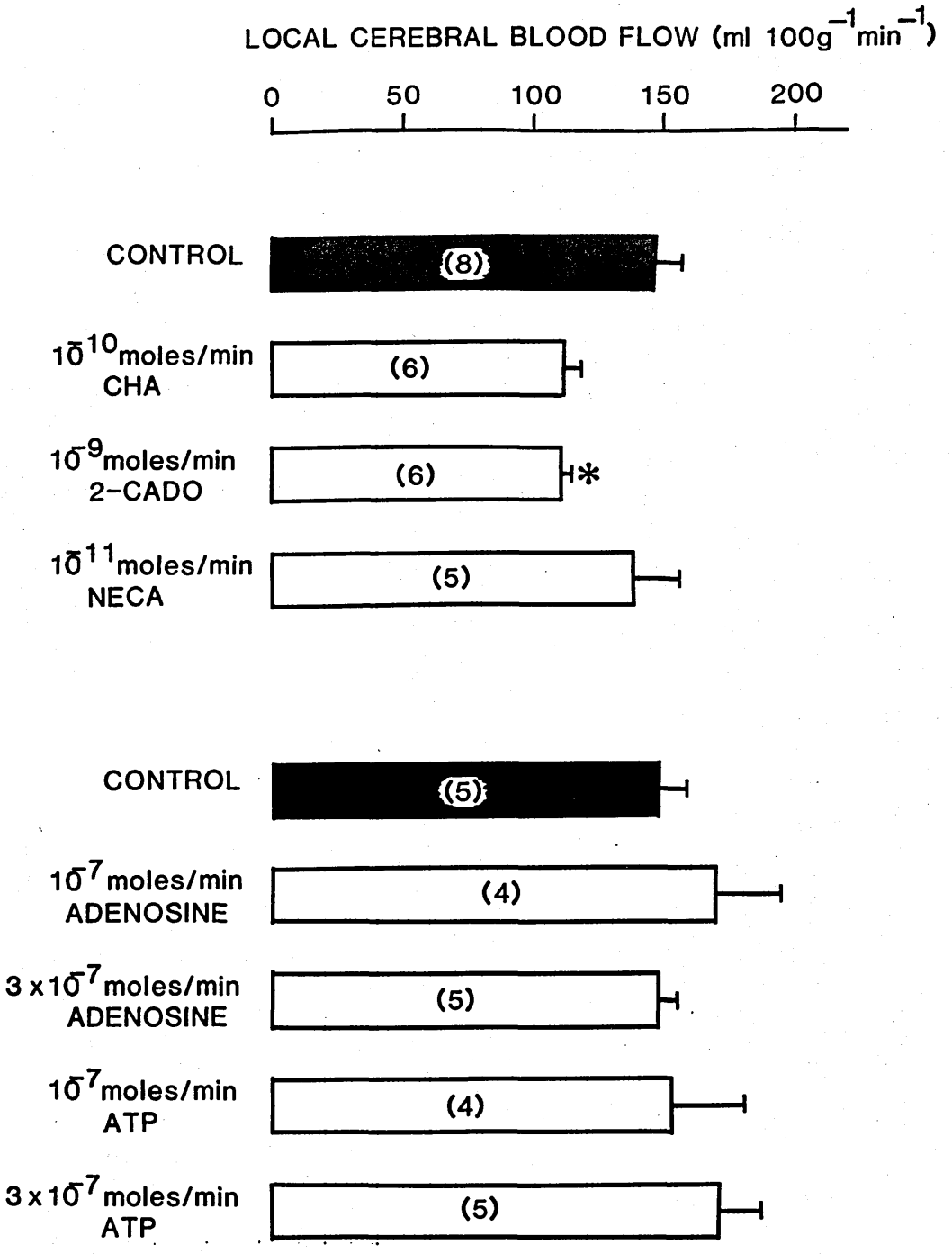


All bars indicate mean ± standard error of mean.

Figures in parentheses indicate n value

* $P < 0.05$ (Students t-test with Bonferroni correction factor)

Fig. Ⅲ 24 LCBF IN THE IPSILATERAL RED NUCLEUS

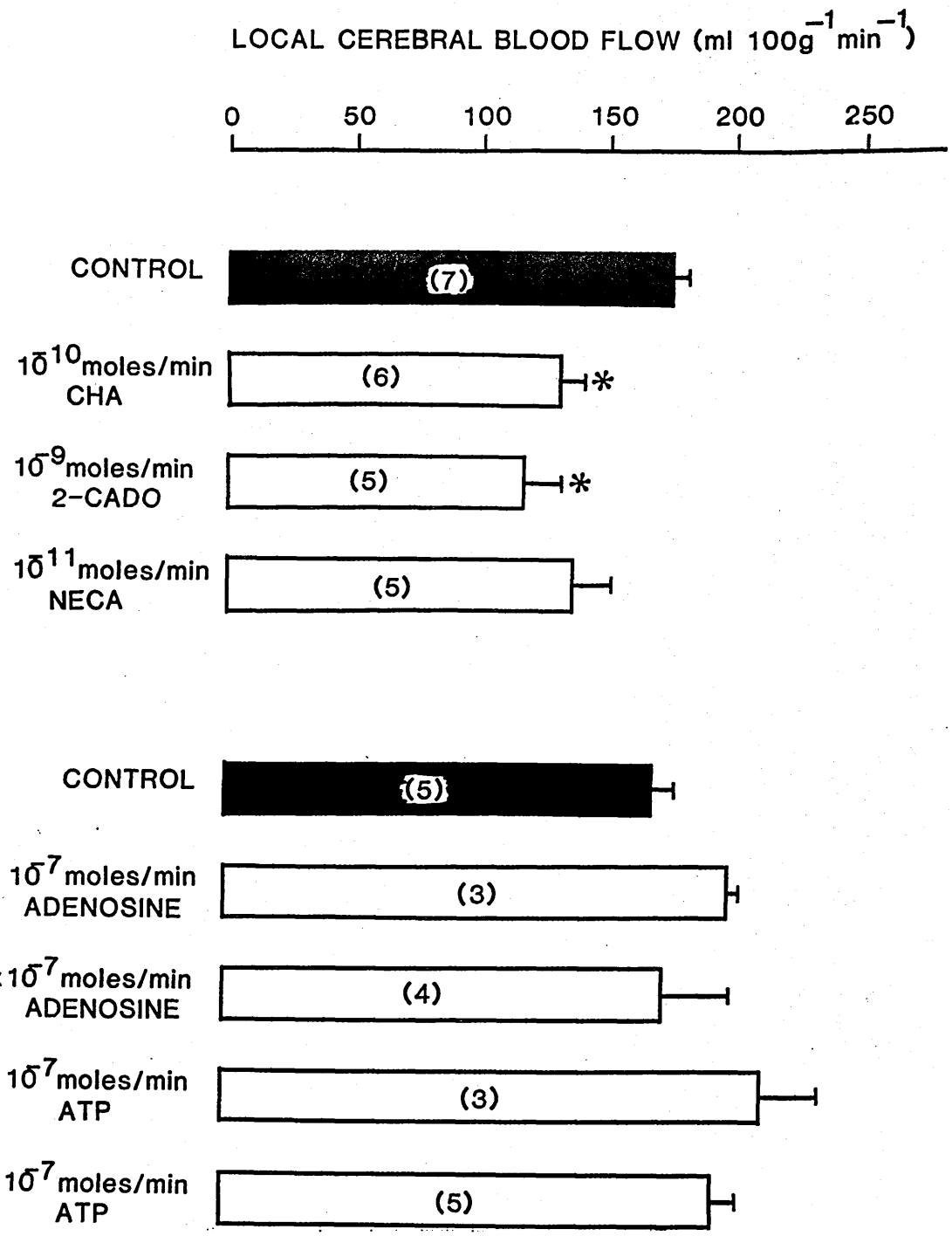


All bars indicate mean ± standard error of mean

Figures in parentheses indicate n value

* $P < 0.05$(Students t-test with Bonferroni correction factor)

Fig.III 25 LCBF IN THE IPSILATERAL CEREBELLUM NUCLEUS

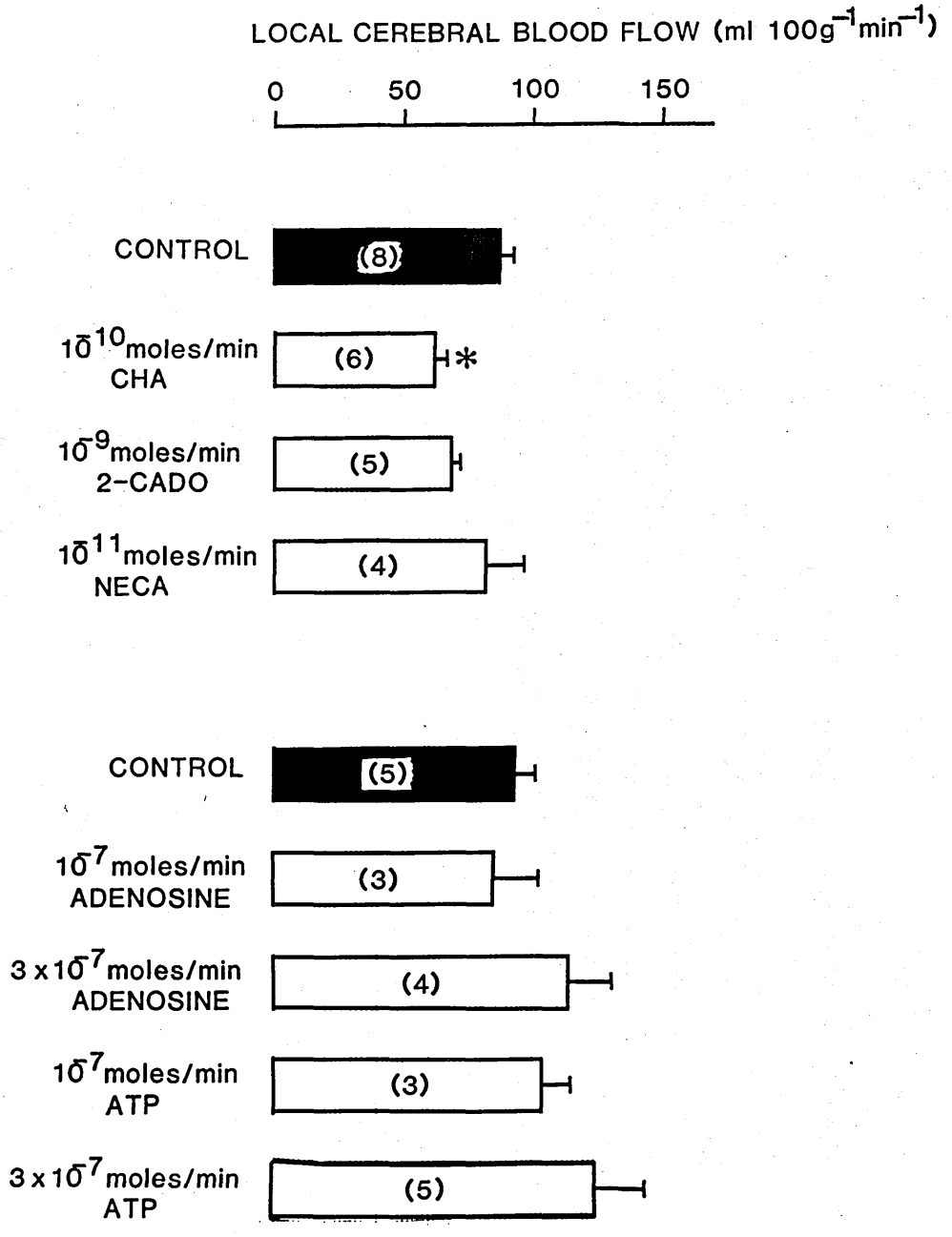


All bars indicate mean ± standard error of mean .

Figures in parentheses indicate n value

* $P < 0.05$(Students t-test with Bonferroni correction factor)

Fig.III 26 LCBF IN THE IPSILATERAL CEREBELLUM HEMISPHERE



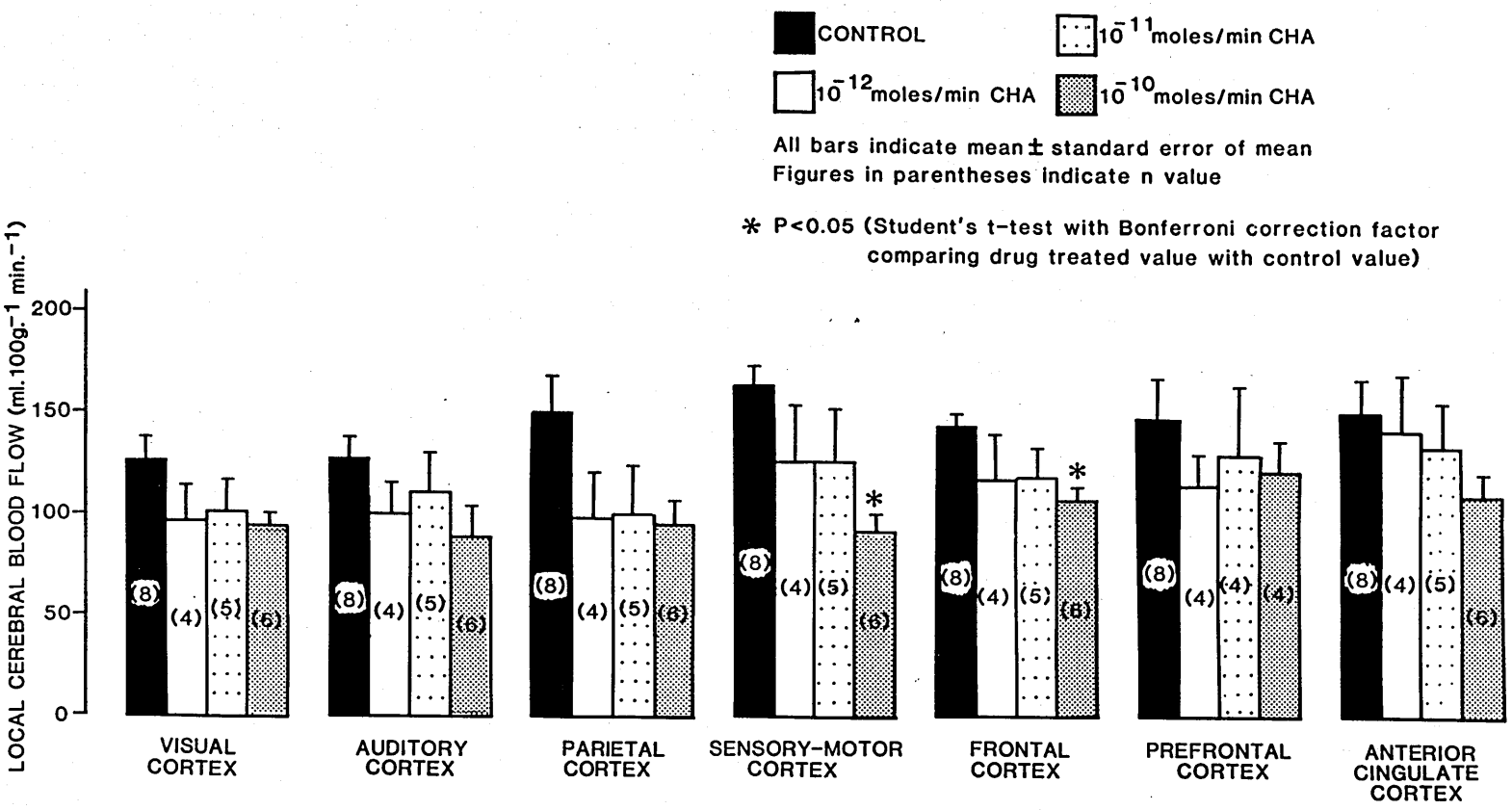
All bars indicate mean ± standard error of mean .

Figures in parentheses indicate n value

* $P < 0.05$(Students t-test with Bonferroni correction factor)

Fig.III-27

CHANGES IN LOCAL CEREBRAL BLOOD FLOW IN THE IPSILATERAL CORTICAL STRUCTURES PRODUCED BY VARIOUS CONCENTRATIONS OF CHA



may produce i.e. CHA and 2-CADO could be vasodilatators but also be producing a decrease in local cerebral glucose utilisation and, therefore, what we are seeing is a decrease in local cerebral blood flow in response to a decreased metabolic demand. It is this aspect of the possible reasons for the reductions in blood flow which is examined in the next section, Section 5, of the results.

The results show that there is no apparent difference between the effects of the analogues on the cortical and non-cortical brain regions, since significant changes were apparent in both. The changes in local cerebral blood flow observed were not only apparent in those areas supplied by the internal carotid artery. There is no physiological significance to the areas affected by by the analogues i.e. the effect appeared to be randomised.

4.3. Summary of the 15 minute infusion results

(i) Adenosine, ATP and NECA

No significant effect on LCBF. All show a tendency to increase LCBF.

(ii) CHA

Decreases LCBF. Significant decreases were observed in the following regions:

Structures	Ipsilateral	Contralateral
Cortical	Sensory-motor cx. Frontal	Sensory-motor Cx.
Diencephalic	Medial geniculate Lateral geniculate Lateral habenula Caudate nucleus Globus pallidus	Globus pallidus
Hindbrain	Superior olive	—
Mesencephalic	—	Red nucleus
Telencephalic	Dentate gyrus Cerebellum nucleus Cerebellum hem.	Cerebellum nucleus Cerebellum hem.
Fibre tracts	—	—

(iii) 2-CADO

Decreases LCBF. Significant decreases were observed in the following regions:

Structures	Ipsilateral	Contralateral
Cortical	—	Sensory-motor cx.
Diencephalic	Globus pallidus	—
Hindbrain	—	Cochlear nucleus
Mesencephalic	Red nucleus	—
Telencephalic	Cerebellum nucleus	Cerebellum nucleus
Fibre tracts	—	—

5. [^{14}C]-2-DEOXYGLUCOSE AUTORADIOGRAPHY

5.1. Effects of a 15 minute infusion of adenosine, ATP and some adenosine analogues on local cerebral glucose utilisation (LCGU) in the rat as measured by [^{14}C]-2-deoxyglucose (2-DG) autoradiography

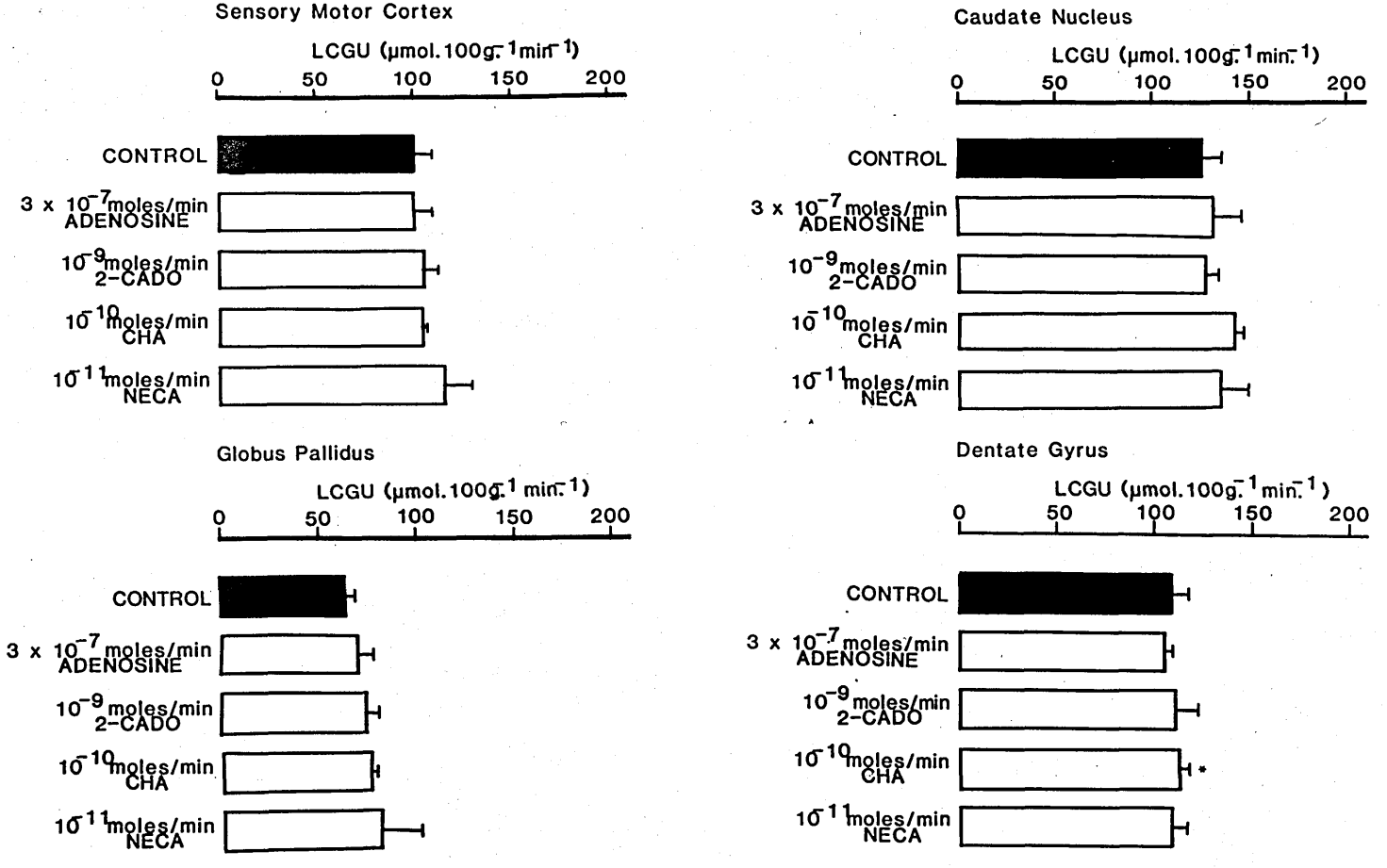
No significant alterations in local cerebral glucose utilisation were observed with any of the analogues used in either the ipsi- or contralateral hemispheres (see Tables III.18 and III.19). If any changes were apparent, however, they showed a tendency to produce increases, and these increases were most marked with CHA. Fig.III.28 shows how the compounds studied (i.e. adenosine, 2-CADO, CHA and NECA) have little effect on local cerebral glucose utilisation in four brain areas which showed significant alterations in local cerebral blood flow.

The following physiological parameters were monitored throughout the course of all the experiments : PCO_2, PO_2, mean arterial blood pressure, core body temperature, bicarbonate (HCO_3^-) concentration, base excess and pH. No significant changes in any of these parameters were found with any of the experiments reported in this section (see Table III.20).

5.2. Discussion

The results obtained using [^{14}C]-2-DG autoradiography show that none of the four compounds used produced any significant changes in LCGU. We have a situation, therefore, whereby 2-CADO and CHA are producing significant decreases in local cerebral blood flow and having no significant effect on glucose utilisation. The results/..

Fig. III .28 LOCAL CEREBRAL GLUCOSE UTILISATION (LCGU) IN THE IPSILATERAL HEMISPHERE



All bars indicate mean ± standard error of mean.

n=5 in all cases, except * where n=4

Figures in parentheses indicate n value.

results obtained indicate that the vascular effects of CHA and 2-CADO observed in 'Results Section 4' are not as a result of any alterations in metabolic demand.

6. THEOPHYLLINE STUDY (1)

6.1. Effects of an intraperitoneal (i.p.) injection of theophylline (30mg/kg) on the changes in local cerebral blood flow (LCBF) produced by a 15 minute infusion of the adenosine analogue, N^6-cyclohexyladenosine (CHA)

The following parameters were monitored throughout the course of all the experiments: PCO_2, PO_2, mean arterial blood pressure, core body temperature, bicarbonate (HCO_3^-) concentration, base excess and pH. No significant alterations in any of these parameters were evident in any of the experimental groups mentioned (see Table III.23).

When administered via the internal carotid artery CHA alone (at 10^{-10}moles/min) (plus saline i.p.) significantly decreased LCBF in the sensory motor cortex and caudate nucleus of the ipsilateral hemisphere (see Table III.21). CHA also showed a tendency to decrease LCBF in a number of other ipsilateral brain regions e.g. frontal cortex, ventrolateral thalamus, globus pallidus, internal capsule and hippocampus molecular layer (see Table III.21). In the contralateral hemisphere CHA had no significant effect on LCBF, however, it did show a tendency to decrease LCBF in a number of the contralateral brain regions e.g. visual and sensory motor cortices, globus pallidus (see Table III.22).

When theophylline alone (30mg/kg) was given intraperitoneally (with saline via the internal carotid artery) there was a significant increase in LCBF in the ipsilateral sensory-motor cortex. Theophylline also significantly decreased LCBF in the internal capsule and the inferior olive of the ipsilateral hemisphere. Theophylline also showed a tendency to increase LCBF in all of the other ipsilateral cortical structures except the visual cortex, and a tendency to/..

to decrease LCBF in a number of the other non-cortical ipsilateral brain regions e.g. globus pallidus, pons(see Table III.21). Theophylline significantly increased the LCBF in the contralateral frontal cortex and also showed a tendency to increase LCBF in all of the other contralateral cortical areas, except the visual cortex. Theophylline also significantly decreased LCBF in the contralateral inferior olive and showed a tendency to decrease LCBF in a number of the other contralateral non-cortical brain regions e.g. superior olive, pons (see Table III.22).

When theophylline (30mg/kg intraperitoneally) and CHA (10^{-10} moles/min via the internal carotid artery) were administered in the same animal they significantly decreased LCBF in the ipsilateral internal capsule and inferior olive. They also showed a tendency to decrease LCBF in a number of the other ipsilateral brain regions e.g. ventrolateral thalamus, globus pallidus (see Table III.21). In all of the ipsilateral cortical structures, except the visual and auditory cortices, CHA and theophylline together had almost no effect on LCBF (see Table III.21). Theophylline and CHA together significantly decreased the LCBF in the contralateral inferior olive and showed a tendency to decrease LCBF in a number of the other contralateral brain regions e.g. globus pallidus, corpus callosum (see Table III.22). In all of the contralateral cortical areas, except the auditory and parietal cortices, CHA and theophylline together had almost no effect on LCBF (see Table III.22).

As was the case with the agonist 15 minute infusion study the effects of the compounds are not only observed in those brain regions being supplied by the internal carotid artery, nor is/..

is there any physiological significance in the areas affected.

6.2. Discussion

The results obtained in this study appear to show an obvious difference between the cortical and non-cortical brain areas. In the majority of the cortical structures CHA alone decreases LCBF, theophylline alone increases LCBF, and CHA and theophylline together have little or no effects. Since CHA alone and theophylline alone have opposite effects in the cortical areas it can not be determined conclusively whether the minimal effects of CHA and theophylline together are due to an antagonism of the CHA effect by theophylline or due to the effects of each compound cancelling each other out.

In the majority of the non-cortical structures CHA alone decreases LCBF, theophylline alone decreases LCBF, and CHA and theophylline together decrease LCBF. It appears, therefore, that theophylline has no effect on the response to CHA in the non-cortical structures.

Since it appears that theophylline can not antagonise the effect of CHA, it is unlikely, therefore, that the decreases in LCBF are mediated via an A_1 or A_2 adenosine receptor interaction since both are antagonised by theophylline. As stated above, however, there may be some evidence to suggest an antagonism by theophylline in the cortical areas and if this is the case it would be likely that the changes produced by CHA in these areas are mediated via an A_1 or A_2 receptor interaction.

6.3. Summary of theophylline/15 minute CHA results

Ipsilateral hemisphere

	Effect on local cerebral blood flow		
Structures	CHA	Theophylline	CHA+Theophylline
Cortical	↓	↑	-
Diencephalic	↓	↓	↓
Hindbrain	↓	↓	↓
Mesencephalic	↓	↓	↓
Telencephalic	↓	↓	↓
Fibre Tracts	↓	↓	↓

Contralateral hemisphere

	Effect on local cerebral blood flow		
Structures	CHA	Theophylline	CHA+Theophylline
Cortical	↓	↑	-
Diencephalic	↓	↓	↓
Hindbrain	↓	↓	↓
Mesencephalic	↓	↓	↓
Telencephalic	↓	↓	↓
Fibre Tracts	↓	↓	↓

- indicates that the LCBF in the majority of structures is unaffected.

↓ indicates that the LCBF in the majority of structures is decreased.

↑ indicates that the LCBF in the majority of structures is increased.

7. SHORT-TERM INFUSION STUDY

Since the [^{14}C]-IAP autoradiographic experiemnts only allow the LCBF measurement to be made at one time point (15.5 minutes) during the infusion, a shorter infusion was used to test if different changes were evident earlier in the infusion. Two minutes was the minimum time that could be used since it took that length of time for the compounds being infused at 50µl/min. to reach the cerebral tissue via the length of internal carotid cannula.

7.1. Effects of a 2 minute infusion of adenosine and the adenosine analogue N^6-cyclohexyladenosine (CHA) on local cerebral blood flow (LCBF) in the rat as measured by [^{14}C]-iodoantipyrine (IAP) autoradiography

The following physiological parameters were monitored throughout the course of all the experiments: PCO_2, PO_2, mean arterial blood pressure, core body temperature, bicarbonate (HCO_3^-) concentration, base excess and pH. No significant changes were found in any of these parameters with any of the experimental groups (see Table III.26).

Adenosine, at a concentration of 3×10^{-7}moles/min., produced significant increases in LCBF in 5 of the 35 ipsilateral brain regions studied: anterior cingulate cortex, ventrolateral thalamus, red nucleus, substantia nigra and corpus callosum (see Table III.24). Adenosine also showed a tendency to increase LCBF in a number of the other ipsilateral brain regions e.g. prefrontal cortex, mediodorsal thalamus (see Table III.24).

Adenosine produced significant increases in LCBF in 2 of the 36 contralateral brain regions studied: anterior cingulate cortex and pons (see Table III.25). Adenosine also showed a tendency to/..

to increase LCBF in a number of the other contralateral brain regions e.g. mediodorsal thalamus, corpus callosum (see Table III.25).

N^6-cyclohexyladenosine (CHA), at a concentration of 10^{-10} moles/min., did not produce any significant changes in LCBF either ipsilateral or contralateral to the side of infusion (See Tables III.24 and III.25). CHA, however, did show a tendency to increase LCBF in a number of the ipsilateral brain regions e.g.frontal cortex, nucleus accumbens (see Table III.24).

CHA also showed a tendency to increase LCBF in a number of the contralateral brain regions e.g. visual cortex, corpus callosum (see Table III.25).

7.2.Discussion

As has already been seen in Sections 1 and 2 of the results section both adenosine and CHA are potent vasodilatators and one would expect them as has been shown in this section to increase LCBF. Both adenosine, as was the case with the 15 minute infusion of adenosine, and CHA, unlike the 15 minute infusion of CHA, either significantly increase or at least show a tendency to increase LCBF. It appears, therefore, that both adenosine and CHA, when infused for a period of two minutes, are able to increase LCBF by an action on a receptor mediating vasodilatation e.g. the A_2 adenosine receptor.

As was the case with the 15 minute infusion agonist study there is no apparent difference between the effects of the compounds on the cortical and non-cortical areas. The effects are not only limited to those areas supplied by the internal carotid artery, nor is there any physiological significance in the areas affected.

7.3. Summary of the 2 minute infusion results

(i) CHA

No significant effect on LCBF, but showed a tendency to increase LCBF.

(ii) Adenosine

Increases LCBF. Significant increases were observed in the following regions:

Structures	Ipsilateral	Contralateral
Cortical	Anterior cingulate cx.	Anterior cingulate cx.
Diencephalic	Ventrolat. thalamus	—
Hindbrain	—	Pons
Mesencephalic	Red nucleus	—
	Substantia nigra	
Telencephalic	—	—
Fibre Tracts	Corpus callosum	—

8. THEOPHYLLINE STUDY (2)

8.1. Effects of an intraperitoneal (i.p.) injection of theophylline (30mg/kg) on the changes in local cerebral blood flow (LCBF) produced by a 2 minute infusion of the adenosine analogue N^6-cyclohexyladenosine (CHA)

The following physiological parameters were monitored throughout the course of the experiments: PCO_2, PO_2, mean arterial blood pressure, core body temperature, bicarbonate (HCO_3^-) concentration, base excess and pH. No significant alterations in any of these parameters were evident in any of the experimental groups mentioned (see Table III.29).

When administered via the internal carotid artery CHA alone (10^{-10}moles/min.) (plus saline i.p.) produced no significant alterations in LCBF either ipsilateral or contralateral to the side of infusion (see Tables III.27 and III.28). CHA did show a tendency to increase LCBF in almost all of the ipsilateral and contralateral brain regions e.g.anterior cingulate cortex and corpus callosum (see Tables III.27 and III.28).

When theophylline alone (30mg/kg) was given intraperitoneally (Plus saline via the internal carotid artery) it produced no significant changes in LCBF either ipsilateral or contralateral to the side of infusion (see Tables III.27 and III.28). In the ipsilateral cortical structures theophylline had little or no effect on LCBF, except on the parietal cortex, sensory motor cortex and frontal cortex where it showed a tendency to increase LCBF. Theophylline also showed a tendency to decrease LCBF in a number of the other ipsilateral non-cortical brain regions e.g. nucleus/..

accumbens, globus pallidus (see Table III.27). In the contralateral cortical structures theophylline had little or no effect on LCBF, except in the parietal, sensory motor and frontal cortices where it showed a tendency to increase LCBF. Theophylline also showed a tendency to decrease LCBF in a number of the other contralateral non-cortical brain regions e.g. lateral geniculate, nucleus accumbens (see Table III.28).

When CHA (10^{-10}moles/min via the internal carotid) and theophylline (30mg/kg intraperitoneally) were administered in the same animal they significantly increased LCBF in the ipsilateral visual cortex. CHA and theophylline together also showed a tendency to increase LCBF in the ipsilateral cortical structures. With the exception of the ipsilateral ventrolateral thalamus CHA and theophylline together produced very little change in the other ipsilateral diencephalic areas. In most of the other non-cortical, non-diencephalic, ipsilateral brain regions, CHA and theophylline together had very little effect on LCBF (see Table III.27).

CHA and theophylline together significantly increased LCBF in 2 of the 36 contralateral brain regions: sensory motor cortex and cochlear nucleus. CHA and theophylline together also showed a tendency to increase LCBF in a number of the other contralateral brain regions e.g. all the cortical structures, caudate nucleus. CHA and theophylline together had little or no effect on LCBF in a number of other contralateral brain regions e.g. globus pallidus, hippocampus molecular alyer (see Table III.28)

8.2.Discussion/..

8.2.Discussion

As was the case in Results Section 6, the results obtained in this section appear to show an obvious difference between the cortical and non-cortical brain regions. In the majority of the cortical structures CHA alone increases LCBF, theophylline alone increases LCBF, and CHA and theophylline together produce an even greater increase in LCBF. In the cortical areas CHA increases LCBF, suggesting an A_2 receptor mediated vasodilatation. Since theophylline (an A_1/A_2 adenosine receptor antagonist) does not antagonise this increase in LCBF this puts doubt on whether the CHA effect is indeed A_2 receptor mediated.

In a number of the non-cortical areas CHA increases LCBF, theophylline alone decreases LCBF, and CHA and theophylline together have no effect. This seems to suggest an antagonism of the A_2 receptor mediated vasodilatation of CHA by the A_1/A_2 receptor antagonist, theophylline. This antagonism is particularly evident in the following ipsilateral brain regions: medial geniculate, amygdala, internal capsule, substantia nigra, superior colliculus, hippocampus molecular layer, dentate gyrus, septal nucleus, cerebellum white and corpus callosum.

As was the case in all of the previous LCBF experiments the changes in LCBF were not restricted to the brain regions supplied by the internal carotid artery, nor was there any physiological significance in those areas affected.

8.3. Summary of theophylline/2 minute CHA results

Antagonism evident in the following ipsilateral brain regions:

(i) Cortical	——
(ii) Diencephalic	medial geniculate
	amygdala
	internal capsule
(iii) Hindbrain	——
(iv) Mesencephalic	substantia nigra
	superior colliculus
(v) Telencephalic	hippocampus molecular layer
	dentate gyrus
	septal nucleus
(vi) Fibre Tracts	cerebellum white
	corpus callosum

SECTION IV

GENERAL DISCUSSION

1. PURINERGIC RECEPTOR SUB-TYPES

As previously mentioned in the 'INTRODUCTION' section the effects of adenosine and the adenosine analogues are mediated via an action on specific extracellularly located receptors. Experiments have shown that the binding of adenosine to compounds of high molecular weight or glass beads does not affect the vasodilator activity and this indicates that the receptors mediating the vasodilation are located on the cell surface (Schrader, Nees & Gerlach,1977; Ghai & Mustafa,1983).

The idea of two groups of purinergic receptors was first put forward in 1978 (Burnstock,1978). The two purinergic receptors were the P_1 receptor, linked to the adenylate cyclase system, and the P_2 receptor, which is not linked to the adenylate cyclase system. The two purinoceptors can be differentiated by the order of potency of a group of purine-related compounds at them i.e.

P_1 receptor: adenosine $\geqslant$ AMP > ADP $\geqslant$ ATP

P_2 receptor: ATP $\geqslant$ ADP > AMP $\geqslant$ adenosine

The P_1 group of purinoceptors can be subdivided into two classes: the A_1 (or R_i) receptor, which is coupled to adenylate cyclase in an inhibitory manner (van Calker et al.,1979; Londos et al.,1980) and the A_2 (or R_a) receptor which is coupled to adenylate cyclase in a stimulatory manner (Bruns,1980). As was the case with the P_1/P_2 purinergic receptors, the A_1/A_2 adenosine receptors can be differentiated by the rank order of potency of adenosine and the various adenosine analogues i.e.

A_1 receptor/...

A_1 receptor:

CHA, L-PIA > 2-CADO > adenosine > NECA

A_2 receptor:

NECA > 2-CADO, adenosine > L-PIA > CHA

The P_1 group of purinoceptor receptors are located on the cell surface of the vascular smooth muscle rather than on the vascular endothelium. The endothelium-independency of the observed adenosine-induced vascular relaxation has been shown in studies involving rabbit vascular smooth muscle, which showed that the relaxation produced by adenosine was unaffected by removal of the endothelium (Furchgott,1983). Although it has been stated that P_1 receptors are located on the cell surface of the vascular smooth muscle, intracellular sites of action for adenosine have also been located (Londos & Wolff,1977; Collis & Brown,1983).

The P_2 group of purinoceptors have been subdivided into two further groups: an excitatory receptor, P_{2x}, located on the vascular smooth muscle; and an inhibitory receptor, P_{2y}, located on the the vascular endothelium. These findings came from studies which showed that ATP was able to act at P_2 receptors at two locations in the isolated rat femoral artery: one on the endothelium mediating vasodilation and one on the smooth muscle mediating vasoconstriction (Kennedy, Delbro & Burnstock,1985). Kennedy et al. (1985) showed that in the isolated rat femoral artery preparation low concentrations of ATP produced a relaxation of vessels which had their tone raised with 10^{-6}M noradrenaline (NA), whereas in the same preparation with the endothelium removed ATP produced a contraction of vessels which also had their tone raised with 10^{-6}M NA, and these/..

contractions were often produced by concentrations of ATP which had no effect when the endothelium was intact. It was these studies led to the introduction of the P_2 receptor classification of P_{2x} and P_{2y}.

2. ISOLATED PORCINE VESSEL STUDY

The rank order of potency of adenosine and the adenosine analogues in this study was as follows:

NECA > 2-CADO, adenosine > L-PIA > CHA

and as stated earlier this order is in keeping with that of the A_2 adenosine receptor sub-type.

No specific efforts were made to either keep the endothelium intact or remove the endothelium since it has been shown that the adenosine-induced relaxation of vascular smooth muscle is endothelium-independent (Furchgott,1983).

The antagonist data, however, shows that the picture is perhaps not as simple as an A_2 receptor-mediated vasodilation. Although the A_1/A_2 adenosine receptor antagonist 8-phenyltheophylline competitively antagonises the vasodilatory effects of NECA it is unable to block the effects of any of the other agonists. The adenosine receptor agonists adenosine, 2-CADO, CHA and NECA, in the presence of 8-phenyltheophylline cause a constriction of the vessels in the dose range of 10^{-9}M to 10^{-6}M agonist. A number of possible explanations can be put forward to explain these results. Firstly, it could be that the antagonist 8-phenyltheophylline is indeed blocking both the A_1 and A_2 adenosine receptors but in the dose range 10^{-9}M to 10^{-6}M the agonists are perhaps acting on another distinct receptor to mediate the vasoconstriction. A second/..

second possible explanation could be that the 8-phenyltheophylline, in this preparation, is a more potent antagonist at the A_2 receptor than at the A_1 receptor and, therefore, in the low dose range of 10^{-9}M to 10^{-6}M the agonists are acting on the A_1 adenosine receptor to mediate a vasoconstriction.

It can be determined from these studies involving isolated porcine vessels that the A_2 adenosine receptor is indeed involved in mediating the vasodilatory effects of the various agonists. Although certain aspects of the results suggest that the mechanism of action is perhaps not as straightforward as a simple A_2 receptor interaction, these experiments with the isolated porcine vessels do not allow us to determine what other receptor(s), if any, is (are) involved in the action of adenosine and the adenosine analogues on the cerebrovasculature.

3. IN VIVO FELINE PIAL VESSEL STUDY

These studies show that adenosine and its analogues are indeed potent vasodilators, thus supporting the results from the in vitro isolated porcine vessel study. The results in this study also confirm the results obtained by other workers using the same preparation i.e. 10^{-5}M adenosine gives a 22% dilation at a P_{CO_2} of 30mmHg which compared favourably with the findings of Gregory et al.(1980) (14% dilation at a P_{CO_2} of 25mmHg and a 29% dilation at P_{CO_2} of 34mmHg) and Wahl & Kuschinsky (1976) (24% dilation at a P_{CO_2} of 31mmHg).

Since it has already been shown that it is the A_2 receptor which mediates vasodilation, both in the previous section discussing the in vitro isolated vessels and in the experiments/..

experiments carried out by Edvinsson & Fredholm (1983), one can propose that the observed dilations in vivo are as a result of an A_2 adenosine receptor interaction. The A_1/A_2 adenosine receptor antagonist 8-phenyltheophylline is able to block the vasodilatory actions of adenosine, CHA and NECA. As was the case with the in vitro vessels, however, the agonists, particularly adenosine and NECA, cause a constriction of the vessels in the presence of 8-phenyltheophylline, suggesting once again that when the A_1/A_2 adenosine receptors are blocked by 8-phenyltheophylline the agonists are perhaps acting on another non A_1/A_2 receptor to mediate a vasoconstriction.

The overall picture to be obtained from the vessel work is that adenosine and the adenosine analogues act on the A_2 receptor to dilate the cerebral vessels and that although other receptor mechanisms, which become apparent on blockade of the A_1/A_2 receptors, may be involved their identity or existence can not be discovered using the aforementioned results.

4. LCBF MEASUREMENT USING LASER-DOPPLER FLOWMETRY

Since it is known that adenosine and the adenosine analogues dilate the cerebral vessels it would be expected that they would in turn cause an increase in local cerebral blood flow (LCBF). When cerebral blood flow measurements are made using laser-Doppler flowmetry, however, they show that one of the adenosine analogues, namely CHA, decreases cerebral blood flow.

When laser-Doppler flowmetry is used to monitor the changes in cerebral blood flow produced during a fifteen minute infusion of CHA at 10^{-10}moles/min. via the right internal carotid artery, it shows CHA producing a monophasic decrease in cerebral blood flow throughout the course of the infusion.

5. PHARMACOKINETICS OF ADENOSINE AND THE ADENOSINE ANALOGUES

It has been shown that a blood-brain barrier transport system exists in the rat for the following compounds: adenine, adenosine, inosine, guanosine and uridine (Cornford & Oldendorf, 1975). These studies show that approximately 9% of the adenosine administered intravascularly will cross to the cerebral side of the blood-brain barrier. There is no evidence for the existence of a carrier for ATP in the rat, however it may cross the blood-brain barrier after it has been metabolised to adenosine.

We shall now look at two of the compounds infused via the internal carotid artery in the autoradiographic experiments (adenosine and CHA) and calculate roughly, making a few assumptions, what concentrations are reaching the cerebral circulation:

5.1.Adenosine/..

5.1.Adenosine

Blood flow to the rat brain approximately 70ml/100g/min.

Weight of rat brain approximately 2g.

Therefore, flow to the rat brain is approximately 1.4ml/min.

1/3 of this goes to the brain via the carotid i.e. 0.47ml/min.

2/3 of this goes to the brain via the basilar i.e. 0.93ml/min.

Dose of adenosine administered via the right internal carotid artery

$= 3 \times 10^{-7}$ moles/min.

Approximately 9% of this will cross the blood-brain barrier

$= 9/100 \times 3 \times 10^{-7}$

$= 2.7 \times 10^{-8}$ moles/min.

Therefore, the concentration reaching the brain $= \frac{2.7 \times 10^{-8} \text{ moles/min}}{0.47 \text{ ml/min}}$

$= 5.7 \times 10^{-8}$ moles/ml

$= 5.7 \times 10^{-5}$ M

Therefore, the total concentration of adenosine reaching the brain over 15 minutes assuming minimal further metabolism

$= 15 \times 5.7 \times 10^{-5}$ M

$= 8.6 \times 10^{-4}$ M

The actual concentration of adenosine reaching the brain over 15 minutes will probably be less than this since it is unlikely that there will be no further adenosine metabolism once it has crossed to the cerebral side of the blood-brain barrier.

Due to its rapid degradation by adenosine deaminase the concentration of adenosine recirculating and passing to the left hemisphere of the rat brain will probably be almost zero.

5.2.CHA

Dose of CHA administered via the right internal carotid artery $=10^{-10}$moles/min.

Assume approximately 9% (as was the case with adenosine since actual CHA figure not known) of this will cross the blood-brain barrier $= 9/100 \times 10^{-10}$moles/min

$= 9 \times 10^{-12}$moles/min.

Therefore, concentration reaching the brain $= \frac{9 \times 10^{-12}\text{moles/min}}{0.47\text{ml/min}}$

$= 19 \times 10^{-12}$moles/min

$= 1.9 \times 10^{-8}$M.

Therefore, the total concentration of adenosine reaching the brain over 15 minutes assuming minimal further metabolism

$= 15 \times 1.9 \times 10^{-8}$M

$= 2.8 \times 10^{-7}$M.

Rate of CHA administration = 50µl/min

Therefore, total volume of CHA given over 15 minutes = 750µl

= 0.75ml.

Therefore, if the CHA recirculates to the left hemisphere it will be dissolved in total rat blood volume of approximately 25ml.

Therefore, the concentration of CHA reaching left carotid

$=0.75/25 \times 2.8 \times 10^{-7}$M

$= 8.4 \times 10^{-9}$M.

Assume/..

Assume 9% crosses the blood-brain barrier

. . concentration of CHA reaching left hemisphere

$= 9/100 \times 8.4 \times 10^{-9}M$

$= 7.6 \times 10^{-10}M.$

Assumptions made in this calculation are that there is no metabolism of the stable analogue CHA during the recirculation, and that the CHA is dissolved only in the total animal blood volume (approximately 25ml.) rather than in the total body water volume (approximately 200-300ml.).

6. [^{14}C]-IODOANTIPYRINE AUTORADIOGRAPHY - 15 MINUTE INFUSION

When the LCBF is measured using [^{14}C]-iodoantipyrine autoradiography 15 minute infusions of CHA (10^{-10}moles/min.) and 2-CADO (10^{-9}moles/min) significantly decrease LCBF in a number of discrete brain regions, whereas 15 minute infusions of adenosine (10^{-7} and 3×10^{-7}moles/min.), ATP (10^{-7} and 3×10^{-7}moles/min.) and NECA (10^{-11}moles/min.) show a tendency to increase LCBF. Although the compounds are administered via the right internal carotid artery the significant decreases in LCBF produced by CHA and 2-CADO are not only observed in those regions supplied by the right internal carotid artery, namely, the cortical areas, caudate nucleus, hippocampus molecular layer, dentate gyrus, globus pallidus, nucleus accumbens, subthalamic nucleus, red nucleus and substantia nigra of the right hemisphere (Yamori et al.,1976). One possible reason for changes being seen in areas other than those supplied by the right internal carotid artery could be that the compounds are circulating round the body and passing into the/..

the brain via other arteries supplying the brain i.e. the left internal carotid artery and the right and left basilar arteries.

Although 15 minute infusions of adenosine, ATP and NECA all show a tendency to increase LCBF, as expected of known dilators, none of them is able to produce a significant increase in LCBF. Adenosine has already been shown to produce significant increases in cerebral blood flow in the dog (Kozniewska et al.,1975; Heistad et al.,1981) and the baboon (Forrester et al.,1979).

A number of reasons can be put forward to explain this lack of a significant effect. In the case of NECA it could be that at the low concentration used (10^{-11}moles/min.) there is an insufficient amount of NECA reaching the site of action to produce a significant effect. As stated earlier, however, higher concentrations of NECA can not be used due to their hypotensive effects. The lack of a significant effect with adenosine and ATP could be attributed to the fact that they are degraded by various enzymes, including adenosine deaminase, therefore the concentration of both of these compounds reaching the site of action are not sufficient to elicit a significant alteration in LCBF.

Previous studies in other species such as the young rat (Hoffman et al.,1984) and the dog (Boarini et al.,1984) have shown infusions of adenosine to have no effect on cerebral blood flow. Boarini et al. (1984) proposed that it is the route of administration of adenosine that determines the effect i.e. the intravascular route of administration of the compounds used in their study produces no cerebrovascular effects.

A reason still has to be found for the vasoconstrictive effect of CHA and 2-CADO resulting in the significant decreases in LCBF observed. Adenosine has been seen to produce vasoconstriction in a number of/..

of previous studies, however, in these studies the observed effects have been attributed to the secondary release of serotonin (5-HT) in rat femoral vascular bed and tail artery (Sakai,1978; Brown & Collis,1981) and to a relationship between adenosine and angiotensin II in the kidney. It has been found that the adenosine-induced constriction in the kidney is blocked by angiotensin antagonists (Spielman & Oswald,1979), suggesting that either angiotensin formation is increased or that there is enhanced vascular reactivity to angiotensin. The exact mechanism, however, by which adenosine acts to unmask the vasoconstrictive effect of angiotensin remains to be elucidated. It could be possible that CHA and 2-CADO are acting through similar mechanisms in the brain to produce the observed constrictions e.g. the effects of CHA and 2-CADO on LCBF could be monitored in the presence of some 5-HT antagonists, such as methysergide or cyproheptadine, to determine whether or not the secondary release of 5-HT is involved in producing the constrictions observed. Decreases in flow have been observed when adenosine was injected into rabbit hypothalamus (Livemore & Mitchell,1983). In this study injections of 10^{-4}M and 10^{-3}M adenosine produced the increases in flow expected of a vasodilator, however, an injection of 10^{-6}M adenosine produced a significant 25% decrease in hypothalamic blood flow. This vasoconstriction of the hypothalamic blood vessels was a consequence of the direct action of adenosine on adenosine receptors on the vessels, since neither adrenergic blockade nor the inhibition of neuronal activity were able to block the effect. The identity of receptor in the hypothalamus was not determined in this study by Livemore & Mitchell,1983), however, it may be possible that 2-CADO and CHA are acting through similar receptors in the rat to produce the significant decreases in LCBF obtained in my own study/..

study.

One possible reason for the observed decreases in flow could be that the vascular effects observed with 2-CADO and CHA are secondary to their metabolic actions i.e. although the compounds are vasodilators they also have the effect of decreasing the metabolic demand and this, in turn, leads to the observed decrease in local cerebral blood flow. This explanation for the decreases in LCBF, however, is not a possibility since studies carried out to monitor the effects of 15 minute infusions of adenosine, CHA, 2-CADO and NECA on local cerebral glucose utilisation, using [^{14}C]-2-deoxy-glucose (2-DG) autoradiography, showed that they have no significant effect on LCGU.

A second possible reason to explain the vascular effects of CHA and 2-CADO could be the involvement of receptors other than the A_2 receptor known to mediate vasodilation. Possible receptors involved could be the A_1 adenosine receptor, a recently identified third (A_3) adenosine receptor or even a completely different receptor type.

7. A THIRD (A_3) ADENOSINE RECEPTOR

The idea of a third (A_3) adenosine receptor, not coupled to adenylate cyclase system but possibly linked to calcium channels, was propsed by Ribeiro & Sebastiao (1986). The order of potency of adenosine analogues at this receptor site is: L-PIA,CHA,NECA>2-CADO. Two speculative hypotheses have been put forward to explain the identity of this A_3 receptor. Firstly, it could be a voltege dependent calcium channel which changes its conformation after bimding adenosine. Secondly it could be that activation of the A_3 adenosine receptor induces a conformational change in the membrane in such a way that the calcium receptor sitr becomes altered. This A_3 receptor, like the A_1/..

and A_2 receptors, is also xanthine sensitive. A third adenosine receptor associated with calcium channels has also been proposed by Chin & DeLorenzo (1986) with a rank order of potency for the adenosine analogues of: 2-CADO>NECA>L-PIA. Since the orders of potency for the analogues are different it seems likely that the A_3 receptor of Ribeiro & Sebastiao and the A_3 receptor of Chin & DeLorenzo are not the same receptor. These proposals for a third adenosine receptor are in continuity with previous suggestions (Phillis & Wu,1981; Stone,1985) concerning the need for a third adenosine receptor mediating the electrophysiological effects of adenosine on the central nervous system.

8. [^{14}C]-IODOANTIPYRINE AUTORADIOGRAPHY - 2 MINUTE INFUSIONS

When adenosine and the analogue CHA were infused for 2 minutes via the right internal carotid artery at concentrations of 3×10^{-7}moles/min. and $10^{-}10$moles/min., respectively, they both increased local cerebral blood flow. The changes in local cerebral blood flow produced by adenosine are significant in a number of brain regions e.g. anterior cingulate cortex, ventrolateral thalamus, red nucleus, substantia nigra, corpus callosum. Although CHA did not produce any significant changes in local cerebral blood flow it did show a tendency to increase flow in a number of brain regions e.g. frontal and anterior cingulate cortices, nucleus accumbens, substantia nigra, cerebellum white, corpus callosum. Since it is know that the A_2 receptor mediates dilation and that a vaso-dilation would result in an increase in local cerebral blood flow it could be proposed that the increases observed in flow produced by CHA and adenosine are mediated via an A_2 receptor mechanism.

9. DIFFERENCES/..

9. DIFFERENCES BETWEEN TWO AND FIFTEEN MINUTE INFUSIONS

The decreases in local cerebral blood flow produced by CHA and 2-CADO are not what one would expect with known vasodilators, however it still seems likely that these changes are real and not due to some experimental artefact, which can be ruled out for two reasons. Firstly, it would be unlikely that any artefact would only be evident in the 15 minute infusion experiments and even then only in the experiments involving CHA and 2-CADO. Secondly, the unexpected results obtained with CHA and 2-CADO are not due to damage as a result of the route of administration i.e. injecting compounds via the internal carotid artery resulting in thrombi or emboli leaving the cannula tip and damaging the brain. Experiments carried out using FAM fixation and pathological examination showed no evidence of damage to the cerebral tissue caused by air emboli or thrombi (see Appendix).

Unlike the increases in LCBF produced by a 15 minute infusion of adenosine, the increases produced by a 2 minute adenosine infusion are significant increases. One possible reason which may explain this difference in the response could be linked to adenosine metabolism. After a 2 minute infusion of adenosine there would be an amount of the adenosine metabolite, inosine, present in addition to the adenosine. After a 15 minute infusion, however, there would be a much larger concentration of the metabolite inosine present, but the adenosine concentration would not be that much greater. In studies involving vasal (dog), cardiac (frog), intestinal (rat) and uterine musculature inosine has been shown to be inactive by itself, but is able to increase tha activity of adenosine (Rossi et al.,1982), and this could explain why the 2 minute adenosine infusion gives significant changes in blood flow i.e. the inosine present enhances the /..

the adenosine action in a 2 minute infusion but in a 15 minute infusion too much of the inactive inosine is present in relation to the adenosine levels.

10. AUTORADIOGRAPHIC STUDIES INVOLVING THE ADENOSINE RECEPTOR ANTAGONIST THEOPHYLLINE

Studies were carried out involving the intraperitoneal administration of the A_1/A_2 adenosine receptor antagonist, theophylline, in animals receiving 15 minute infusions of the adenosine analogue, CHA, via the right internal carotid artery. These studies seem to show no evidence of an antagonism of the decreases in local cerebral blood flow produced by the 15 minute infusion of CHA, therefore it seems unlikely that the decreases in flow are mediated by an A_1 or A_2 receptor interaction. The A_3 receptor proposed by Ribeiro & Sebastiao (1986) is also xanthine sensitive, therefore it is unlikely that the decreases are caused by an A_3 receptor interaction.

When the A_1/A_2 adenosine receptor antagonist, theophylline was given in animals receiving a 2 minute infusion of CHA there appeared to be an antagonism of the increases in local cerebral blood flow produced by CHA in a number of the non-cortical brain regions studied e.g. amygdala, caudate nucleus, internal capsule, substantia nigra, hippocampus molecular layer, dentate gyrus, septal nucleus and corpus callosum. In the cortical brain areas, however, there appears to be a degree of synergism i.e. the increase in local cerebral blood flow produced by CHA and theophylline together is greater than the sum of the increases produced by CHA alone and theophylline alone.

In all of the autoradiographic experiments theophylline increases local cerebral blood flow in the cortical areas but decreases local cerebral blood flow in the other non-cortical brain areas. When the theophylline is given to animals receiving a 2 minute infusion of CHA the synergistic effect in the cortical areas could be due to the theophylline blocking the A_1 receptor, which CHA would act on to/..

to constrict the vessels, thus allowing the A_2 receptor mediated vasodilatory effect of CHA to become more noticeable.

11. CONCLUSION

The significant decreases in local cerebral blood flow produced by a 15 minute infusion of CHA do not appear to occur either as a result of decreases in metabolic demand, since CHA has no effect on local cerebral glucose utilisation as measured by [^{14}C]-2-deoxy-glucose autoradiography, or as a result of an interaction with any of the known xanthine sensitive adenosine (A_1,A_2,A_3)receptors, since the xanthine derivative, theophylline, was not able to block the observed decreases in local cerebral blood flow. Theophylline exerts some of its pharmacological effects via , among others, the inhibition of cyclic AMP phosphodiesterase and also via the release of catecholamines from neurones, however, it is unlikely that after a dose of 30mg/kg i.p. theophylline (as used in this study) has any effect on either phosphodiesterase activity or catecholamine release (Winn et al.,1985). It could be possible that the decreases in LCBF occur as a result of an interaction with another, as yet unknown, receptor type, however, the results obtained in this study do not allow us to identify what this receptor may be. One factor that can not be used to explain the observed decreases in is that of cerebral damage since FAM fixation pathological studies showed no evidence of embolic or thrombic damage following the administration of solutions via the internal carotid artery. As discussed earlier adenosine has produced vasoconstrictions in the rat femoral vascular bed and rat tail artery (Salai,1978; Brown & Collis,1981) which have been attributed to the secondary release of/..

of serotonin and it could be that CHA and 2-CADO are producing their constrictions via a similar mechanism. Adenosine has been shown to produce a vasoconstriction of the hypothalamic blood vessels Livemore & Mitchell,1983) to cause a 25% decrease in blood flow, and it was proposed that the effect was due to a direct action on adensosine receptors on the blood vessels, however the exact identity of the receptor was not proposed.

It is known that the A_2 receptor mediates vasodilation (Edvinsson & Fredholm,1983), which results in increased blood flow, and that the same receptor is blocked by xanthine derivatives, of which theophylline is one,(Daly,1982), therefore it seems likely that the increases in LCBF produced by a 2 minute infusion of CHA, which are blocked by theophylline (30mg/kg i.p.), are mediated via an A_2 receptor interaction.

Although the isolated porcine vessel experiments support the role of adenosine and the adenosine analogues as cerebral vasodilators, the unmasking of a vasoconstrictive action in the agonist dose range of 10^{-9} to 10^{-6}M following the application of the A_1/A_2 receptor blocker 8-phenyltheophylline (a xanthine derivative), suggests that receptors other than the classical A_1/A_2 adenosine receptors may be involved in producing the observed responses in vitro.

In summary, the isolated porcine vessel and cat pial vessel studies confirm the vasidilatory properties of adenosine and the adenosine analogues and the LCBF autoradiographic experiments involving 2 minute infusions of adensoine and CHA and 15 minute infusions of adenosine, ATP and NECA show the <u>increases</u> in LCBF expected of known vasodilators. The <u>decreases</u> in LCBFproduced by 15 minute infusions of the known vasodilators CHA and 2-CADO ,/..

2-CADO, however, are without doubt the most interesting and original findings within the thesis. Studies carried out using [^{14}C]-2-deoxyglucose autoradiography, FAM fixation and the adenosine antagonist theophylline showed that the decreases in LCBF were not a consequence of either changes in metabolic demand, damage to the cerebral tissue during the infusion or an interaction with one of the known, xanthine-sensitive adenosine (A_1,A_2,A_3) receptors, respectively. The range of techniques available to me, however, did not allow me to identify what factors were involved in producing the vasoconstrictions observed with the vasodilators CHA and 2-CADO.

12. FURTHER STUDIES

There are a number of other studies which could be carried out to help clarify the picture of what is happening. Although the lack of antagonism by theophylline of the decreases in LCBF produced by CHA suggest that none of the adenosine receptors is involved in the mechanism of action, one could test specific A_1 and A_2 receptor antagonists to monitor the involvement of each adenosine receptor independently e.g. the specific A_1 receptor antagonist 8-cyclopentyl-3,7-dihydro-1,3-dipropyl-1H-purine-2,6-dione (PD,116,948)(Haleen,Steffen & Hamilton,1987) or the specific A_2 receptor antagonist N-[2-(dimethylamin)ethyl]-N-methyl-4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl) benzenesulfonamide (PD,115,199)(Bruns et al.,1987).

The A_3 adenosine receptor is thought to be linked in some way to a calcium channel, therefore, perhaps the use of a calcium channel blocker, such as nifedipine or nimodipine, would help to determine/..

determine if the A_3 receptor was involved in any way.

The concentrations of adenosine and the adenosine analogues used were chosen, as stated earlier, since they were the highest that could be used without producing hypotensive effects. Higher concentrations could be tested, however, however, to see how they would differ from those already used if the peripheral vascular effects of adenosine could be antagonised without affecting the central actions. The adenosine receptor antagonist 8-p-sulfophenyltheophylline (8-SPT) is a polar analogue of theophylline and as such does not gain access to the central nervous system across the blood brain barrier (Evoniuk, von Borstel &Wurtman, 1987). 8-SPT is able, therefore, to block the peripheral hypotensive effects of adenosine and the adenosine analogues without affecting their central effects, thus allowing higher concentrations of adenosine and the adenosine analogues to be used.

SECTION V

A P P E N D I X

1. EFFECT OF CHANGES IN MEAN ARTERIAL BLOOD PRESSURE (MABP) ON RAT LOCAL CEREBRAL BLOOD FLOW (LCBF)

1.1. Experimental Protocol

All the rats used in this experimental group received 15 minute infusions of 0.9% saline via the right internal carotid artery in order to replicate the conditions under which the earlier autoradiographic experiments were carried out. The MABP of the animals was reduced by haemorrhage and maintained for approximately 15-20 minutes at this new level before the [^{14}C]-IAP experiments were carried out to determine the LCBF.

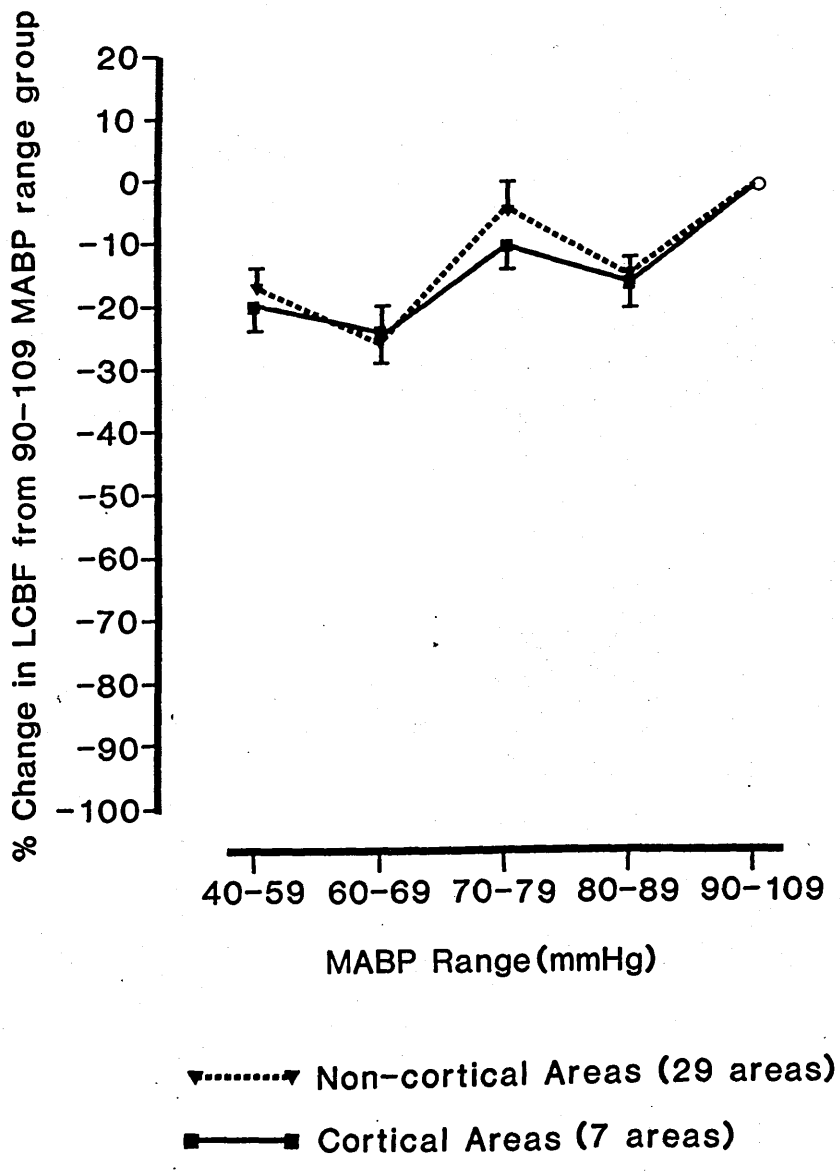
1.2. Results

Comparing the LCBF of each MABP range with that of the upper MABP range of 90-109 mmHg (for each of the 36 discrete brain regions studied), no significant alterations in LCBF were evident at any of the MABP ranges, either contralateral or ipsilateral to the side of saline infusion (see Tables V.1. and V.2.).

If we express the change in LCBF at each MABP range from the LCBF of the 90-109 mmHg range as a percentage (for each of the 36 discrete brain regions) and then calculate the mean percent change from all of the cortical and non-cortical regions, we can plot the graphs of "% change in LCBF against MABP range" shown in Figs. V.1. and V.2. Figures V.1 and V.2 show that in both the ipsilateral and contralateral hemispheres and for both the cortical and non-cortical areas there is no significant alteration in LCBF when the MABP is taken down below 90mmHg, even when it is taken as low as 40mmHg.

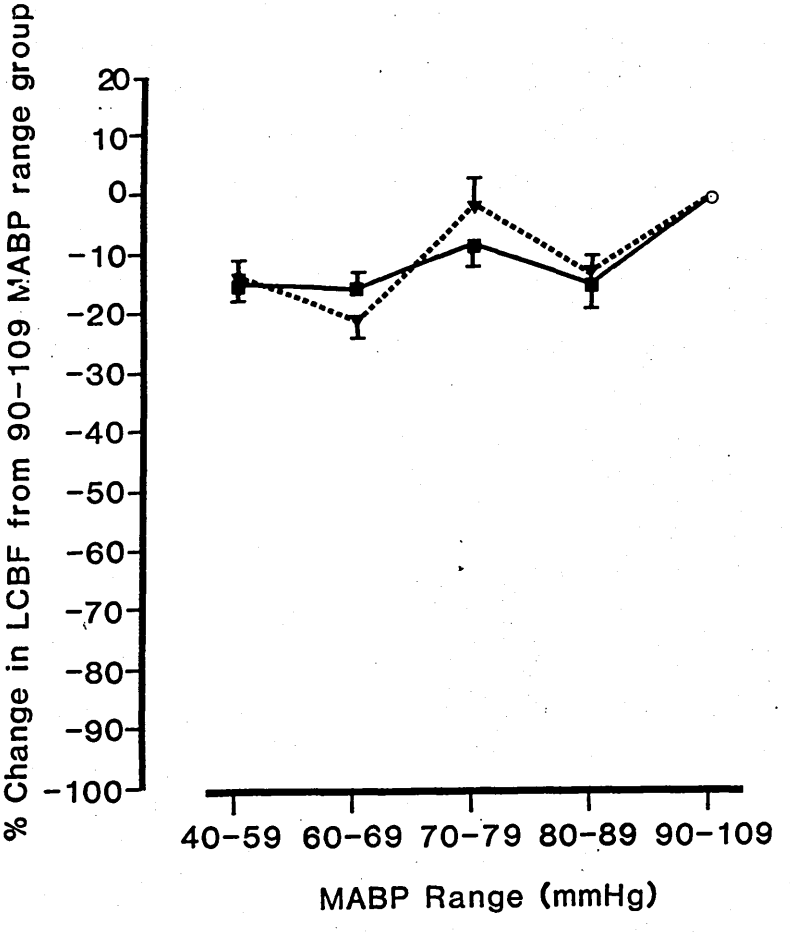
With the obvious exception of the MABP there were no significant alterations in any of the physiological parameters monitored from/..

Fig. V .1 CHANGES IN LCBF PRODUCED BY CHANGES IN MABP - IPSILATERAL HEMISPHERE



All bars indicate mean ± standard error of mean

Fig. V .2 CHANGES IN LCBF PRODUCED BY CHANGES IN MABP - CONTRALATERAL HEMISPHERE



Cortical Areas (7 areas)

Non-cortical Areas (29 areas)

All bars indicate mean ± standard error of mean

from range to range of blood pressure (see Table V.3).

1.3.Discussion

One possible problem that can be experienced with lowering the animal's blood pressure is that the flow rate of the blood flowing from the femoral cannula can be so low that it results in a false calculation of blood flow. This problem was overcome by using a small screw clamp which was tightened round the femoral cannula at higher blood pressures and loosened as the blood pressure was reduced, thus ensuring a similar flow rate through the femoral cannula at all the blood pressure ranges.

The results obtained in this study show that there is no significant alteration in LCBF in the range 40-109mmHg. No alterations in LCBF were evident due to autoregulation i.e. when the MABP is reduced the blood flow also decreases, therefore the vessels dilate to counteract this and increase flow. The lower the MABP is taken the greater the vasodilatation of vessels required to compensate for the decrease. Since vasodilatatory compounds are being tested one does not want the vessels to be dilated too much initially or the effect of the compound may not be observed. Although the MABP can be taken as low as 40mmHg and still not affect the LCBF, the higher it is kept the better the preparation is to show a vasodilatatory effect, therefore, in all of the autoradiographic experiments no animal was used if the MABP fell below 70mmHg.

2. PATHOLOGICAL EXAMINATION OF THE RAT BRAIN USING FAM FIXATION

2.1.Results and Discussion/..

2.1. Results and Discussion

As stated in the Material and Methods section all of the animals used in this experimental group received a 15 minute infusion of saline via the right internal carotid artery in order to replicate the conditions under which the earlier autoradiographic experiments were carried out. Four hours after the end of the 15 minute infusion the rats underwent FAM (formaldehyde/acetic acid/ methanol) fixation. The brains of all the rats used in this study then underwent pathological examination and in all cases there was no evidence of embolic or thrombic damage to the cerebral tissue. The results obtained in this section show that the changes observed in the earlier autoradiographic experiments are not as a result of damage to the brain caused by the administration of the compounds.

SECTION VI

TABLES

Table III.1 pD_2 VALUES AS DETERMINED FROM THE AGONIST DOSE RESPONSE CURVES

Agonist	n	Relative Emax (mean ± s.e.m.)	Relative EC_{50} (mean)	Relative pD_2
NECA	13	88.1 ± 7.1	7.1×10^{-7} M	6.15
Adenosine	8	145.1 ± 14.4	3.9×10^{-6} M	5.40
2-CADO	9	157.6 ± 20.8	4.5×10^{-6} M	5.35
L- PIA	7	79.3 ± 10.7	1.1×10^{-5} M	4.95
CHA	8	89.5 ± 8.6	2.0×10^{-5} M	4.70

Table III.2 p^{D_2} VALUES AS DETERMINED FROM THE HILL PLOT

Agonist	p^{D_2} (mean ± s.e.m.)	Slope of Hill Plot (Hill co-efficient)
NECA	6.00 ± 0.47	0.55
2-CADO	5.43 ± 0.15	1.16
Adenosine	5.36 ± 0.19	0.87
L-PIA	5.31 ± 0.80	0.45
CHA	4.85 ± 0.25	1.24

Table III.3 EFFECTS OF MOCK CSF, 10mM K+, THEOPHYLLINE (THEO) AND 8-PHENYLTHEOPHYLLINE (8-PT) on PIAL VESSEL DIAMETER

Compound	n_1 (n_2)	Effect (%change ±standard deviation)	Mean Arterial Blood Pressure (mmHg)		Initial Vessel Calibre (μm)	
			Range	Mean±s.d.	Range	Mean±s.d.
Mock CSF	28(23)	+1.5.±6.8	71-135	99±16	36-243	105±44
10mM K+	28(23)	+35.8±12.6*	72-140	99±16	46-194	98±40
10^{-9}M 8-PT	10(6)	+5.1±6.1	61-120	92±21	65-198	123±39
10^{-7}M 8-PT	8(5)	+5.9±6.9	88-119	94±10	68-165	97±37
10^{-9}M THEO	9(5)	-0.8±7.0	85-102	93±6	36-165	101±36
10^{-7}M THEO	7(5)	-0.7±9.4	85-100	92±6	53-174	113±54

Positive values indicate dilatation; negative values indicate constriction. *P<0.05 (Student's t-test, incorporating Bonferroni correction factor, comparing each compound with the mock CSF control). P_{CO_2} level maintained at approximately 30mmHg (Range 28-33mmHg). n_1 = number of observations; n_2 = number of cats in which n_1 observations were made.

Table III.4 EFFECTS OF ADENOSINE (ADO) ON PIAL VESSEL DIAMETER AND THEIR ANTAGONISM BY 10^{-9}M 8-PHENYLTHEOPHYLLINE (8-PT)

Compound	n_1 (n_2)	Effect (% change ±standard deviation)	Mean Arterial Blood Pressure (mmHg)		Initial Vessel Calibre (μm)	
			Range	Mean±s.d.	Range	Mean±s.d.
Mock CSF	28(23)	+1.5±68	71-135	99±16	36-243	105±44
10^{-9}M ADO	7(3)	-7.0±13.0	77-142	107±25	58-123	92±26
10^{-9}M ADO/10^{-9}M8-PT	9(5)	-1.8±7.8	78-148	106±24	45-142	100±32
10^{-7}M ADO	9(3)	+13.0±5.6*	75-132	102±19	34-161	88±40
10^{-7}M ADO/10^{-9}M8-PT	13(6)	-3.9±9.0#	89-130	104±14	69-309	119±67
10^{-5}M ADO	10(3)	+22.3±18.2*	85-125	101±11	40-223	123±56
10^{-5}M ADO/10^{-9}M8-PT	8(3)	-8.2±13.1*#	81-100	95±8	43-149	94±35

Positive values indicate dilation; negative value indicate constriction. *P<0.05 (Student's t-test, incorporating Bonferroni correction factor, comparing each compound with the mock CSF control). #P<0.05 (Student's t-test, incorporating Bonferroni correction factor, comparing each compound in the absence and presence of 10^{-9}M 8-PT). P_{CO_2} level maintained at approximately 30mmHg (Range 28-30mmHg). n_1 = number of observations; n_2 = number of cats in which n_1 observations were made.

Table III.5 EFFECTS OF N^6-CYCLOHEXYLADENOSINE (CHA) ON PIAL VESSEL DIAMETER AND THEIR ANTAGONISM BY 10^{-9}M 8-PHENYLTHEOPHYLLINE (8-PT)

Compound	n_1 (n_2)	Effect (% change ±standard deviation)	Mean Arterial Blood Pressure (mmHg)		Initial Vessel Calibre (μm)	
			Range	Mean±s.d.	Range	Mean±s.d.
Mock CSF	28(23)	±1.5±6.8	71-135	99±16	36-243	105±44
10^{-9}M CHA	9(5)	+9.8±13.5	88-112	103±7	66-248	139±67
10^{-9}M CHA/10^{-9}M8-PT	5(3)	+4.0±8.8	80-138	110±23	49-164	98±43
10^{-7}M CHA	14(6)	+8.2±5.3*	76-104	88±11	46-251	110±63
10^{-7}M CHA/10^{-9}M8-PT	5(3)	-2.0±7.6#	102-155	126±24	69-244	158±78
10^{-5}M CHA	11(5)	+20.7±9.8*	70-110	86±14	68-189	127±42
10^{-5}M CHA/10^{-9}M8-PT	5(3)	+2.2±4.5#	94-145	121±20	73-236	161±75

Positive values indicate dilatation; negative values indicate constriction. *P<0.05 (Student's t-test, incorporating Bonferroni correction factor, comparing each compound with the mock CSF control). #P<0.05 (Student's t-test, incorporating Bonferroni correction factor, comparing each compound in the absence and presence of 10^{-9}M 8-PT). P_{CO_2} level maintained at approximately 30mmHg (Range 28-33mmHg). n_1 = number of observations; n_2 = number of cats in which n_1 observations were made.

Table III.6 EFFECTS OF 5'-(N-ETHYL)CARBOXAMIDOADENOSINE (NECA) ON PIAL VESSEL DIAMETER AND THEIR ANTAGONISM BY 10^{-9}M 8-PHENYLTHEOPHYLLINE (8-PT)

Compound	$n_1(n_2)$	Effect (% change ±standard deviation)	Mean Arterial Blood Pressure (mmHg)		Initial Vessel Calibre (μm)	
			Range	Mean±s.d.	Range	Mean±s.d.
Mock CSF	28(23)	+1.5±6.8	71-135	99±16	36-243	105±44
10^{-9}M NECA	10(6)	+9.4±8.8*	75-115	93±14	48-182	111±42
10^{-9}M NECA/10^{-9}M8-PT	3(3)	-0.3±11.8	92-115	107±13	101-161	122±34
10^{-7}M NECA	13(5)	+15.0±7.5*	70-112	91±15	63-179	119±43
10^{-7}M NECA/10^{-9}M8-PT	3(3)	-0.7±5.7#	88-108	101±11	96-129	107±19
10^{-5}M NECA	10(5)	+17.4±11.3*	75-105	90±9	57-192	114±51
10^{-5}M NECA/10^{-9}M8-PT	4(3)	-8.2±12.3#	105-118	111±5	44-97	65±23

Positive values indicate dilation; negative values indicate constriction. *P<0.05 (Student's t-test, incoporating Bonferroni correction factor, comparing each compound with the mock CSF control). #P<0.05 (Student's t-test incorporating Bonferroni correction factor, comparing each compound in the absence and presence of 10^{-9}M8-PT). P_{CO_2} level maintained at approximately 30mmHg (Range 28-33mmHg). n_1 = number of observations; n_2 = number of cats in which n_1 observations were made.

Table III.7 EFFECT OF CHA ON CEREBRAL BLOOD FLOW AS MEASURED BY LASER DOPPLER FLOWMETRY

Time (minutes)	% change in CBF from baseline level	
	0.9% saline	10^{-10} moles|min CHA
1	-2.8±6.5(6)	-3.5±5.9(6)
2	0.1±8.6(6)	-2.8±6.0(6)
3	1.6±11.4(6)	-5.8±11.4(6)
4	6.2±11.2(6)	-5.4±17.0(6)
5	4.2±7.4(6)	-9.9±7.4(6) *
10	7.2±7.8(6)	-18.8±14.4(6)*
15	10.3±7.5(6)	-20.0±16.0(5)*
15.5	10.6±7.3(6)	-17.6±12.9(5)*

All values are expressed as mean±standard deviation

Figures in parentheses indicate n value

* $P<0.05$ (Student's t-test comparing CHA value to the corresponding saline control value at each time point)

Table III.8 PHYSIOLOGICAL PARAMETERS MONITORED IN LASER - DOPPLER EXPERIMENTS

Parameter	Pre-saline infusion	Pre-CHA infusion
P_{CO_2} (mmHg)	44.6±4.4	43.1±6.9
PO_2 (mmHg)	129.1±17.9	134.0±15.4
MABP (mmHg)	105±17	87±19
Temp. (°C)	36.6±0.9	36.1±0.8
$[HCO_3^-]$ (mmol/l)	24.4±2.8	24.5±2.4
Base excess (mmol/l)	-1.1±4.1	-0.9±3.6
pH	7.360±0.078	7.375±0.087

All values are expressed as mean±standard deviation.

n = 6 in all cases.

No significant differences in any of the parameters between CHA and saline control.

Table III.9 IPSILATERAL HEMISPHERE: CHANGES IN LCBF PRODUCED BY ADENOSINE AND ATP

STRUCTURE	0.9% Saline CONTROL	10^{-7} moles/min ADENOSINE	$3x10^{-7}$ m/m ADENOSINE	10^{-7} moles/min ATP	$3x10^{-7}$ m/m ATP
Cortical Areas					
Visual Cortex	140±4 (4)	154±32 (4)	147±16 (5)	152±42 (4)	220±53 (5)
Auditory Cortex	178±72 (5)	185±56 (4)	181±34 (5)	270±112 (4)	186±56 (5)
Parietal Cortex	157±31 (5)	245±156 (4)	165±66 (5)	187±60 (4)	143±20 (5)
Sensory Motor Cortex	178±65 (5)	164±62 (4)	142±27 (5)	269±94 (4)	174±23 (5)
Frontal Cortex	150±59 (5)	150±54 (4)	184±29 (5)	189±47 (4)	199±46 (5)
Prefrontal Cortex	166±5 (3)	202±47 (4)	175±23 (5)	208±67 (3)	230±49 (4)
Anterior Cingulate Cortex	196±64 (5)	253±75 (4)	165±26 (4)	296±123 (4)	200±80 (5)
Diencephalic Areas					
Medial Geniculate	159±45 (5)	188±50 (4)	165±14 (5)	222±86 (3)	188±102 (5)
Lateral Geniculate	120±40 (5)	169±49 (4)	182±78 (5)	198±68 (4)	159±43 (5)
Thalamus, Medio-dorsal	148±56 (5)	185±85 (4)	154±33 (4)	197±58 (4)	182±60 (5)
Thalamus, Ventro-lateral	125±42 (5)	134±54 (4)	139±49 (5)	158±71 (4)	129±36 (5)
Hypothalamus	121±44 (5)	138±65 (4)	113±38 (5)	174±128 (4)	109±24 (5)
Lateral Habenula	154±25 (5)	189±53 (4)	204±93 (5)	208±78 (4)	176±62 (5)
Amygdala	99±31 (5)	138±1 (2)	114±41 (3)	92±35 (2)	102±18 (3)
Caudate Nucleus	233±71 (5)	176±58 (4)	156±29 (5)	208±66 (4)	190±43 (5)
Nucleus Accumbens	164±91 (5)	185±69 (4)	174±60 (5)	164±12 (4)	154±21 (5)
Globus Pallidus	104±34 (5)	120±85 (4)	91±18 (5)	161±120 (4)	126±42 (5)
Internal Capsule	90±35 (4)	106±12 (2)	94±48 (3)	67±3 (2)	92±23 (2)
Subthalamic Nucleus	145±34 (5)	140±38 (4)	160±18 (4)	177±69 (4)	188±48 (5)
Hindbrain Areas					
Vestibular Nucleus	193±49 (4)	209±8 (3)	192±45 (4)	256±68 (3)	263±112 (3)
Superior Olive	227±121 (5)	196±54 (4)	204±42 (5)	233±100 (3)	203±49 (3)
Inferior Olive	205±62 (5)	210±22 (2)	130±37 (2)	426±181 (2)	286±20 (3)
Pons	135±44 (5)	101±13 (4)	89±14 (5)	90±32 (2)	176±113 (5)

Table III.9 (continued)

STRUCTURE	0.9% Saline CONTROL	10^{-7} moles/min ADENOSINE	$3x10^{-7}$ m/m ADENOSINE	10^{-7} moles/min ATP	$3x10^{-7}$ m/m ATP
Mesencephalic Areas					
Red Nucleus	147±28 (5)	170±49 (4)	147±11 (4)	152±48 (3)	171±34 (5)
Substantia Nigra	103±26 (5)	122±38 (4)	101±15 (5)	134±31 (3)	124±27 (5)
Superior Colliculus	156±21 (5)	196±102 (4)	161±36 (5)	202±48 (3)	203±46 (5)
Inferior Colliculus	204±47 (5)	246±118 (4)	229±38 (5)	251±60 (3)	282±98 (4)
Telencephalic Areas					
Hippocampus, Molecular layer	139±57 (5)	116±13 (4)	133±17 (5)	140±18 (4)	178±17 (5)
Dentate Gyrus	143±50 (5)	122±41 (4)	134±16 (5)	164±50 (4)	156±51 (5)
Septal Nucleus	163±67 (5)	108±25 (4)	110±23 (5)	122±49 (4)	140±58 (5)
Cerebellum Nucleus	169±19 (5)	197±10 (3)	173±53 (4)	212±38 (3)	193±21 (5)
Cerebellum Hemisphere	93±18 (5)	85±30 (3)	115±31 (4)	104±19 (3)	124±43 (5)
Fibre Tracts					
Cerebellum White	55±15 (5)	53±12 (3)	66±29 (4)	84±17 (3)	70±29 (5)
Genu	69±32 (5)	67±15 (4)	76±36 (5)	86±30 (4)	64±11 (5)

All values for local cerebral blood flow ($ml.100g^{-1}.min^{-1}$.) are expressed as mean ± standard deviation.

Figures in parentheses indicate n value.

Table III.10 CONTRALATERAL HEMISPHERE: CHANGES IN LCBF PRODUCED BY ADENOSINE AND ATP

STRUCTURE	0.9% Saline CONTROL	10^{-7} moles/min ADENOSINE	$3x10^{-7}$ m/m ADENOSINE	10^{-7} moles/min ATP	$3x10^{-7}$ m/m ATP
Cortical Structures					
Visual Cortex	156±50 (5)	145±32 (4)	133±17 (5)	138±41 (4)	155±94 (5)
Auditory Cortex	150±47 (5)	170±60 (4)	152±33 (5)	237±124 (4)	150±42 (5)
Parietal Cortex	157±24 (5)	160±29 (4)	162±78 (5)	146±42 (4)	137±30 (5)
Sensory motor Cortex	167±45 (5)	139±25 (4)	133±15 (5)	164±59 (4)	160±48 (5)
Frontal Cortex	174±66 (5)	142±32 (4)	132±19 (5)	164±42 (4)	143±48 (5)
Prefrontal Cortex	159±7 (3)	173±42 (4)	147±41 (5)	183±43 (3)	180±40 (4)
Anterior Cingulate Cortex	189±56 (5)	244±78 (4)	160±19 (4)	275±121 (4)	180±74 (5)
Diencephalic Areas					
Medial Geniculate	164±46 (5)	178±56 (4)	183±42 (5)	159±78 (3)	181±62 (5)
Lateral Geniculate	124±34 (5)	142±23 (4)	173±81 (5)	192±82 (4)	164±70 (5)
Thalamus, Medio-dorsal	152±57 (5)	156±46 (4)	127±10 (4)	184±71 (4)	159±35 (5)
Thalamus, Ventro-lateral	121±37 (5)	126±47 (3)	128±38 (5)	163±121 (4)	140±50 (5)
Hypothalamus	107±19 (5)	103±30 (4)	104±45 (5)	160±127 (4)	105±23 (5)
Lateral Habenula	154±24 (5)	166±36 (4)	180±70 (5)	212±107 (4)	163±40 (5)
Amygdala	106±28 (5)	109±3 (2)	73±4 (3)	80±22 (2)	91±6 (3)
Caudate Nucleus	234±70 (5)	138±22 (4)	135±7 (5)*	189±101 (4)	187±80 (5)
Nucleus Accumbens	174±88 (5)	154±56 (4)	128±16 (5)	162±35 (4)	148±17 (5)
Globus Pallidus	106±41 (5)	80±26 (4)	82±22 (5)	182±138 (4)	129±79 (5)
Internal Capsule	96±31 (4)	86±8 (2)	62±11 (3)	80±29 (2)	82±4 (2)
Subthalamic Nucleus	148±35 (5)	123±30 (4)	159±11 (4)	156±67 (4)	185±50 (5)
Hindbrain Areas					
Vestibular Nucleus	186±40 (4)	193±19 (3)	189±44 (4)	233±84 (3)	129±79 (4)
Superior Olive	235±142 (5)	204±52 (4)	216±46 (5)	222±76 (3)	206±36 (4)
Inferior Olive	194±55 (3)	216±12 (2)	124±12 (2)	246±100 (2)	264±30 (3)
Pons	129±40 (5)	96±15 (4)	94±14 (5)	83±28 (2)	152±110 (5)

Table III.10 (continued)

STRUCTURE	0.9% Saline CONTROL	10^{-7} moles/min ADENOSINE	$3x10^{-7}$ m/m ADENOSINE	10^{-7} moles/min ATP	$3x10^{-7}$ m/m ATP
Mesencephalic Area					
Red Nucleus	146±26 (5)	146±38 (4)	141±13 (4)	148±52 (3)	173±26 (5)
Substantia Nigra	102±21 (5)	99±10 (4)	93±10 (5)	123±38 (3)	113±33 (5)
Superior Colliculus	158±23 (5)	176±67 (4)	153±14 (5)	157±51 (3)	183±28 (5)
Inferior Colliculus	202±49 (5)	199±50 (4)	235±60 (5)	192±32 (3)	236±106 (5)
Telencephalic Areas					
Hippocampus, Molecular layer	124±19 (5)	118±16 (4)	140±14 (5)	121±32 (4)	146±20 (5)
Dentate Gyrus	120±26 (5)	116±11 (4)	131±8 (5)	125±34 (4)	126±31 (5)
Septal Nucleus	156±54 (5)	107±21 (4)	100±13 (5)	116±38 (4)	127±59 (5)
Cerebellum Nucleus	166±35 (4)	180±9 (3)	160±35 (4)	209±55 (3)	200±35 (5)
Cerebellum Hemisphere	92±14 (5)	81±8 (3)	116±31 (4)	101±24 (3)	110±31 (5)
Fibre Tracts					
Cerebellum White	55±20 (5)	44±5 (3)	60±18 (4)	63±14 (3)	72±24 (5)
Genu	69±31 (5)	62±15 (4)	50±11 (5)	82±22 (4)	56±17 (5)

All values for local cerebral blood flow ($ml.100g^{-1}.min^{-1}$.) are expressed as mean ± standard deviation.

Figures in parentheses indicate n value

*$P<0.05$ (Student's t-test, incorporating Bonferroni correction factor comparing each compound to control).

Table III.11 IPSILATERAL HEMISPHERE: CHANGES IN LCBF PRODUCED BY CHA, 2CADO and NECA

STRUCTURE	0.9% Saline CONTROL	10^{-10} moles/min CHA	10^{-9} moles/min 2-CADO	10^{-11} moles/min NECA
Cortical Structures				
Visual Cortex	126±34 (8)	94±17 (6)	114±25 (6)	190±94 (5)
Auditory Cortex	128±27 (8)	89±37 (6)	122±38 (6)	180±88 (5)
Parietal Cortex	151±52 (8)	95±30 (6)	124±24 (6)	147±43 (5)
Sensory Motor Cortex	165±32 (8)	93±18 (6)*	136±36 (6)	159±35 (5)
Frontal Cortex	144±17 (8)	108±16 (6)*	126±26 (6)	168±40 (5)
Prefrontal Cortex	148±48 (6)	122±30 (4)	132±49 (4)	186±52 (3)
Anterior Cingulate	151±48 (8)	110±30 (6)	131±30 (6)	196±85 (5)
Diencephalic Areas				
Medial Geniculate	131±36 (8)	86±16 (6)*	115±39 (6)	141±37 (5)
Lateral Geniculate	106±36 (8)	68±11 (6)*	113±16 (6)	109±44 (5)
Thalamus, Mediodorsal	145±72 (8)	99±25 (6)	98±43 (5)	150±54 (5)
Thalamus, Ventrolateral	145±72 (8)	82±17 (6)	101±49 (5)	106±35 (5)
Hypothalamus	86±27 (8)	76±22 (6)	68±12 (6)	94±32 (5)
Lateral Habenula	133±34 (7)	91±18 (6)*	118±31 (6)	137±50 (5)
Caudate Nucleus	153±29 (8)	99±34 (6)*	128±28 (6)	129±26 (5)
Nucleus Accumbens	120±25 (8)	93±28 (6)	113±49 (6)	141±52 (5)
Globus Pallidus	109±26 (8)	77±10 (6)*	67±18 (6)*	94±35 (5)
Subthalamic Nucleus	153±26 (8)	122±18 (6)	128±18 (6)	157±49 (5)
Hindbrain Areas				
Vestibular Nucleus	169±40 (8)	122±28 (6)	140±14 (3)	176±37 (4)
Superior Olive	174±38 (8)	131±6 (6)*	139±20 (6)	163±47 (5)
Inferior Olive	158±46 (8)	127±23 (5)	119±24 (5)	160±48 (3)
Pons	102±29 (8)	76±12 (6)	78±10 (6)	86±18 (4)

Table III.11 (continued.)

STRUCTURE	0.9% Saline CONTROL	10^{-10} moles/min CHA	10^{-9} moles/min 2-CADO	10^{-11} moles/min NECA
Mesencephalic Areas				
Red Nucleus	146±29 (8)	111±17 (6)	110±8 (6)*	138±41 (5)
Substantia Nigra	109±22 (8)	86±13 (6)	82±15 (6)	113±44 (5)
Superior Colliculus	143±34 (8)	106±13 (6)	137±36 (6)	142±46 (5)
Inferior Colliculus	196±63 (8)	136±25 (6)	132±17 (6)	196±59 (4)
Telencephalic Areas				
Hippocampus, Molecular layer	122±29 (8)	87±26 (6)	105±32 (6)	104±30 (5)
Dentate Gyrus	115±24 (8)	83±14 (6)*	107±39 (6)	104±30 (5)
Septal Nucleus	91±18 (8)	71±10 (6)	70±26 (6)	98±30 (5)
Cerebellum Nucleus	175±17 (7)	131±25 (6)*	117±33 (5)*	136±33 (5)
Cerebellum Hemisphere	86±17 (8)	62±9 (6)*	69±7 (5)	82±28 (4)
Fibre Tracts				
Cerebellum White	50±21 (8)	38±12 (6)	46±7 (4)	52±17 (5)
Genu	50±21 (8)	34±8 (6)	47±9 (6)	61±15 (5)

All values for local cerebral blood flow (ml.100g^{-1}. min^{-1}) are expressed as mean ± standard deviation.

Figures in parentheses indicate n value.

*P<0.05 (Student's t-test, incorporating Bonferroni correction factor, comparing each compound to control.)

Table III.12 CONTRALATERAL HEMISPHERE: CHANGES IN LCBF PRODUCED BY CHA, 2-CADO and NECA

STRUCTURE	0.9% Saline CONTROL	10^{-10} moles/min CHA	10^{-9} moles/min 2-CADO	10^{-11} moles/min NECA
Cortical Structures				
Visual Cortex	142±44 (8)	103±24 (6)	92±35 (6)	157±97 (5)
Auditory Cortex	126±32 (8)	105±29 (6)	95±50 (6)	151±78 (5)
Parietal Cortex	147±44 (8)	103±17 (6)	97±24 (6)	148±71 (5)
Sensory Motor Cortex	174±47 (8)	108±10 (6)*	105±30 (6)*	136±64 (5)
Frontal Cortex	151±40 (8)	120±11 (6)	108±27 (6)	147±58 (5)
Prefrontal Cortex	148±46 (6)	128±16 (4)	120±24 (4)	175±39 (3)
Anterior Cingulate Cortex	145±43 (8)	117±28 (6)	101±46 (6)	218±92 (5)
Diencephalic Areas				
Medial Geniculate	131±41 (8)	92±9 (6)	103±53 (6)	144±45 (5)
Lateral Geniculate	121±38 (8)	81±18 (6)	106±36 (6)	126±52 (5)
Thalamus, Mediodorsal	151±75 (8)	103±25 (6)	101±52 (5)	139±55 (5)
Thalamus, Ventrolateral	128±39 (8)	84±11 (6)	86±24 (5)	105±45 (3)
Hypothalamus	87±20 (8)	65±16 (6)	62±10 (6)	95±38 (5)
Lateral Habenula	129±28 (7)	93±23 (6)	113±36 (6)	137±48 (5)
Caudate Nucleus	148±53 (8)	109±9 (5)	105±22 (6)	112±48 (5)
Nucleus Accumbens	120±38 (8)	106±31 (6)	101±24 (6)	138±60 (5)
Globus Pallidus	112±30 (8)	66±17 (6)*	79±12 (6)	91±34 (5)
Subthalamic Nucleus	154±37 (8)	134±15 (6)	120±20 (6)	162±68 (5)
Hindbrain Areas				
Vestibular Nucleus	169±39 (8)	117±25 (6)	133±23 (3)	182±48 (4)
Cochlear Nucleus	148±16 (6)	134±11 (2)	100±4 (2)*	166±32 (2)
Superior Olive	179±43 (8)	135±14 (6)	131±29 (6)	155±60 (5)
Inferior Olive	162±37 (8)	129±21 (5)	115±30 (5)	157±46 (3)
Pons	102±26 (8)	76±14 (6)	77±8 (6)	89±29 (4)

Table III.12 (continued)

STRUCTURE	0.9% Saline CONTROL	10^{-10} moles/min CHA	10^{-9} moles/min 2-CADO	10^{-11} moles/min NECA
Mesencephalic Areas				
Red Nucleus	138±26 (8)	105±13 (6)*	108±11 (6)	137±42 (5)
Substantia Nigra	100±19 (8)	89±11 (6)	77±11 (6)	114±39 (5)
Superior Colliculus	144±31 (8)	108±9 (6)	118±19 (6)	140±49 (5)
Inferior Colliculus	205±77 (8)	142±25 (6)	139±26 (6)	173±57 (4)
Telencephalic Areas				
Hippocampus, Molecular Layer	123±42 (8)	90±25 (6)	97±31 (6)	109±48 (5)
Dentate Gyrus	114±31 (8)	91±19 (6)	97±36 (6)	111±46 (5)
Septal Nucleus	93±15 (8)	74±12 (6)	75±29 (6)	97±36 (5)
Cerebellum Nucleus	166±15 (8)	133±28 (6)*	116±33 (5)*	128±38 (5)
Cerebellum Hemisphere	85±17 (8)	64±5 (6)*	70±7 (5)	82±28 (5)
Fibre Tracts				
Cerebellum White	51±22 (8)	38±4 (6)	52±13 (4)	51±16 (5)
Genu	53±12 (8)	42±11 (6)	45±8 (6)	62±25 (5)

All values for local cerebral blood flow ($ml.100g^{-1}.min^{-1}$) are expressed as mean±standard deviation.

Figures in parentheses indicated n value

*$P<0.05$ (Student's t-test, incorporating Bonferroni correction factor, comparing each compound to control).

Table III.13 IPSILATERAL HEMISPHERE: CHANGES IN LCBF PRODUCED BY VARIOUS CONCENTRATIONS OF CHA

STRUCTURE	0.9% Saline CONTROL	10^{-10} moles/min CHA	10^{-11} moles/min CHA	10^{-12} moles/min CHA
Cortical Structures				
Visual Cortex	126±34 (8)	94±17 (6)	101±35 (5)	97±34 (4)
Auditory Cortex	128±27 (8)	89±37 (6)	111±45 (5)	100±33 (4)
Parietal Cortex	151±52 (8)	95±30 (6)	100±53 (5)	99±43 (4)
Sensory Motor Cortex	165±32 (8)	93±18 (6)*	126±59 (5)	126±56 (4)
Frontal Cortex	144±17 (8)	108±16 (6)*	119±53 (5)	118±45 (4)
Prefrontal Cortex	148±48 (6)	122±30 (4)	130±69 (4)	115±30 (4)
Anterior Cingulate Cortex	151±48 (8)	110±30 (6)	134±49 (5)	142±55 (4)
Diencephalic Areas				
Medial Geniculate	131±36 (8)	86±16 (6)*	118±42 (5)	86±26 (4)
Lateral Geniculate	106±36 (8)	68±11 (6)*	79±43 (5)	75±21 (4)
Thalamus, Mediodorsal	145±72 (8)	99±25 (6)	104±28 (5)	109±37 (4)
Thalamus, Ventrolateral	145±72 (8)	82±17 (6)	85±34 (5)	75±25 (4)
Hypothalamus	86±27 (8)	76±22 (6)	61±19 (5)	56±24 (4)
Lateral Habenula	133±34 (7)	91±18 (6)*	105±34 (5)	120±45 (4)
Amygdala	85±28 (6)	60±21 (2)	59±22 (5)	47±16 (3)
Caudate Nucleus	153±29 (8)	99±34 (6)*	128±59 (5)	139±65 (4)
Nucleus Accumbens	120±25 (8)	93±27 (6)	93±27 (5)	94±30 (4)
Globus Pallidus	109±26 (8)	77±10 (6)*	76±17 (5)	89±32 (4)
Internal Capsule	65±12 (6)	51±23 (2)	42±16 (5)	42±15 (3)
Subthalamic Nucleus	153±26 (8)	122±18 (6)	126±54 (5)	148±37 (4)
Hindbrain Areas				
Vestibular Nucleus	169±40 (8)	122±28 (6)	142±47 (5)	135±38 (4)
Superior Olive	174±38 (8)	131±6 (6)*	152±37 (5)	164±48 (4)
Inferior Olive	158±46 (8)	127±23 (5)	136±19 (5)	144±16 (4)
Pons	102±29 (8)	76±12 (6)	85±18 (5)	76±16 (4)

Table III.13 (continued)

STRUCTURE	0.9% Saline CONTROL	10^{-10} moles/min CHA	10^{-11} moles/min CHA	10^{-12} moles/min CHA
Mesencephalic Areas				
Red Nucleus	146±29 (8)	111±17 (6)	125±43 (5)	116±37 (4)
Substantia Nigra	109±22 (8)	86±13 (6)	89±9 (5)	86±24 (4)
Superior Colliculus	143±34 (8)	106±13 (6)	120±22 (5)	125±19 (4)
Inferior Colliculus	196±63 (8)	136±25 (6)	142±41 (5)	136±44 (4)
Telencephalic Areas				
Hippocampus, Molecular Layer	122±29 (8)	87±26 (6)	96±30 (5)	95±25 (4)
Dentate Gyrus	115±24 (8)	83±14 (6)*	90±28 (5)	86±27 (4)
Septal Nucleus	91±18 (8)	71±10 (6)	79±28 (5)	81±33 (4)
Cerebellum Nucleus	175±17 (7)	131±25 (6)*	134±53 (5)	148±45 (4)
Cerebellum Hemisphere	86±17 (8)	62±9 (6)*	67±18 (5)	68±19 (4)
Fibre Tracts				
Cerebellum White	50±21 (8)	38±12 (6)	44±14 (5)	34±10 (4)
Genu	50±21 (8)	34±8 (6)	39±14 (5)	45±15 (4)

All values for local cerebral blood flow (ml.100g^{-1}.min^{-1}) are expressed as mean ± standard deviation.

Figures in parentheses indicate n value.

*P<0.05 (Student's t-test, incorporating Bonferroni correction factor, comparing each compound to control.

Table III.14 CONTRLATERAL HEMISPHERE: CHANGES IN LCBF PRODUCED BY VARIOUS CONCENTRATIONS OF CHA

STRUCTURE	0.9% Saline CONTROL	10^{-10} moles/min CHA	10^{-11} moles/min CHA	10^{-12} moles/min CHA
Cortical Structures				
Visual Cortex	142±44 (8)	103±24 (6)	116±36 (5)	106±43 (4)
Auditory Cortex	126±32 (8)	105±29 (6)	128±58 (5)	107±38 (4)
Parietal Cortex	147±44 (8)	103±17 (6)	114±49 (5)	117±54 (4)
Sensory Motor Cortex	174±47 (8)	108±10 (6)	141±66 (5)	140±68 (4)
Frontal Cortex	151±40 (8)	120±11 (6)	131±62 (5)	140±43 (4)
Prefrontal Cortex	148±46 (6)	128±16 (4)	142±84 (4)	126±40 (4)
Anterior Cingulate Cortex	145±43 (8)	117±28 (6)	143±52 (5)	148±55 (4)
Diencephalic Areas				
Medial Geniculate	131±41 (8)	92±9 (6)	107±28 (5)	89±28 (4)
Lateral Geniculate	121±38 (8)	81±18 (6)	82±46 (5)	78±28 (4)
Thalamus, Mediodorsal	151±75 (8)	103±25 (6)	104±29 (5)	114±44 (4)
Thalamus, Ventrolateral	128±39 (8)	84±11 (6)	96±39 (5)	90±35 (4)
Hypothalamus	87±20 (8)	65±16 (6)	62±19 (5)	65±24 (4)
Lateral Habenula	129±28 (7)	92±23 (6)	110±36 (5)	130±58 (4)
Amygdala	86±25 (6)	65±16 (2)	72±27 (5)	59±23 (3)
Caudate Nucleus	148±53 (6)	109±9 (5)	145±45 (5)	142±69 (4)
Nucleus Accumbens	120±38 (8)	106±31 (6)	102±22 (5)	104±23 (4)
Globus Pallidus	112±30 (8)	66±17 (6)	98±27 (5)	96±34 (4)
Internal Capsule	66±12 (6)	55±17 (2)	50±16 (5)	47±19 (3)
Subthalamic Nucleus	154±37 (8)	134±15 (6)	166±32 (5)	148±46 (4)
Hindbrain Areas				
Vestibular Nucleus	169±39 (8)	117±25 (6)*	145±52 (5)	134±32 (4)
Cochlear Nucleus	148±16 (6)	134±11 (2)	142±51 (3)	151±51 (4)
Superior Olive	179±43 (8)	135±14 (6)	161±20 (5)	183±57 (4)
Inferior Olive	162±37 (8)	129±21 (5)	129±19 (5)	144±36 (4)
Pons	102±26 (8)	76±14 (6)	83±16 (5)	85±20 (4)

Table III.14 (continued)

STRUCTURE	0.9% Saline CONTROL	10^{-10} moles/min CHA	10^{-11} moles/min CHA	10^{-12} moles/min CHA
Mesencephalic Areas				
Red Nucleus	138±26 (8)	105±13 (6)*	116±33 (5)	118±34 (4)
Substantia Nigra	100±19 (8)	89±11 (6)	90±10 (5)	97±25 (4)
Superior Colliculus	144±31 (8)	108±9 (6)	133±27 (5)	136±37 (4)
Inferior Colliculus	205±77 (8)	142±25 (6)	147±52 (5)	150±48 (4)
Telencephalic Areas				
Hippocampus, Molecular Layer	123±42 (8)	90±25 (6)	102±34 (5)	104±26 (4)
Dentate Gyrus	114±31 (8)	91±19 (6)	94±25 (5)	92±30 (4)
Septal Nucleus	93±15 (8)	74±12 (6)	84±28 (5)	85±35 (4)
Cerebellum Nucleus	166±15 (8)	133±28 (6)*	133±72 (5)	146±45 (4)
Cerebellum Hemisphere	85±17 (8)	64±5 (6)*	68±21 (5)	71±19 (4)
Fibre Tracts				
Cerebellum White	51±22 (8)	38±4 (6)	45±15 (5)	34±9 (4)
Genu	53±12 (8)	42±11 (6)	41±16 (5)	44±14 (4)

All values for local cerebral blood flow ($ml.100g^{-1}.min^{-1}$) are expressed as mean ± standard deviation.

Figures in parentheses indicate n value

*$P<0.05$ (Student's t-test, incorporating Bonferroni correction factor, comparing each compound to control).

Table III.15 PHYSIOLOGICAL PARAMETERS MONITORED IN IAP EXPERIMENTS INVOLVING ADENOSINE AND ATP

Parameter	0.9% Saline CONTROL	10^{-7} moles/min ADENOSINE	$3x10^{-7}$ moles/min ADENOSINE	10^{-7} moles/min ATP	$3x10^{-7}$ moles/min ATP
P_{CO^2} (mmHg)	40.6±3.0	39.0±1.7	39.7±1.5	40.4±3.1	38.8±1.6
PO_2 (mmHg)	181.5±27.7	146.8±45.7	173.8±9.7	198.6±54.4	174.9±17.1
MABP (mmHg)	88±8	100±20	86±22	74±5	84±10
Temp(°C)	37.0±1.8	36.4±1.2	37.7±1.6	36.8±0.9	37.5±0.5
$[HCO_3-]$ (mmol|l)	22.8±2.7	24.6±2.1	23.4±3.3	25.0±1.0	24.0±4.0
Base excess (mmol|l)	-1.8±4.1	-0.2±2.2	-0.7±3.8	0.2±1.9	-0.2±5.5
pH	7.373±0.079	7.388±0.050	7.378±0.055	7.345±0.121	7.394±0.088
n	5	4	5	4	5

All values are expressed as mean±standard deviation

Table III.16 PHYSIOLOGICAL PARAMETERS MONITORED IN IAP EXPERIMENTS INVOLVING CHA, 2-CADO AND NECA

Parameter	0.9% Saline CONTROL	10^{-10} moles/min CHA	10^{-9} moles/min 2-CADO	10^{-11} moles/min NECA
P_{CO_2} (mmHg)	39.8±0.9	40.3±1.8	38.5±1.2	39.3±0.8
PO_2 (mmHg)	141.9±18.1	138.9±25.7	141.6±18.6	160.9±21.2
MABP (mmHg)	87±11	76±6	89±11	97±16
Temp ($^{\circ}$C)	37.6±0.8	37.6±0.8	37.2±1.2	38.2±0.4
$[HCO_3^-]$ (mmol|l)	24.1±1.9	25.6±2.8	27.1±1.7	25.9±1.7
Base excess (mmol|l)	0.1±2.2	0.7±2.2	4.0±1.9	2.9±2.0
pH	7.392±0.034	7.411±0.047	7.457±0.026	7.433±0.029
n	8	6	6	5

All values are expressed as mean±standard deviation

Table III.17 PHYSIOLOGICAL PARAMETERS MONITORED IN IAP EXPERIMENTS INVOLVING CHA

Parameter	0.9% Saline CONTROL	10^{-10} moles/min CHA	10^{-11} moles/min CHA	10^{-12} moles/min CHA
P_{CO_2} (mmHg)	39.8±0.9	40.3±1.8	41.5±1.4	40.6±1.0
PO_2 (mmHg)	141.9±18.1	138.9±25.7	128.3±32.9	137.4±28.4
MABP (mmHg)	87±11	76±6	76±9	74±5
Temp ($^{\circ}$C)	37.6±0.8	37.6±0.8	37.5±0.4	37.9±0.8
$[HCO_3^-]$ (mmol|l)	24.1±1.9	25.6±2.8	25.5±2.0	26.4±3.2
Base excess (mmol|l)	0.1±2.2	0.7±2.2	1.6±2.5	3.0±4.1
pH	7.392±0.034	7.411±0.047	7.401±0.034	7.424±0.057
n	8	6	5	4

all values are expressed as mean±standard deviation

Table III.18 IPSILATERAL HEMISPHERE: CHANGES IN LCGU PRODUCED BY ADENOSINE, 2-CADO, CHA AND NECA

STRUCTURE	0.9% Saline CONTROL	3×10^{-7} moles/min ADENOSINE	10^{-9} moles/m 2-CADO	10^{-10} moles/m CHA	10^{-11} moles/m NECA
Cortical Areas					
Visual Cortex	108±15 (5)	110±20 (5)	140±34 (5)	132±25 (4)	130±19 (5)
Auditory Cortex	112±14 (5)	111±17 (5)	138±30 (5)	150±22 (4)	128±15 (5)
Parietal Cortex	101±26 (5)	98±18 (5)	115±22 (5)	120±20 (5)	120±28 (5)
Sensory Motor Cortex	101±21 (5)	101±20 (5)	106±16 (5)	105±5 (5)	116±30 (5)
Frontal Cortex	101±22 (5)	102±13 (5)	107±16 (5)	116±10 (5)	117±23 (5)
Prefrontal Cortex	117±20 (5)	117±21 (5)	122±28 (5)	143±21 (5)	136±14 (5)
Anterior Cingulate Cortex	130±35 (5)	128±28 (5)	142±14 (5)	145±25 (5)	140±21 (5)
Diencephalic Areas					
Medial Geniculate	84±16 (5)	95±12 (5)	97±33 (5)	98±15 (5)	89±11 (5)
Lateral Geniculate	75±23 (5)	78±16 (5)	87±22 (5)	99±16 (5)	88±24 (5)
Thalamus, Medio-dorsal	96±17 (5)	96±25 (5)	101±19 (5)	103±21 (5)	110±12 (5)
Thalamus, Ventro-lateral	82±10 (5)	85±24 (5)	82±16 (5)	85±8 (5)	92±17 (5)
Hypothalamus	64±12 (5)	62±17 (5)	78±19 (5)	70±15 (5)	69±15 (5)
Lateral Habenula	98±29 (5)	106±31 (5)	101±18 (5)	119±18 (5)	110±20 (5)
Amygdala	53±15 (5)	54±15 (4)	66±11 (4)	60±6 (5)	65±13 (5)
Caudate Nucleus	126±22 (5)	132±32 (5)	127±16 (5)	142±9 (5)	135±32 (5)
Nucleus Accumbens	109±32 (5)	96±25 (5)	103±11 (5)	119±24 (5)	116±24 (5)
Globus Pallidus	64±11 (5)	70±19 (5)	74±14 (5)	76±6 (5)	81±23 (5)
Internal Capsule	40±16 (5)	44±11 (4)	36±17 (4)	53±15 (5)	46±10 (5)
Subthalamic Nucleus	91±15 (5)	103±26 (5)	101±14 (5)	107±18 (5)	117±25 (5)
Hindbrain Areas					
Vestibular Nucleus	109±21 (5)	112±20 (5)	117±13 (5)	131±12 (5)	121±12 (5)
Superior Olive	95±26 (5)	92±12 (5)	97±15 (5)	113±18 (5)	112±14 (5)
Inferior Olive	103±21 (5)	98±18 (5)	108±15 (5)	110±10 (5)	106±22 (5)
Pons	64±12 (5)	72±14 (5)	85±10 (5)	82±14 (4)	72±13 (5)

Table III.18 (continued)

STRUCTURE	0.9% Saline CONTROL	$3x10^{-7}$ moles/min ADENOSINE	10^{-9} moles/m 2-CADO	10^{-10} moles/m CHA	10^{-11} moles/m NECA
Mesencephalic Areas					
Red Nucleus	84±22 (5)	89±12 (4)	104±30 (4)	95±5 (4)	91±17 (5)
Substantia Nigra Compacta	86±19 (5)	94±15 (5)	114±38 (5)	101±28 (4)	91±18 (5)
Substantia Nigra Reticulata	70±18 (5)	74±16 (5)	84±28 (5)	81±12 (4)	80±23 (5)
Superior Colliculus	98±13 (5)	106±18 (5)	110±24 (5)	120±7 (5)	107±22 (5)
Inferior Colliculus	104±23 (5)	111±23 (5)	128±31 (5)	131±22 (4)	132±15 (5)
Telencephalic Areas					
Hippocampus Molecular layer	127±16 (5)	128±14 (5)	145±27 (5)	138±15 (4)	124±21 (5)
Dentate Gyrus	109±21 (5)	106±10 (5)	111±27 (5)	113±10 (4)	109±17 (5)
Septal Nucleus	70±10 (5)	83±18 (5)	87±14 (5)	88±14 (5)	88±12 (5)
Cerebellum Nucleus	103±28 (5)	114±17 (5)	133±28 (5)	127±11 (5)	122±28 (5)
Cerebellum Hemisphere	63±14 (5)	63±13 (5)	79±10 (5)	66±8 (5)	70±10 (5)
Fibre Tracts					
Cerebellum White	46±13 (5)	44±13 (5)	53±10 (5)	42±9 (5)	44±12 (5)
Corpus Callosum	48±12 (3)	54±22 (4)	60±22 (5)	46±9 (3)	60±10 (5)
Genu	49±16 (5)	58±21 (5)	67±10 (5)	67±15 (5)	68±17 (5)

All values for local cerebral glucose utilisation ($\mu mol.100g^{-1}.min^{-1}$) are expressed as mean±standard deviation.

Figures in parentheses indicate n value

Table III.19 CONTRALATERAL HEMISPHERE: CHANGES IN LCGU PRODUCED BY ADENOSINE, 2-CADO, CHA AND NECA

STRUCTURE	0.9% Saline CONTROL	$3x10^{-7}$ moles/min ADENOSINE	10^{-9} moles/m 2-CADO	10^{-10} moles/m CHA	10^{-11} moles/min NECA
Cortical Areas					
Visual Cortex	109±25 (5)	111±25 (5)	139±43 (5)	120±23 (4)	122±17 (5)
Auditory Cortex	108±20 (5)	106±18 (5)	127±38 (5)	132±8 (4)	122±16 (5)
Parietal Cortex	104±32 (5)	106±20 (5)	107±25 (5)	114±25 (5)	123±29 (5)
Sensory Motor Cortex	89±29 (5)	98±15 (5)	103±13 (5)	95±6 (5)	110±21 (5)
Frontal Cortex	96±23 (5)	90±13 (5)	101±21 (5)	102±11 (5)	116±23 (5)
Prefrontal Cortex	101±18 (2)	114±24 (4)	115±23 (5)	139±17 (5)	136±12 (4)
Anterior Cingulate Cortex	126±33 (5)	123±28 (5)	138±24 (5)	142±25 (5)	141±9 (5)
Diencephalic Areas					
Medial Geniculate	79±17 (5)	92±13 (5)	102±29 (5)	96±9 (4)	90±12 (5)
Lateral Geniculate	79±28 (5)	81±20 (5)	94±16 (5)	96±23 (5)	81±17 (5)
Thalamus, Medio-dorsal	95±18 (5)	93±19 (5)	102±20 (5)	100±22 (5)	113±11 (5)
Thalamus, Ventro-lateral	82±9 (5)	81±28 (5)	85±36 (5)	85±14 (5)	89±20 (5)
Hypothalamus	63±11 (5)	63±16 (5)	80±19 (5)	71±11 (5)	70±16 (5)
Lateral Habenula	94±31 (5)	104±25 (5)	104±14 (5)	117±17 (5)	108±22 (5)
Amygdala	53±18 (5)	59±14 (4)	66±6 (4)	60±8 (5)	63±16 (5)
Caudate Nucleus	116±10 (5)	123±22 (5)	117±22 (5)	139±19 (5)	134±26 (5)
Nucleus Accumbens	101±23 (5)	100±25 (5)	105±19 (5)	110±15 (5)	113±23 (5)
Globus Pallidus	55±28 (5)	69±20 (5)	76±8 (5)	73±5 (5)	85±24 (5)
Internal Capsule	39±16 (5)	36±22 (4)	44±6 (4)	51±12 (5)	44±9 (5)
Subthalamic Nucleus	96±13 (5)	101±27 (5)	105±15 (5)	104±18 (5)	116±24 (5)
Hindbrain Areas					
Vestibular Nucleus	106±16 (5)	109±15 (5)	122±21 (5)	131±10 (5)	121±12 (5)
Superior Olive	92±23 (5)	90±10 (5)	96±15 (5)	118±21 (5)	112±14 (5)
Inferior Olive	114±14 (3)	97±19 (5)	108±23 (5)	116±9 (5)	106±22 (5)
Pons	66±14 (5)	68±15 (5)	85±12 (5)	78±13 (4)	72±13 (5)

Table III.19 (continued)

STRUCTURE	0.9% Saline CONTROL	$3x10^{-7}$ moles/min ADENOSINE	10^{-9} moles/m 2-CADO	10^{-10} moles/m CHA	10^{-11} moles/min NECA
Mesencephalic Areas					
Red Nucleus	84±23 (5)	87±14 (4)	105±32 (4)	97±8 (4)	92±22 (5)
Substantia Nigra Compacta	88±15 (5)	93±17 (5)	113±38 (5)	105±28 (4)	91±22 (5)
Substantia Nigra Reticulata	71±16 (5)	68±16 (5)	79±28 (5)	81±18 (5)	81±27 (5)
Superior Colliculus	97±15 (5)	104±14 (5)	114±28 (5)	121±10 (5)	107±19 (5)
Inferior Colliculus	104±21 (5)	113±30 (5)	128±30 (5)	126±16 (4)	130±17 (5)
Telencephalic Areas					
Hippocampus Molecular layer	122±23 (5)	114±19 (5)	119±27 (5)	133±22 (5)	122±18 (5)
Dentate Gyrus	110±20 (5)	99±16 (5)	107±24 (5)	115±15 (5)	103±17 (5)
Septal Nucleus	69±12 (5)	84±17 (5)	83±18 (5)	84±13 (5)	88±16 (5)
Cerebellum Nucleus	101±27 (5)	100±14 (5)	138±29 (5)	133±16 (5)	125±34 (5)
Cerebellum Hemisphere	60±12 (5)	63±10 (5)	83±12 (5)	72±10 (5)	67±11 (5)
Fibre Tracts					
Cerebellum White	43±11 (5)	41±13 (5)	57±13 (5)	43±20 (5)	42±13 (5)
Corpus Callosum	48±16 (5)	55±21 (4)	62±20 (5)	52±12 (3)	57±8 (4)
Genu	45±12 (5)	51±20 (5)	66±9 (5)	60±5 (5)	65±18 (5)

All values for local cerebral glucose utilisation ($\mu mol.100g^{-1}.\ min^{-1}$) are expressed as mean ± standard deviation.

Figures in parentheses indicate n value.

Table III.20 PHYSIOLOGICAL PARAMETERS MONITORED IN 2-DG EXPERIMENTS INVOLVING ADENOSINE, CHA, 2-CADO AND NECA

Parameter	0.9% Saline CONTROL	3×10^{-7} moles/min ADENOSINE	10^{-10} moles/min CHA	10^{-9} moles/min 2-CADO	10^{-11} moles/min NECA
P_{CO_2} (mmHg)	39.0±2.0	40.0±2.0	40.2±1.0	39.7±2.7	42.0±2.9
P_{O_2} (mmHg)	140.7±15.6	131.7±24.7	122.7±11.1	137.2±26.2	130.9±20.7
MABP (mmHg)	84±5	85±23	82±12	72±6*	80±6
Temp. (°C)	37.4±0.4	37.2±0.3	37.3±0.4	37.1±0.4	37.4±0.5
$[HCO_3^-]$ (mmol|l)	23.1±2.8	25.0±3.0	23.4±2.8	24.6±2.7	23.7±1.9
Base excess (mmol|l)	1.5±3.0	2.6±1.6	-1.1±3.5	0.5±3.1	-1.1±2.7
pH	7.38±0.049	7.428±0.025	7.373±0.049	7.401±0.034	7.365±0.053
n	5	5	5	5	5

All values are expressed as mean±standard deviation. *P<0.01 (Student's t-test incorporating Bonferroni correction factor comparing drug treated value with control value.

Table III.21 THE EFFECT OF THEOPHYLLINE ON THE CHANGES IN LCBF PRODUCED BY A 15 MINUTE INFUSION OF CHA (IPSILATERAL HEMISPHERE

Structure	0.9% Saline i.c+5ml/kg saline i.p.	10^{-10}moles/min CHA i.c.+5ml/kg saline i.p.	0.9% Saline i.c.+30mg/kg theophylline i.p.	10^{-10}moles/min CHA i.c.+30mg/kg theophylline i.p.
Cortical Areas				
Visual cortex	126±32 (16)	106±23 (12)	122±20 (9)	137±63 (9)
Auditory cortex	130±27 (16)	111±39 (12)	147±37 (9)	157±56 (9)[e]
Parietal cortex	140±40 (16)	117±36 (12)	165±40 (9)[d]	139±49 (9)
Sensory-motor cortex	156±35 (16)	114±31 (12)[a]	178±37 (9)[d]	154±45 (9)[e]
Frontal cortex	147±22 (16)	117±22 (12)	167±30 (9)[d]	146±48 (9)[e]
Prefrontal cortex	143±37 (14)	123±33 (9)	160±16 (7)	141±34 (8)
Anterior cingulate cortex	143±42 (16)	130±37 (12)	170±38 (9)	138±33 (9)
Diencephalic Area				
Medial geniculate	120±38 (16)	101±25 (12)	117±31 (9)	130±45 (9)
Lateral geniculate	98±30 (16)	80±20 (12)	92±23 (9)	93±22 (9)
Thalamus, Medio-dorsal	129±55 (16)	114±36 (12)	119±28 (9)	104±17 (9)
Thalamus, ventro-dorsal	122±57 (16)	88±20 (12)	106±25 (9)	92±18 (9)
Hypothalamus	79±22 (16)	76±17 (12)	73±19 (9)	68±16 (9)
Lateral habenula	120±32 (15)	104±21 (12)	113±29 (9)	105±18 (9)
Amygdala	82±24 (14)	64±11 (7)	72±13 (8)	65±11 (8)
Caudate nucleus	152±33 (16)	114±30 (12)[a]	133±24 (9)	125±41 (9)
Nucleus accumbens	117±41 (16)	102±28 (12)	116±34 (9)	94±27 (9)
Globus pallidus	103±25 (16)	81±16 (12)	88±18 (9)	81±26 (9)
Internal capsule	66±15 (14)	52±9 (7)	54±11 (8)[b]	49±11 (8)[c]
Subthalamic nucleus	152±23 (16)	134±29 (12)	142±49 (9)	121±31 (9)
Hindbrain Areas				
Vestibular nucleus	163±37 (16)	139±31 (12)	141±32 (9)	174±37 (9)
Cochlear nucleus	154±30 (7)	128±47 (5)	136±27 (8)	151±49 (7)
Superior olive	166±35 (16)	136±17 (12)	135±31 (8)	140±50 (8)
Inferior olive	150±36 (16)	134±22 (12)	114±21 (9)[b]	118±22 (8)[c]
Pons	96±24 (16)	82±13 (12)	77±13 (9)	92±49 (9)

Table III.21 (continued)

Structure	0.9% saline i.c.+5ml/kg saline i.p.	10^{-10} moles/min CHA i.c.+5ml/kg saline i.p.	0.9% saline i.p.+30mg/kg theophylline i.p.	10^{-10} moles/min CHA i.c.+30mg/kg theophyllline i.p.
Mesencephalic Areas				
Red Nucleus	134±29 (16)	122±23 (12)	114±26 (9)	131±81 (9)
Substantia nigra	98±21 (16)	89±16 (12)	82±20 (9)	82±25 (9)
Superior colliculus	136±32 (16)	118±22 (12)	115±27 (9)	136±65 (9)
Inferior colliculus	175±57 (16)	151±26 (12)	159±36 (9)	202±104 (9)
Telencephalic Areas				
Hippocampus, Molecular layer	117±32 (16)	95±26 (12)	106±25 (9)	108±52 (9)
Dentate gyrus	108±27 (16)	92±17 (12)	98±22 (9)	101±42 (9)
Septal nucleus	88±18 (16)	85±21 (12)	84±20 (9)	80±25 (9)
Cerebellum nucleus	163±32 (15)	141±36 (12)	158±33 (9)	163±58 (9)
Cerebellum hemisphere	78±16 (16)	69±13 (12)	79±17 (9)	80±32 (9)
Fibre Tracts				
Cerebellum white	47±16 (16)	42±11 (12)	43±12 (9)	41±14 (9)
Corpus callosum	50±14 (16)	56±25 (12)	60±27 (9)	41±8 (9)
Genu	47±16 (16)	39±19 (12)	46±9 (9)	42±9 (9)

All values are for local cerebral blood flow ($ml.100g^{-1}.min^{-1}$) expressed as mean ± standard deviation.

Figures in parentheses indicate n value.

i.c. indicates that the compound was administered via the right internal carotid artery.

i.p. indicates that the compound was administered intraperitoneally.

a, b and c-indicates significant difference from saline i.c./saline i.p. groups;

d and e - significant difference from CHA i.c./saline i.p. group;and

f - significant difference from saline i.c./theophylline i.p. group

($P<0.05$ - one way analysis of variance with Newman-Keuls multiple range test)

Table III.22 THE EFFECT OF THEOPHYLLINE ON THE CHANGES IN LCBF PRODUCED BY A 15 MINUTE INFUSION OF CHA (CONTRALATERAL HEMISPHERE)

STRUCTURE	0.9% saline i.c.+5ml/kg saline i.p.	10^{-10} moles/min CHA i.c.+5ml/kg saline i.p.	0.9% saline i.c.+30mg/kg theophylline i.p.	10^{-10} moles/min CHA i.c.+30mg/kg theophylline i.p.
Cortical Areas				
Visual cortex	133±40 (16)	113±28 (12)	132±23 (9)	140±77 (9)
Auditory cortex	126±26 (16)	128±38 (12)	154±40 (9)	163±59 (8)
Parietal cortex	138±38 (16)	123±26 (12)	175±41 (9)[d]	152±52 (9)
Sensory-motor cortex	163±41 (16)	128±26 (12)	191±44 (9)[d]	162±41 (9)[e]
Frontal cortex	147±37 (16)	128±17 (12)	184±39 (9)[b,d]	151±43 (9)[f]
Prefrontal cortex	141±37 (14)	136±23 (12)	175±20 (7)	144±34 (8)[f]
Anterior cingulate cortex	140±42 (16)	140±44 (12)	171±41 (9)	137±31 (9)
Diencephalic Areas				
Medial geniculate	114±36 (16)	113±31 (12)	116±39 (9)	132±55 (9)
Lateral geniculate	104±35 (16)	91±20 (12)	96±25 (9)	94±21 (9)
Thalamus, Medio-dorsal	128±59 (16)	116±35 (12)	116±32 (9)	101±17 (9)
Thalamus, ventro-lateral	110±38 (16)	95±20 (12)	100±28 (9)	91±16 (9)
Hypothalamus	79±20 (16)	76±23 (12)	76±18 (9)	67±12 (9)
Lateral habenula	118±27 (16)	103±20 (12)	119±37 (9)	106±17 (9)
Amygdala	86±24 (14)	76±12 (8)	79±16 (8)	70±12 (8)
Caudate nucleus	151±40 (16)	128±22 (12)	135±28 (9)	124±26 (9)
Nucleus accumbens	116±39 (16)	113±30 (12)	112±25 (9)	100±29 (9)
Globus pallidus	106±26 (16)	90±18 (9)	90±18 (9)	85±27 (9)
Internal capsule	67±14 (14)	60±8 (7)	62±11 (8)	52±9 (8)
Subthalamic nucleus	154±34 (16)	138±28 (12)	138±41 (9)	125±31 (9)
Hindbrain Areas				
Vestibular nucleus	156±36 (16)	138±31 (12)	146±37 (9)	170±79 (9)
Cochlear nucleus	161±32 (12)	153±30 (7)	147±41 (9)	151±47 (5)
Superior olive	184±41 (16)	145±23 (12)	148±25 (9)	154±51 (8)
Inferior olive	156±33 (16)	139±26 (12)	119±27 (9)[b]	120±27 (8)[c]
Pons	94±22 (16)	84±16 (12)	79±13 (9)	91±50 (9)

Table III.22 (continued)

STRUCTURE	0.9% saline i.c.+5ml/kg saline i.p.	10^{-10} moles/min CHA i.c.+5ml/kg saline i.p.	0.9% saline i.c.+30mg/kg theophylline i.p.	10^{-10} moles/min CHA i.c.+30mg/kg theophylline i.p.
Mesencephalic Areas				
Red nucleus	131±26 (16)	120±26 (12)	108±22 (9)	129±73 (9)
Substantia nigra	95±19 (16)	94±13 (12)	79±16 (9)	78±25 (9)
Superior colliculus	134±31 (16)	124±25 (12)	116±27 (9)	135±73 (9)
Inferior colliculus	184±66 (16)	162±30 (12)	168±35 (9)	198±98 (9)
Telencephalic Areas				
Hippocampus, Molecular layer	115±36 (16)	98±28 (12)	109±24 (9)	115±52 (9)
Dentate Gyrus	108±27 (16)	101±22 (12)	99±25 (9)	107±50 (9)
Septal nucleus	89±17 (16)	89±21 (12)	88±18 (9)	82±24 (9)
Cerebellum nucleus	163±30 (16)	145±42 (12)	160±43 (9)	163±49 (9)
Cerebellum hemisphere	80±14 (16)	70±12 (12)	80±18 (9)	79±28 (9)
Fibre Tracts				
Cerebellum white	50±17 (16)	40±9 (12)	40±10 (9)	45±12 (9)
Corpus callosum	66±32 (16)	60±27 (12)	56±23 (9)	41±8 (9)
Genu	50±10 (16)	45±10 (12)	46±12 (9)	43±10 (9)

All values are for local cerebral blood flow ($ml.100g^{-1}.min^{-1}$) expressed as mean ± standard deviation.

Figures in parentheses indicate n value.

i.c. indicates that the compound was administered via the right interal carotid artery.

i.p. indicates that the compound was administered intraperitoneally.

b and c – indicates a significant difference from saline i.c./saline i.p. group;

d and e – significant difference from CHA i.c./saline i.p.; and

f significant difference from saline i.c./theophylline i.p. group

($P<0.05$ – one way analysis of variance with Newman-Keuls multiple range test).

Table III.23 PHYSIOLOGICAL PARAMETERS MONITORED DURING THE THEOPHYLLINE/15 MINUTE CHA INFUSION STUDY

Parameter	0.9% Saline i.c. + 5ml/kg saline i.p.	10^{-10}moles/min CHA i.c. + 5ml/kg saline i.p.	0.9% Saline i.c. + 30mg/kg theophylline i.p.	10^{-10}moles/min i.c. + 30mg/kg theophylline i.p.
PCO_2 (mmHg)	39.5±1.6(16)	39.9±2.0(12)	39.8±1.8(9)	39.8±1.8(9)
PO_2 (mmHg)	151.7±20.2(16)	143.4±22.3(12)	151.4±30.6(9)	151.9±18.5(9)
MABP (mmHg)	83±10(16)	76±14(12)	78±8(9)	79±13(9)
Temp (°C)	37.8±0.8(16)	37.6±0.6(12)	37.7±0.8(9)	37.7±0.4(9)
$[HCO_3^-]$ (mmol|l)	23.4±2.1(16)	24.2±3.0(12)	23.3±2.0(9)	24.5±3.0(9)
Base excess (mmol|l)	-0.6±2.6(26)	-0.4±2.8(12)	-0.8±2.6(9)	0.6±3.5(9)
pH	7.384±0.037(16)	7.391±0.046(12)	7.378±0.038(9)	7.398±0.047(9)

All values are expressed as mean±standard deviation. Figures in parentheses indicate n value.

i.c. indicates that the compound was administered via the internal carotid artery.

i.p. indicates that the compound was administered intraperitoneally.

Table III.24 IPSILATERAL HEMISPHERE CHANGES IN lCBF PRODUCED BY 2 MINUTES INFUSIONS OF ADENOSINE AND CHA

SIRUCUIRE	0.9% Saline	$3x10^{-7}$ moles/min ADENOSINE	10^{-10} moles/min CHA
Cortical Areas			
Visual cortex	113±20 (6)	137±14 (4)	143±51 (7)
Auditory cortex	129±31 (6)	156±26 (4)	160±45 (7)
Parietal cortex	121±21 (6)	150±30 (4)	158±57 (7)
Sensory-motor cortex	122±23 (6)	143±20 (4)	162±51 (7)
Frontal cortex	122±20 (6)	146±24 (4)	163±64 (7)
Prefrontal cortex	142±50 (6)	189±26 (4)	174±66 (7)
Anterior cingulate cortex	138±28 (6)	260±80 (4) *	255±184 (7)
Diencephalic Areas			
Medial geniculate	109±32 (6)	135±21 (4)	140±40 (7)
Lateral geniculate	85±16 (6)	109±14 (4)	102±30 (7)
Thalamus, mediodorsal	102±52 (6)	145±12 (4)	116±48 (7)
Thalamus, ventrolateral	76±20 (6)	109±12 (4)*	95±25 (7)
Hypothalamus	69±20 (6)	84±9 (4)	75±21 (7)
Lateral habenula	101±36 (6)	136±28 (4)	107±36 (7)
Amygdala	65±13 (6)	81±18 (4)	84±27 (7)
Caudate nucleus	123±24 (6)	164±21 (4)	160±50 (7)
Nucleus accumbens	106±32 (6)	132±15 (4)	146±62 (7)
Globus pallidus	98±20 (6)	102±16 (4)	103±41 (7)
Internal capsule	55±7 (6)	64±13 (4)	64±17 (7)
Subthalamic nucleus	146±45 (6)	186±13 (4)	183±67 (7)
Hindbrain Areas			
Vestibular nucleus	132±13 (6)	155±39 (4)	157±35 (7)
Cochlear nucleus	142±20 (4)	-	171±46 (3)
Superior olive	153±11 (6)	171±47 (4)	182±32 (7)
Inferior olive	128±13 (6)	132±22 (4)	158±40 (7)
Pons	80±10 (6)	99±12 (4)	96±24 (7)

Table III.24 (continued)

STRUCTURE	0.9% Saline	3×10^{-7} moles/min ADENOSINE	10^{-10} moles/min CHA
Mesencephalic Areas			
Red nucleus	111±14 (6)	148±15 (4)*	135±5 (7)
Substantia nigra	79±15 (6)	108±17 (4)*	107±40 (7)
Superior colliculus	122±22 (6)	158±35 (4)	149±39 (7)
Inferior colliculus	144±18 (6)	180±35 (4)	159±35 (7)
Telencephalic Areas			
Hippocampus, molecular layer	96±17 (6)	121±15 (4)	122±41 (7)
Dentate gyrus	92±10 (6)	108±9 (4)	112±28 (7)
Septal nucleus	82±19 (6)	102±28 (4)	106±37 (7)
Cerebellum nucleus	148±21 (6)	156±20 (4)	163±23 (7)
Cerebellum hemisphere	67±12 (6)	76±12 (4)	78±14 (7)
Fibre Tracts			
Cerebellum white	35±10 (6)	44±14 (4)	47±22 (7)
Corpus callosum	44±7 (6)	66±16 (4)*	62±23 (7)
Genu	41±8 (6)	54±11 (4)	50±21 (7)

All values for local cerebral blood flow ($ml.100g^{-1}.min^{-1}$) are expressed as mean ± standard deviation.

Figrues in parentheses indicate n value.

* $P<0.05$ (Student's t-test incorporating Bonferroni correction factor)

Table III.25 CONTRALATERAL HEMISPHERE: CHANGES IN lCBF PRODUCED BY 2 MINUTE INFUSIONS OF ADENOSINE AND CHA

STRUCTURE	0.9% Saline	3×10^{-7} moles/min ADENOSINE	10^{-10} moles/min CHA
Cortical Areas			
Visual cortex	109±20 (6)	130±12 (4)	140±42 (7)
Auditory cortex	128±28 (6)	136±23 (4)	159±52 (7)
Parietal cortex	117±14 (6)	134±23 (4)	154±64 (7)
Sensory-motor cortex	123±19 (6)	135±33 (4)	148±42 (7)
Frontal cortex	119±20 (6)	136±22 (4)	147±56 (7)
Prefrontal cortex	151±69 (6)	177±29 (4)	176±74 (7)
Anterior cingulate cortex	146±86 (6)	255±60 (4)*	228±149 (7)
Diencephalic Areas			
Medial geniculate	109±36 (6)	135±19 (4)	134±42 (7)
Lateral geniculate	87±20 (6)	100±24 (4)	99±28 (7)
Thalamus, mediodorsal	106±49 (6)	150±19 (4)	124±62 (7)
Thalamus, ventrolateral	80±26 (6)	105±12 (4)	97±36 (7)
Hypothalamus	66±17 (6)	86±15 (4)	70±20 (7)
Lateral habenula	101±30 (6)	129±26 (4)	108±41 (7)
Amygdala	60±6 (6)	70±10 (4)	76±26 (7)
Caudate nucleus	125±30 (6)	149±26 (4)	149±54 (7)
Nucleus accumbens	110±36 (6)	129±18 (4)	132±55 (7)
Globus pallidus	95±19 (6)	96±10 (4)	98±33 (7)
Internal capsule	54±15 (6)	62±12 (4)	59±17 (7)
Subthalamic nucleus	152±32 (6)	173±20 (4)	162±48 (7)
Hindbrain Areas			
Vestibular nucleus	134±11 (6)	158±29 (4)	160±33 (7)
Cochlear nucleus	147±16 (5)	157±43 (4)	141±22 (6)
Superior olive	141±10 (6)	150±38 (4)	171±36 (7)
Inferior olive	134±18 (6)	133±24 (4)	162±42 (7)
Pons	78±10 (6)	101±9 (4)*	94±28 (7)

Table III.25 (continued)

STRUCTURE	0.9% Saline	3×10^{-7} moles/min ADENOSINE	10^{-10} moles/min CHA
Mesencephalic Areas			
Red nucleus	116±24 (6)	147±12 (4)	139±48 (7)
Substantia nigra	92±22 (6)	101±19 (4)	104±42 (7)
Superior colliculus	120±26 (6)	149±20 (4)	147±42 (7)
Inferior colliculus	135±9 (6)	162±32 (4)	156±44 (7)
Telencephalic Areas			
Hippocampus, molecular layer	100±25 (6)	108±18 (4)	113±25 (7)
Dentate gyrus	97±20 (6)	99±15 (4)	102±25 (7)
Septal nucleus	81±18 (6)	102±26 (4)	102±28 (7)
Cerebellum nucleus	145±16 (6)	149±18 (4)	160±34 (7)
Cerebellum hemisphere	66±7 (6)	71±6 (4)	76±14 (7)
Fibre Tracts			
Cerebellum white	38±5 (6)	44±12 (4)	44±16 (7)
Corpus callosum	44±9 (6)	62±17 (4)	63±26 (7)
Genu	44±10 (6)	57±8 (4)	50±20 (7)

All values for local cerebral blood flow ($ml.100g^{-1}.min^{-1}$) are expressed as mean ± standard deviation.
Figures in parentheses indicate n value.

Table III.26 PHYSIOLOGICAL PARAMETERS MONITORED DURING EXPERIMENTS WITH A 2 MINUTE INFUSION OF ADENOSINE AND CHA

Parameter	0,9% Saline	$3x10^{-7}$ moles/min ADENOSINE	10^{-10} moles/min CHA
PCO_2 (mmHg)	39.7±2.1(6)	39.5±0.9(4)	41.4±2.8(7)
PO_2 (mmHg)	174.1±48.6(6)	154.6±41.1(4)	142.5±26.7(7)
MABP (mmHg)	71±7(6)	75±10(4)	79±15(7)
Temp (°C)	37.5±0.6(6)	37.9±0.7(4)	37.5±0.8(7)
$[HCO_3^-]$ (mmol|l)	22.9±4.0(6)	23.9±4.3(4)	23.1±2.9(7)
Base excess (mmol|l)	-1.3±5.4(6)	-0.1±5.2(4)	-2.1±2.9(7)
pH	7.370±0.079(6)	7.388±0.079(4)	7.352±0.032(7)

All values are expressed as mean ± standard deviation. Figures in parentheses indicate n value.

Table III.27 THE EFFECT OF THEOPHYLLINE ON THE CHANGES IN LCBF PRODUCED BY A 2 MINUTE INFUSION OF CHA (IPSILATERAL HEMISPHERE)

STRUCTURE	0.9% saline i.c.+5ml/kg saline i.p.	10^{-10} moles/min CHA i.c.+5ml/kg saline i.p.	0.9% saline i.c.+30mg/kg theophylline i.p.	10^{-10} moles/min CHA i.c.+30mg/kg theophylline i.p.
Cortical Areas				
Visual cortex	113±20 (6)	143±51 (7)	114±17 (4)	145±63 (4)
Auditory cortex	129±31 (6)	160±45 (7)	124±48 (4)	181±52 (4)
Parietal cortex	121±21 (6)	158±57 (7)	134±38 (4)	187±61 (4)
Sensory-motor cortex	122±23 (6)	162±51 (7)	148±44 (4)	233±104 (4)[a]
Frontal cortex	122±20 (6)	163±64 (7)	149±45 (4)	207±92 (4)
Prefrontal cortex	142±50 (6)	174±66 (7)	146±54 (4)	178±44 (4)
Anterior cingulate cortex	138±28 (6)	255±184 (7)	141±46 (4)	269±182 (4)
Diencephalic Areas				
Medial geniculate	109±32 (6)	140±40 (7)	95±26 (4)	118±21 (4)
Lateral geniculate	85±16 (6)	102±30 (7)	76±22 (4)	90±16 (4)
Thalamus, Medio-dorsal	102±52 (6)	116±48 (7)	92±38 (4)	112±48 (4)
Thalamus, Ventro-lateral	77±20 (6)	95±28 (7)	94±35 (4)	103±27 (4)
Hypothalamus	69±20 (6)	75±21 (7)	62±24 (4)	76±22 (4)
Lateral habenula	101±36 (6)	107±36 (7)	93±35 (4)	109±29 (4)
Amygdala	65±13 (6)	84±27 (7)	59±14 (4)	70±13 (4)
Caudate nucleus	123±24 (6)	160±50 (7)	125±26 (4)	137±43 (4)
Nucleus accumbens	106±32 (6)	146±62 (7)	83±21 (4)	116±30 (4)
Globus pallidus	98±20 (6)	103±41 (7)	77±19 (4)	90±12 (4)
Interal capsule	55±7 (6)	64±17 (7)	55±18 (4)	53±15 (4)
Subthalamic nucleus	146±45 (6)	183±67 (7)	116±34 (4)	127±30 (4)
Hindbrain Areas				
Vestibular nucleus	132±13 (6)	157±35 (7)	130±33 (4)	149±31 (4)
Cochlear nucleus	142±20 (4)	171±46 (5)	111±11 (2)	133±20 (3)
Superior olive	153±11 (6)	182±32 (7)	125±28 (4)[b]	138±28 (4)[c]
Inferior olive	128±13 (6)	158±40 (7)	120±28 (4)	150±27 (4)
Pons	80±10 (6)	96±24 (7)	73±18 (4)	87±27 (4)

Table III.27 (continued)

STRUCUIRE	0.9% saline i.c.+5ml/kg saline i.p.	10^{-10}moles/min CHA i.c.+5ml/kg saline i.p.	0.9% saline i.c.+30mg/kg theophylline i.p.	10^{-10}moles/min CHA i.c.+30mg/kg theophylline i.p.
Mesencephalic Areas				
Red nucleus	111±14 (6)	135±50 (7)	96±37 (4)	102±16 (4)
Substantia nigra	79±15 (6)	107±40 (7)	74±21 (4)	78±9 (4)
Superior colliculus	122±22 (6)	149±39 (7)	103±21 (4)	109±10 (4)
Inferior colliculus	144±18 (6)	159±35 (7)	142±32 (4)	164±54 (4)
Telencephalic Areas				
Hippocampus, Molecular layer:	96±17 (6)	122±41 (7)	90±26 (4)	98±17 (4)
Dentate gyrus	92±10 (6)	112±28 (7)	88±32 (4)	90±14 (4)
Septal nucleus	82±19 (6)	106±37 (7)	75±22 (4)	84±20 (4)
Cerebellum nucleus	148±21 (6)	163±23 (7)	125±28 (4)	151±26 (4)
Cerebellum hemisphere	67±12 (6)	78±14 (7)	69±20 (4)	76±16 (4)
Fibre Tracts				
Cerebellum white	35±10 (6)	47±23 (7)	36±13 (4)	42±17 (4)
Corpus callosum	44±7 (6)	62±23 (7)	44±11 (4)	45±17 (4)
Genu	41±8 (6)	50±21 (7)	50±17 (4)	56±21 (4)

All values for local cerebral blood flow ($ml.100g^{-1}.min^{-1}$) are expressed as mean ± standard deviation.

Figures in parentheses indicate n value.

i.c. indicates that the compound was administered via the right internal carotid artery.

i.p. indicates that the compounds was administered intraperitoneally.

a - indicates a significant difference from saline i.c./saline i.p. group; and

b and c - significant difference from CHA i.c./saline i.p. group.

($P<0.05$ - one way analysis of variance with Newman-Keuls multiple range test.)

Table III.28 THE EFFECT OF THEOPHYLLINE ON THE CHANGES IN LCBF PRODUCED BY A 2 MINUTE INFUSION OF CHA (CONTRALATERAL HEMISPHERE)

STRUCTURE	0.9% saline i.c.+5ml/kg SALINE i.p.	10^{-10} moles/min CHA i.c.+5ml/kg saline i.p.	0.9% saline i.c.±30mg/kg theophylline i.p.	10^{-10} moles/min CHA i.c.+30mg/kg theophylline i.p.
Cortical Areas				
Visual cortex	109±20 (6)	140±42 (7)	117±35 (4)	152±59 (4)
Auditory cortex	128±28 (6)	159±52 (7)	126±45 (4)	187±56 (4)
Parietal cortex	117±14 (6)	154±64 (7)	130±41 (4)	182±63 (4)
Sensory-motor cortex	123±19 (6)	148±42 (7)	144±47 (4)	232±106 (4)[a, c]
Frontal cortex	119±20 (6)	147±56 (7)	151±48 (4)	220±105 (4)
Prefrontal cortex	151±69 (6)	176±74 (7)	140±53 (4)	196±51 (4)
Anterior cingulate cortex	146±86± (6)	228±149 (7)	134±33 (4)	238±127 (4)
Diencephalic Areas				
Medial geniculate	109±36 (6)	134±42 (7)	98±26 (4)	127±36 (4)
Lateral geniculate	87±20 (6)	99±28 (7)	73±17 (4)	95±13 (4)
Thalamus, Medio-dorsal	106±49 (6)	124±62 (7)	95±35 (4)	118±50 (4)
Thalamus, Ventro-lateral	80±26 (6)	97±36 (7)	95±40 (4)	113±34 (4)
Hypothalamus	66±17 (6)	70±20 (7)	66±22 (4)	77±23 (4)
Lateral habenula	101±30 (6)	108±41 (7)	93±34 (4)	124±26 (4)
Amygdala	60±6 (6)	76±26 (7)	62±21 (4)	72±14 (4)
Caudate nucleus	125±30 (6)	149±54 (7)	110±25 (4)	157±70 (4)
Nucleus accumbens	110±36 (6)	132±55 (7)	82±14 (4)	120±31 (4)
Globus pallidus	95±19 (6)	98±33 (7)	74±26 (4)	93±18 (4)
Internal capsule	54±5 (6)	59±17 (7)	54±19 (4)	58±14 (4)
Subthalamic nucleus	152±32 (6)	162±48 (7)	119±30 (4)	130±35 (4)
Hindbrain Areas				
Vestibular nucleus	134±11 (6)	160±33 (7)	138±31 (4)	160±39 (4)
Cochlear nucleus	147±16 (6)	141±22 (6)	131±34 (4)	202±73 (4)[a, d]
Superior olive	141±10 (6)	171±36 (7)	127±32 (4)	135±26 (4)
Inferior olive	134±18 (6)	162±42 (7)	122±35 (4)	146±37 (4)
Pons	78±10 (6)	94±28 (7)	76±21 (4)	90±29 (4)

Table III.28 (continued)

STRUCUIRE	0.9% saline i.c.+5ml/kg saline i.p.	10^{-10} moles/min CHA i.c.+5ml/kg saline i.p.	0.9% saline i.c.+30mg/kg theophylline i.p.	10^{-10} moles/min CHA i.c.+30mg/kg theophylline i.p.
Mesencephalic Areas				
Red nucleus	116±24 (6)	139±48 (7)	102±32 (4)	108±25 (4)
Substantia nigra	92±22 (6)	104±42 (7)	75±23 (4)	81±16 (4)
Superior colliculus	120±26 (6)	147±42 (7)	99±27 (4)	110±17 (4)
Inferior colliculus	135±9 (6)	156±44 (7)	144±28 (4)	156±58 (4)
Telencephalic Areas				
Hippocampus, Molecular layer	100±25 (6)	113±25 (7)	90±27 (4)	104±34 (4)
Dentate gyrus	97±20 (6)	102±25 (7)	82±29 (4)	96±28 (4)
Septal nucleus	81±18 (6)	102±28 (7)	73±23 (4)	88±21 (4)
Cerebellum nucleus	145±16 (6)	160±34 (7)	141±49 (4)	140±28 (4)
Cerebellum hemisphere	66±7 (6)	76±14 (7)	70±22 (4)	76±16 (4)
Fibre Tracts				
Cerebellum white	38±5 (6)	44±16 (7)	46±13 (4)	42±12 (4)
Corpus callosum	44±9 (6)	63±26 (7)	46±11 (4)	44±17 (4)
Genu	44±10 (6)	50±20 (7)	50±17 (4)	56±20 (4)

All values for local cerebral blood flow ($ml.100g^{-1}$) are expressed as mean ± standard deviation.

Figures in parentheses indicate n value.

i.c. indicates that the compound was administered via the right internal carotid artery.

i.p. indicates that the compound was administered intraperitoneally.

a - indicates a significant difference from saline i.c./saline i.p.;

c - significant difference from CHA i.c./saline i.p. group; and d - significant difference from saline i.c./theophylline i.p. group.

($P<0.05$; one way analysis of variance with Newman-Keuls multiple range test).

Table III.29 PHYSIOLOGICAL PARAMETERS MONITORED DURING THE THEOPHYLLINE/2MINUTE CHA INFUSION STUDY

Parameter	0.9% Saline i.c. + 5ml/kg saline i.p.	10^{-10}moles/min CHA i.c. + 5ml/kg saline i.p.	0.9% Saline i.c. + 30mg/kg theophylline i.p.	10^{-10}moles/min CHA i.c + 30mg/kg theophylline i.p.
PCO_2 (mmHg)	39.7±2.1(6)	41.4±2.8(7)	40.0±2.0(4)	40.1±1.1(4)
PO_2 (mmHg)	174.1±48.6(6)	142.5±26.7(7)	153.9±35.7(4)	183.2±38.8(4)
MABP (mmHg)	71±7 (6)	79±15 (7)	80±10 (4)	86±11 (4)
Temp ($^{\circ}$C)	37.5±0.6(6)	37.5±0.8(7)	37.5±0.4(4)	37.2±0.9(4)
$[HCO_3^-]$ (mmol|l)	22.9±4.0(6)	23.1±2.9(7)	24.1±2.5(4)	22.8±2.5(4)
Base excess (mmol|l)	-1.3±5.4(6)	-2.1±2.9(7)	-0.4±2.2(4)	-1.9±3.3(4)
pH	7.370±0.079(6)	7.352±0.032(7)	7.374±0.032(4)	7.362±0.039(4)

All values are expressed as mean ± standard deviation. Figures in parentheses indicate n value.

i.c. indicates that the compound was administered via the internal carotid artery.

i.p. indicates that the compound was administerd intraperitoneally.

Table V.1 EFFECT OF CHANGES IN MEAN ARTERIAL BLOOD PRESSURE (MABP) ON LOCAL CEREBRAL BLOOD FLOW (LCBF) - IPSILATERAL HEMISPHERE

STRUCTURE	MABP 40-59mmHg	MABP 60-69mmHg	MABP 70-79mmHg	MABP 80-89mmHg	MABP 90-109mmHg
Cortical Areas					
Visual cortex	117±53 (8)	116±74 (4)	145±64 (4)	123±39 (4)	136±8 (5)
Auditory cortex	132±66 (8)	130±67 (4)	141±78 (4)	121±17 (4)	164±69 (6)
Parietal cortex	118±49 (8)	112±41 (4)	143±66 (4)	145±51 (4)	176±34 (6)
Sensory-motor cortex	130±44 (7)	128±50 (4)	153±54 (4)	162±22 (4)	199±46 (6)
Frontal cortex	127±51 (7)	96±38 (4)	147±58 (4)	147±17 (4)	141±25 (5)
Prefrontal cortex	155±82 (7)	148±92 (3)	155±69 (3)	133±6 (3)	176±40 (5)
Anterior cingulate cortex	163±100 (8)	170±122 (4)	170±47 (4)	149±53 (4)	190±68 (5)
Diencephalic Areas					
Medial geniculate	130±55 (8)	104±45 (4)	154±52 (4)	120±28 (4)	150±41 (6)
Lateral geniculate	97±34 (8)	91±42 (4)	118±46 (4)	92±22 (4)	119±40 (6)
Thalamus, Medio-dorsal	123±58 (8)	108±54 (4)	125±36 (4)	122±56 (4)	165±77 (6)
Thalamus, ventro-lateral	96±27 (8)	88±32 (4)	124±45 (4)	110±31 (4)	134±41 (6)
Hypothalamus	67±35 (8)	70±28 (4)	85±29 (4)	84±33 (4)	104±19 (6)
Lateral habenula	119±38 (8)	104±48 (4)	137±31 (4)	136±48 (5)	150±29 (5)
Amygdala	70±29 (8)	58±17 (4)	81±22 (4)	80±21 (4)	107±32 (5)
Caudate nucleus	137±53 (7)	130±67 (4)	181±37 (4)	141±8 (4)	222±70 (6)
Nucleus accumbens	132±63 (7)	109±59 (4)	128±39 (4)	114±13 (4)	167±80 (6)
Globus pallidus	94±31 (7)	76±35 (4)	115±34 (4)	113±23 (4)	102±30 (6)
Internal capsule	62±25 (8)	45±16 (4)	69±18 (4)	67±12 (4)	89±35 (4)
Subthalamic nucleus	166±81 (8)	144±57 (4)	168±100 (4)	170±21 (4)	149±29 (6)
Hindbrain Areas					
Vestibular nucleus	162±73 (8)	160±56 (4)	201±74 (4)	174±54 (4)	176±25 (5)
Cochlear nucleus	157±43 (7)	130±4 (4)	206±88 (4)	-	194±110 (2)
Superior olive	193±80 (8)	172±49 (4)	282±147 (4)	168±32 (4)	185±57 (6)
Inferior olive	158±66 (8)	147±48 (4)	180±55 (4)	180±57 (4)	181±64 (6)
Pons	115±58 (8)	98±38 (4)	118±38 (4)	99±36 (4)	129±43 (6)

Table V.1 (continued.)

STRUCUTRE	MABP 40-59mmHg	MABP 60-69mmHg	MABP 70-79mmHg	MABP 80-89mmHg	MABP 90-109mmHg
Mesencephalic Areas					
Red nucleus	151±77 (8)	142±56 (4)	153±44 (4)	148±29 (4)	141±25 (6)
Substantia nigra	120±55 (8)	95±32 (4)	116±40 (4)	110±18 (4)	99±17 (6)
Superior colliculus	148±82 (8)	132±49 (4)	139±51 (4)	138±29 (4)	160±25 (6)
Inferior colliculus	177±75 (8)	158±80 (4)	201±62 (4)	176±52 (4)	216±57 (6)
Telencephalic Areas					
Hippocampus, Molecular layer	114±55 (8)	102±46 (4)	134±47 (4)	118±36 (4)	144±48 (6)
Dentate gyrus	106±47 (8)	97±42 (4)	132±42 (4)	114±33 (4)	142±43 (6)
Septal nucleus	96±39 (7)	86±44 (4)	111±24 (4)	84±9 (4)	150±67 (6)
Cerebellum nucleus	180±88 (8)	154±30 (4)	217±82 (4)	159±16 (4)	176±22 (5)
Cerebellum hemisphere	92±41 (8)	81±17 (4)	100±33 (4)	90±23 (4)	86±18 (6)
Fibre Tracts					
Cerebellum white	46±18 (8)	42±15 (4)	55±16 (4)	51±30 (4)	48±16 (6)
Corpus callosum	55±28 (8)	42±17 (4)	76±34 (4)	69±17 (4)	125±54 (6)
Genu	45±16 (7)	42±20 (4)	52±14 (4)	48±6 (4)	72±28 (6)

All values for local cerebral blood flow ($ml.100g^{-1}.min^{-1}$) are expressed as mean ± standard deviation.

Figures in parentheses indicate n value.

Table V.2 EFFECT OF CHANGES IN MEAN ARTERIAL BLOOD PRESSURE (MABP) ON LOCAL CEREBRAL BLOOD FLOW (LCBF) - CONTRALATERAL HEMISPHERE

SIRUCTURE	MABP 40-59mmHg	MABP 60-69mmHg	MABP 70-79mmHg	MABP 80-89mmHg	MABP 90-109mmHg
Cortical Areas					
Visual cortex	123±59 (8)	120±73 (4)	135±50 (4)	132±43 (4)	165±45 (6)
Auditory cortex	144±73 (8)	130±64 (4)	150±58 (4)	122±40 (4)	146±45 (6)
Parietal cortex	129±56 (8)	125±49 (4)	151±62 (4)	142±36 (4)	167±38 (6)
Sensory-motor cortex	142±43 (7)	143±53 (4)	160±38 (4)	181±45 (4)	180±52 (6)
Frontal cortex	135±42 (7)	128±49 (4)	170±62 (4)	148±52 (3)	153±42 (5)
Prefrontal cortex	156±72 (7)	156±80 (3)	147±52 (3)	127±3 (3)	173±38 (5)
Anterior cingulate cortex	165±113 (8)	174±130 (4)	169±42 (4)	141±43 (4)	186±61 (6)
Diencephalic Areas					
Medial geniculate	145±73 (8)	125±52 (4)	143±46 (4)	119±33 (4)	153±44 (6)
Lateral geniculate	98±36 (8)	104±62 (4)	126±54 (4)	108±23 (4)	129±37 (6)
Thalamus, Medio-dorsal	120±58 (8)	111±52 (4)	137±34 (4)	127±58 (4)	171±82 (6)
Thalamus, ventro-lateral	99±29 (8)	94±38 (4)	123±46 (4)	120±36 (4)	128±45 (6)
Hypothalamus	73±40 (8)	66±26 (4)	92±30 (4)	85±25 (4)	103±18 (6)
Lateral habenula	124±49 (8)	104±39 (4)	138±28 (4)	131±38 (4)	150±26 (5)
Amygdala	80±48 (8)	70±23 (4)	86±27 (4)	96±25 (4)	98±31 (5)
Caudate nucleus	156±55 (7)	145±70 (4)	183±49 (4)	136±12 (4)	225±80 (6)
Nucleus accumbens	133±60 (7)	117±55 (4)	138±30 (4)	106±5 (4)	174±86 (6)
Globus pallidus	97±36 (7)	84±37 (4)	127±34 (4)	108±28 (4)	105±37 (6)
Internal capsule	60±29 (8)	60±26 (4)	78±28 (4)	72±9 (4)	83±32 (4)
Subthalamic nucleus	174±83 (8)	147±64 (4)	157±78 (4)	152±24 (4)	165±42 (6)
Hindbrain Areas					
Vestibular nucleus	166±78 (8)	155±56 (4)	202±82 (4)	172±49 (4)	177±35 (5)
Cochlear nucleus	119±11 (3)	145±33 (4)	245±153 (3)	144±16 (4)	178±56 (4)
Superior olive	193±71 (8)	168±32 (4)	283±169 (4)	183±39 (4)	182±55 (6)
Inferior olive	157±59 (8)	134±44 (4)	176±52 (4)	177±45 (4)	178±49 (6)
Pons	113±55 (8)	98±39 (4)	121±40 (4)	96±27 (4)	122±38 (6)

Table V.2 (continued)

STRUCTURE	MABP 40-59mmHg	MABP 60-69mmHg	MABP 70-79mmHg	MABP 80-89mmHg	MABP 90-109mmHg
Mesencephalic Areas					
Red nucleus	145±75 (8)	137±52 (4)	145±44 (4)	136±21 (4)	139±21 (6)
Substantia nigra	114±55 (8)	91±38 (4)	118±40 (4)	98±12 (4)	97±14 (6)
Superior colliculus	143±73 (8)	142±59 (4)	148±51 (4)	138±26 (4)	159±24 (6)
Inferior colliculus	183±77 (8)	183±66 (4)	221±58 (4)	172±60 (4)	206±45 (6)
Telencephalic Areas					
Hippocampus, Molecular layer	124±56 (8)	116±58 (4)	128±36 (4)	128±53 (4)	125±24 (6)
Dentate gyrus	123±59 (8)	109±43 (4)	124±36 (4)	121±41 (4)	120±25 (6)
Septal nucleus	96±34 (7)	87±39 (4)	112±17 (4)	84±8 (4)	146±54 (6)
Cerebellum nucleus	199±95 (7)	176±56 (4)	216±71 (4)	159±16 (4)	162±22 (5)
Cerebellum hemisphere	90±39 (7)	78±19 (4)	96±36 (4)	92±22 (4)	86±14 (6)
Fibre Tracts					
Cerebellum white	48±16 (8)	45±12 (4)	61±12 (4)	52±26 (4)	52±16 (6)
Corpus callosum	52±25 (8)	43±20 (4)	87±46 (4)	68±16 (4)	126±52 (6)
Genu	50±20 (7)	47±23 (4)	58±19 (4)	49±9 (4)	71±27 (6)

All values for local cerebral blood flow ($ml.100g^{-1}.min^{-1}$) are expressed as mean ± standard deviation.

Figures in parentheses indicate n value.

Table V.3 PHYSIOLOGICAL PARAMETERS MONITORED DURING THE MABP ALTERATION STUDY

Parameter	MABP 40-59mmHg	MABP 60-69mmHg	MABP 70-79mmHg	MABP 80-89mmHg	MABP 90-109mmHg
PCO_2 (mmHg)	38.6±1.0(8)	41.2±0.6(4)	41.2±2.3(4)	39.9±1.0(4)	38.6±3.9(6)
PO_2 (mmHg)	176.4±62.0(8)	114.4±8.4(4)	147.7±28.6(4)	134.2±18.6(4)	167.5±34.6(6)
MABP (mmHg)	48±6(8)	60±0(4)	71±2(4)	82±3(4)	95±6(6)
Temp. (°C)	37.8±0.3(8)	37.5±0.9(4)	37.5±0.6(4)	37.3±0.5(4)	37.1±1.8(6)
$[HCO_3-]$ (mmol/l)	21.4±1.3(8)	27.8±4.2(4)	23.3±3.2(4)	24.4±1.8(4)	23.8±2.0(6)
Base excess (mmol/l)	-3.0±1.7(8)	3.8±5.4(4)	-1.4±4.5(4)	0.4±2.6(4)	-0.3±2.7(6)
ph	7.356±0.025(8)	7.421±0.073(4)	7.360±0.078(4)	7.396±0.042(4)	7.396±0.051(6)

All values are expressed as mean ± standard deviation. Figures in parentheses indicate n value.

SECTION VII

REFERENCES

REFERENCES

Astrup,J., Heuser,D., Lassen,N.A., Nilsson,B., Norberg, & Siesjo,B.K. (1978) Evidence against H^+ and K^+ as main factors for the control of cerebral blood flow: a microelectrode study. In Ciba Foundation Symposium Vol.56: Cerebral Vascular Smooth Muscle and its Control, ed. Purves,M.J., pp.313-337. Amsterdam: Elsevier-North Holland.

Berne,R.M. (1963) Cardiac nucleotides in hypoxia: possible role in the regulation of coronary blood flow. American Journal of Physiology, 204, 317-322.

Berne,R.M. (1980) The role of adenosine in the regulation of coronary blood flow. Circulation Research, 46, 807-813.

Berne,R.M. (1985) Some cardiovascular effects of adenosine. In Adenosine: Receptors and Modulation of Cell Function, eds. Stefanovich,V., Rudolphi,K., Schubert,P., pp.357-372. Oxford/ Washington,D.C.: IRL Press.

Berne,R.M., Rubio,R. & Curnish,R.R. (1974) Release of adenosine from ischemic brain. Effect on cerebral vascular resistence and incorporation into cerebral adenine nucleotides. Circulation Research, 35, 262-271.

Boarini,D.J., Kassell,N.F., Sprowell,J.A. & Olin,J.S. (1984) Intra-vertebral artery adenosine fails to alter cerebral blood flow in the dog. Stroke, 15, 1057-1060.

Born,G.V.R. (1964) Strong inhibition by 2-chloroadenosine of the aggregation of blood platelets by adenosine diphosphate. Nature, 202, 95-96.

Brown,C.M. & Collis,M.G. (1981) Adenosine contracts the isolated rat tail artery by releasing endogenous 5-hydroxytryptamine. European Journal of Pharmacology, 76, 275-277.

Bruns,R.F. (1980) Adenosine receptor activation in human fibroblasts: nucleoside agonists and antagonists. Canadian Journal of Physiology and Pharmacology, 58, 673-691.

Bruns,R.F., Fergus,J.H., Badger,E.W., Bristol,J.A., Santay,L.A. & Hays,S.J. (1987) PD,115,199: an antagonist ligand for adenosine A_2 receptors. Naunyn-Schmiedeberg's Archives of Pharmacology, 335, 64-69.

Burnstock,G. (1978) A basis for distinguishing two types of purinergic receptor. In Cell Membrane Receptors For Drugs And Hormones: A Multidisciplinary Approach, eds. Bolis,L. & Straub,R.W., pp.107-118. New York: Raven Press.

Burnstock,G. & Kennedy,C. (1985) Is there a basis for distinguishing two types of P_2-purinoceptor? General Pharmacology, 16, 433-440.

Chin,J.H. & DeLorenzo,R.J. (1985) Cobalt ion enhancement of 2-chloroadenosine binding to a novel class of adenosine receptors in brain: antagonism by calcium. Brain Research, 348, 381-386.

Chin,J.H. & DeLorenzo,R.J. (1986) A new class of adenosine receptors in brain. CHaracterisation by 2-chloro[^{3}H]adenosine binding. Biochemical Pharmacology, 35, 847-856.

Collis,M.G. & Brown,C.M. (1983) Adenosine relaxes the aorta by interacting with an A_2 receptor and an intracellular site. European Journal of Pharmacology, 96, 61-69.

Cornford,E.M. & Oldendorf,W.H. (1975) Independent blood-brain barrier transport systems for nucleic acid precursors. Biochimica et Biophysica Acta, 394, 211-219.

Courtice,F.C. (1941) The effect of oxygen lack on the cerebral circulation. Journal of Physiology, 100, 198-211.

Crawley,J.N., Patel,J. & Marangos,P.J. (1981) Behavioural characterisation of two long lasting adenosine analogs: sedative properties and interaction with diazepam. Life Sciences, 29, 2623-2630.

Cushley,M.J., Tattersfield,A.E. & Holgate,S.T. (1983) Inhaled adenosine and guanosine on air way resistance in normal and asthmatic subjects. British Journal of Clinical Pharmacology, 15, 161-165.

Daly,J.W. (1977) Cyclic Nucleotides In The Nervous System. New York: Plenum.

Daly,J.W. (1982) Adenosine receptors: targets for future drugs. Journal of Medicinal Chemistry, 25, 197-207.

Dolphin,A.C. & Archer,E.R. (1983) An adenosine agonists inhibits and a cyclic AMP analog enhances the release of glutamate but not γ-aminobutyric acid from slices of rat dentate gyrus. Neuroscience Letters, 43, 49-54.

Drury,A.N. & Szent-Gyorgi,A. (1929) The physiological activity of adenosine compounds with especial reference to their action upon the mammalian heart. Journal of Physiology, 68, 213-237.

Edvinsson,L. & Fredholm,B.B. (1983) Characterisation of adenosine receptors in isolated cerebral arteries of cat. British Journal of Pharmacology, 80, 631-637.

Evoniuk,G., von Borstel,R.W. & Wurtman,R.J. (1987) Antagonism of the cardiovascular effects of adenosine by caffeine or 8-(p-sulfophenyl)theophylline. Journal of Pharmacology and Experimental Therapeutics, 240, 428-432.

Fain,J.N. (1973) Biochemical aspects of drug and hormone action on adipose tissue. Pharmacological Reviews, 25, 67-118.

Forrester,T. Harper,A.M., MacKenzie,E.T. & Thomson,E.M. (1979) Effect of adenosine triphosphate and some derivatives on cerebral blood flow and metabolism. Journal of Physiology, 296, 343-355.

Fredholm,B.B., Jonzon,B. & Lindgren,E. (1983) Inhibition of noradrenaline release from hippocampal slices by a stable adenosine analogue. Acta Physiologica Scandinavica, 515, 7-10.

Furchgott,R.F. (1983) Role of endothelium in responses of vascular smooth muscle. Circulation Research, 53, 557-573.

Garcia-Sainz,J.A. & Torner,M.L. (1985) Rat fat cells have three types of adenosine receptors (R_a, R_i and P). Differential effects of pertussis toxin. Biochemical Journal, 35, 847-856.

Ghai,G. & Mustafa,S.J. (1983) Adenosine receptors in blood vessels: direct evidence. In Physiology and Pharmacology of Adenosine Derivatives, eds. Daly,J., Kuroda,Y., Phillis,J., Shimizu,W., Ui,M., pp.71-75. New York: Raven Press.

Gregory,P.C., Boisvert,D.P.J. & Harper,A.M. (1980) Adenosine response on pial arteries of cats and its inhibition by theophylline. Pflügers Archiv. European Journal of Physiology, 362, 55-59.

Haleen,S.J., Steffen,R.P. & Hamilton,H.W. (1987) PD,116,948, a highly selective A_1 adenosine receptor antagonist. Life Sciences, 40, 555-561.

Hardebo,J.E. & Edvinsson,L. (1979) Adenine compounds: cerebrovascular effects in vitro with reference to their possible involvement in migraine. Stroke, 10, 58-62.

Harms,H.H., Wardeh,G. & Mulder,A.H. (1979) Effect of adenosine on depolarisation-induced release of various radiolabelled neurotransmitters from slices of rat corpus striatum. Neuropharmacology, 18, 577-580.

Haslam,R.J. & Cusack,N.J. (1981) Blood platelet receptors for adenylate cyclase. In Purinergic Receptors, ed. Burnstock,G. pp.281-286. London: Chapman & Hall.

Heistad,D.D. & Marcus,M.L. (1980) Effect of acetylcholine, vasoactive intestinal peptide and adenosine on cerebral blood flow. Blood Vessels, 17, 151-152 (abstract).

Heistad,D.D., Marcus,M.L., Gourley,J.K. & Busija,D.W. (1981) Effect of adenosine and dipyridamole on cerebral blood flow American Journal of Physiology, 240, H775-H780.

Hoffman,W.E., Albrecht,R.F. & Miletich,D.J. (1984) The role of adenosine in CBF increases during hypoxia in young vs. aged rats. Stroke, 15, 124-129.

Hollins,C. & Stone,T.W. (1980) Adenosine inhibition of γ-aminobutyric acid release from slices of rat cerebral cortex. British Journal of Pharmacology, 69, 107-112.

Kennedy,C., Delbro,D. & Burnstock,G. (1985) P_2-purinoceptors mediate both vasodilation (via the endothelium) and vasoconstriction of the isolated rat femoral artery. European Journal of Pharmacology, 107, 161-168.

Kontos,H.A., Raper,J.A. & Patterson,J.L. (1977) Analysis of vasoactivity of location pH, PCO2, and bicarbonate on pial vessels. Stroke, 8, 358-360.

Kontos,H.A. & Wei,E.P. (1981) Role of adenosine in cerebral arteriolar dilation from arterial hypoxia. Federation Proceedings, 40, 454 (abstract).

Kontos,H.A., Wei,E.P., Navari,R.M., Levasseur,J.E., Rosenblum, W.I. & Patterson,J.L. (1978) Responses of cerebral arteries and arterioles to acute hypotension and hypertension. American Journal of Physiology, 234, H371-H383.

Kozniewska,E., Trzebski,A. & Zielinski,A. (1975) Comparison of the effects of inorganic phosphate, adenosine and ATP on the cerebral blood flow in dogs. Proceedings of the Physiological Society, Dec.1975, 96P-97P.

Kuroda,Y. & McIlwain,H. (1974) Uptake and release of [^{14}C] adenine derivatives at beds of mammalian cortical synaptosomes in a superfusion system. Journal of Neurochemistry, 22, 691-700.

Kuschinsky,W., Wahl,M., Bosse,O. & Thurau,K. (1972) Perivascular potassium and pH as determinants of local pial arterial diameter in cats. A microapplication study. Circulation Research, 31, 240-274.

Lee,K.S. & Reddington,M. (1986) 1,3-dipropyl-8-cyclopentyl-xanthine (DPCPX) inhibition of [^{3}H]-N-ethylcarboxamido-adenosine (NECA) binding allows the visualisation of putative non-A_1 adenosine receptors. Brain Research, 368, 397-398.

Livemore,P. & Mitchell,G. (1983) Adenosine causes dilatation and constriction of hypothalamic blood vessels. Journal of Cerebral Blood Flow and Metabolism, 3, 529-534.

Londos,C. & Wolff,J. (1977) Two distinctive adenosine-sensitive sites on adenylate cyclase. Proceedings of the National Academy of Science U.S.A., 74, 5482-5486.

Londos,C., Cooper,D.M.F., Schlegel,W. & Rodbell,M. (1978) Adenosine analogs inhibit adipocyte adenylate cyclase by a GTP-dependent process: basis for actions of adenosine and methylxanthines on cyclic AMP production and lipolysis. Proceedings of the National Academy of Science U.S.A., 75, 5362-5366.

Londos,C., Cooper,D.M.F. & Wolff,J. (1980) Subclasses of external adenosine receptors. Proceedings of the National Academy of Science U.S.A., 77, 2551-2554.

Maitre,M., Ciesielski,L., Lehmann,P., Kempf,E. & Mandel,P. (1984) Protective effect of adenosine and nicotinamide against audiogenic seizure. Biochemical Pharmacology, 23, 2807-2816.

Marley,E. & Nistico,G.M. (1972) Effects of catecholamines and adenosine derivatives given into the brain of fowls. British Journal of Pharmacology, 40, 619-636.

Meldrum,B.S. & Nilsson,B. (1976) Cerebral blood flow and metabolic rate early and late in prolonged epileptic seizures induced in rats by bicuculline. Brain, 99, 523-542.

Patel,J., Marangos,P.J. & Boulenger,J.P. (1984) Adenosine: its action and sites of action in the CNS. In Brain Receptor Methodologies, Part B, Ch.15, pp.297-325. New York: Academic Press.

Pedata,F., Antonelli,T., Lambertini,L., Beani,L. & Pepeu,G. (1983) Effect of adenosine, adenosine triphosphate, adenosine deaminase, dipyridamole and aminophylline on acetylcholine release from electrically-stimulated brain slices. Neuropharmacology, 22, 609-614.

Phillis,J.W. & Wu,P.H. (1981) The role of adenosine and its nucleotides in central synaptic transmission. Progress in Neurobiology, 16, 187-239.

Pull,I. & McIlwain,H. (1972) Adenosine derivatives as neuro-humoral agents in the brain. Biochemical Journal, 130, 975-981.

Pull,I. & McIlwain,H. (1977) Adenine mononucleotides and their metabolites liberated from and applied to isolated tissues of the mammalian brain. Neurochemistry Research, 2, 203-216.

Rehncrona,S., Siesjo,B.K. &Westerberg,E. (1978) Adenosine and cyclic AMP in cerebral cortex of rats in hypoxia, status epilepticus and hypercapnia. Acta Physiologica Scandinavica, 104, 453-463.

Ribeiro,J.A. & Sebastiao,A.M. (1986) Adenosine receptors and calcium: basis for proposing a third (A_3) adenosine receptor. Progress in Neurobiology, 26, 179-209.

Rossi,F., Lampa,E., Giordano,L., Marfella,A., Ariello,B., Matera, M.G., DeCarlo,R. & Marmo,E. (1982) Interactions between inosine and adenosine: experimental researches. Research Communications in Chemical Pathology and Pharmacology, 35, 397-404.

Rubio,R., Berne,R., Bockman,E.L. & Curnish,R. (1975) Relationship between adenosine concentration and oxygen supply in rat brain. American Journal of Physiology, 228, 1896-1902.

Rubio,R., Berne,R.M., Winn,H.R. (1978) Production, metabolism and possible function of adenosine in brain tissue in situ. In Ciba Foundation Symposium Vol.56: Cerebral Vascular Smooth Muscle and its Control, ed. Purves,M.J., pp.355-372. Amsterdam: Elsevier-North Holland.

Sakai,K. (1978) Tryptaminergic mechanism participating in induction of vasoconstriction by adenine nucleotides, adenosine, IMP and inosine in the isolated and blood-perfused hindlimb preparation of the rat. Japanese Journal of Pharmacology, 28, 579-587.

Sakurada,O., Kennedy,C., Jehle,J.M,Brown,J.D., Carbin,G.L. & Sokoloff,L. (1978) Measurement of local cerebral blood flow with iodo[^{14}C]antipyrine. American Journal of Physiology, 234, H59-H66.

Schrader,J., Nees,S. & Gerlach,E. (1977) Evidence for a cell surface adenosine receptor on coronary myocytes and atrial muscle cells. Pflugers Archiv. European Journal of Physiology, 369, 251-257.

Schwabe,U. (1985) Classification of adenosine receptors. In Adenosine: Receptors and Modulation of Cell Function, eds. Stefanovich,V., Rudolphi,K., Schubert,P., pp.15-28. Oxford/Washington D.C.: IRL Press.

Schwabe,U., Ebert,R. & Erbler,H.C. (1973) Adenosine release from isolated fat cells and its significance for the effects of hormones on cyclic 3',5'-AMP levels and lipolysis. Naunyn-Schmiedeberg's Archives of Pharmacology, 276, 133-148.

Schwabe,U., Ukena,D. & Lohse,M.J. (1985) Xanthine derivatives as antagonists at A_1 and A_2 adenosine receptors. Naunyn-Schmiedeberg's Archives of Pharmacology, 330, 212-221.

Severinghaus,J.W. & Lassen,N.A. (1967) Step hypocapnia to separate arterial from tissue PCO_2 in the regulation of cerebral blood flow. Circulation Research, 20, 272-278.

Siesjo,B.K. & Zwetnow,N.N. (1970) The effect of hypovolemic hypotension on extra- and intracellular acid-base parameters and energy metabolites in the rat brain. Acta Physiologica Scandinavica, 79, 114-124.

Sokoloff,L., Reivich,M., Kennedy, Des Rosiers,M.H., Patlak,C.S., Pettigrew,K.D., Sakurada,O. & Shinohara,M. (1977) The [^{14}C] deoxyglucose method for the measurement of local cerebral glucose utilisation: theory, procedure, and normal values in the conscious and anaesthetized albino rat. Journal of Neurochemistry, 28, 897-916.

Spielman,W.S. & Oswald,H. (1979) Blockade of postocclusive renal vasoconstriction by an angiotensin II antagonist: evidence for an angiotensin-adenosine interaction. American Journal of Physiology, 237, F463-F467.

Stone,T.W. (1981) Physiological roles for adenosine and adenosine 5'-triphosphate in the nervous system. Neuroscience, 6, 523-555.

Stone,T.W. (1985) Some unresolved problems. In Purines: Pharmacology and Physiological Roles, ed. Stone,T.W., pp.245-251. London: The MacMillan Press.

Trost,T. & Stock,K.(1977) Effects of adenosine derivatives on cAMP accumulation and lipolysis in rat adipocytes and on adenylate cyclase in adipocyte plasma membranes. Naunyn-Schmiedeberg's Archives of Pharmacology, 299, 33-40.

van Calker,D., Müller,M. & Hamprecht,B. (1979) Adenosine regulates via two different types of receptors the accumulation of cyclic AMP in cultured brain cells. Journal of Neurochemistry, 33, 999-1005.

Wahl,M. & Kuschinsky,W. (1976) The dilatatory action of adenosine on pial arteries of cats and its inhibition by theophylline. Pflügers Archiv. European Journal of Physiology, 362, 55-59.

Williams,M. (1984) Adenosine- a selective neuromodulator in the mammalian CNS. Trends in Neuroscience, 7, 164-168.

Winn,H.R., Morii,S., Ngai,A.C. & Berne,R.M. (1983) The effects of theophylline, an adenosine receptor blocker, on cerebral blood flow(CBF). In Adenosine: Receptors and Modulation of Cell Function, eds. Stefanovich,V., Rudolphi,K. &Schubert,P., pp.379-390. Oxford/Washington,D.C.: IRL Press.

Winn,H.R., Rubio,R. & Berne,R.M. (1979) Brain adenosine production during 60 seconds of ischemia. Circulation Research, 45, 486-492.

Winn,H.R., Rubio,R.M. & Berne,R.M. (1981) Brain adenosine concentrations during hypoxia in rat. American Journal of Physiology, 241, H235-H242.

Winn,H.R., Rubio,R. & Berne,R.M. (1981) The role of adenosine in the regulation of cerebral blood flow. Journal of Cerebral Blood Flow and Metabolism, 1, 239-244.

Winn,H.R., Welsh,J., Rubio,R. & Berne,R.M. (1980a) Brain adenosine production in rat during sustained alteration in systemic blood pressure. American Journal of Physiology, 239, H644-H651.

Winn,H.R., Welsh,J., Rubio,R. & Berne,R.M. (1980b) Changes in brain adenosine during bicuculline induced seizures in rats. Effects of hypoxia and altered systemic blood pressure. Circulation Research, 47, 481-491.

Winn,H.R., Park,T.S., Curnish,R.R. &Rubio,R. (1980c) Incorporation of adenosine and its metabolites into brain nucleotides. American Journal of Physiology, 239, H212-H219.

Yamori,Y., Horie,R., Handa,H., Sato, M. & Fukase,M. (1976) Pathogenic similarity of strokes in stroke-prone spontaneously hypertensive rats and humans. Stroke, 7, 46-53.