PURINES IN CO-TRANSMISSION

A thesis presented for the degree of

Doctor of Philosophy

by

Janette MacGillivray Bulloch

October 1987

Institute of Physiology

University of Glasgow.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Section I

Section IV

ACKNOWLEDGMENTS

I would like to thank my supervisor, Dr. J.C. McGrath of the Institute of Physiology, University of Glasgow for his support throughout the project (and for figure 2:23).

Professor S. Jennett for her advice and for giving me the opportunity to carry out research in her department.

The S.E.R.C. for providing the C.A.S.E. award.

Special thanks to Val Alabaster and everyone, especially Ruth, at Pfizer Central Research, Sandwich for all their help during my 'industrial period'.

Craig and Ian for their help with the computers and Charlie for his assistance with the rabbits.

Radio Clyde and Nighean for keeping me awake during the typing of this thesis.

Mum, Dad and Ann for supplying the coffee and hiding the sweets.

Finally to all the postgrads. and 'Lake District people' for making the last three years so much fun.

DECLARATION AND LIST OF PUBLICATIONS

The experimental and research work presented in this thesis was performed by the author, except for some experiments involving pithed rabbits (Section I: figures 1:1, 1:2, and 1:6) which were carried out by Dr C.E. McKean and pithed rats and rabbits (for figure 1:5) which were performed by Dr J.R.Docherty.

Part of the work contained in this thesis has been, or will be, published as follows:

1) Bulloch, J.M., McGrath, J.C. & McKean, C.E. (1985). Comparison of alpha$_2$-adrenoceptor-mediated pressor responses in pithed rats and rabbits. Br. J. Pharmac.,86, 745P.

2) Bulloch, J.M. & McGrath, J.C. (1986). Blockade of vasopressor and vas deferens responses by alpha, beta-methylene ATP in the pithed rat. Br. J. Pharmac.,89, 577P.

3) Alabaster, V.A., Bulloch, J.M. & McGrath, J.C. (1986). Purinergic involvement in the NANC bronchodilatory response in the anaesthetised cat. Br. J. Pharmac.90, 33P.

4) Bulloch, J.M., Docherty, J.R., Flavahan, N.A., McGrath, J.C. & McKean, C.E. (1987). Differennce in the potency of alpha$_2$-adrenoceptor agonists and antagonists between the pithed rabbit and rat. Br. J. Pharmac.,91, 457-466.

5) Bulloch, J. M., McGrath, J.C. & Alabaster, V.A. (1987). Bronchoconstrictory effects of purines. Proceedings of the Xth International Congress of Pharmacology.

6) Bulloch, J.M. & McGrath, J.C. (1987). α-adrenergic and purinergic co-transmission in sympathetic vasopressor responses. Blood Vessels,24, 202.

7) Bulloch, J. M. & McGrath, J.C. (1987). An investigation of purinergic co-transmission in sympathetic vascular control and in the vas deferens motor response. Submitted to Br. J. Pharmac. September 1987.

SUMMARY

The sub-types of alpha-adrenoceptors which mediate pressor responses to sympathomimetic agonists or to nerve stimulation in pithed rabbits have been classified according to the effects of "selective" antagonists and a comparison has been made, for the $alpha_2$ subtype, with corresponding responses in the rat.

In the rabbit the dose-response curve for phenylephrine was shifted to the right in parallel by prazosin (1 mg/kg) and was unaffected by rauwolscine (1mg/kg). The dose-response curve for noradrenaline was shifted to the right by prazosin (1 mg/kg) and was shifted to a smaller extent by rauwolscine (1mg/kg) or imiloxan (10 mg/kg). After rauwolscine, prazosin produced a rightward shift larger than when given alone. After prazosin, rauwolscine produced a rightward shift larger than when given alone.

The responses to pressor nerve stimulation at low frequencies (<1Hz) could be reduced by prazosin, rauwolscine or imiloxan but those at higher frequency could be reduced only by prazosin.

These results indicate that the responses to noradrenaline or to nerve stimulation are mediated by both $alpha_1$- and $alpha_2$-adrenoceptors. Low doses or

frequencies have a proportionately greater component which is alpha$_2$.

Responses to noradrenaline after prazosin (1 mg/kg), were sufficiently sensitive to rauwolscine to be considered as predominantly alpha$_2$. A comparison was therefore made of prazosin-resistant pressor responses to noradrenaline in the rat and rabbit. Pressor responses to noradrenaline were produced by a lower dose per unit body weight in the rat whereas this was less marked for the alpha$_2$-adrenoceptor agonist guanabenz. In the rabbit pressor responses to noradrenaline were more susceptible to blockade by rauwolscine but were less sensitive to Wy 26703 than in the rat. This demonstrates that the alpha$_2$-adrenoceptors mediating pressor responses *in vivo*, like those in other tissues *in vitro*, are different in rat and rabbit, with regard to sensitivity to antagonists.

The fractions of alpha versus non-alpha components of vasopressor responses were assessed in the pithed rat preparation.

Sympathetic pressor nerves exit from the spinal cord over most of the thoraco-lumbar outflows, the optimum region for evoking increases in systemic arterial blood pressure being T_6 - T_8. This region was therefore chosen for the study of the involvement of purinergic co-transmission in sympathetic nerve

transmission. In the case of the vas deferens, maximal responses were obtained by stimulation at regions T_{13} - L_1 (McGrath, 1973).

The involvement of purines in the pressor responses to stimulation of the spinal sympathetic outflow was investigated by the following methods.

Firstly the position was found in the spinal column where the pithing rod electrode should be placed to give large responses suitable for investigating the relative involvement of the components within the response. This allowed determination of the optimum stimulatory parameters for the alpha-blocker - resistant component of the sympathetic pressor response which could be unmasked by giving combined $alpha_1$- and $alpha_2$-adrenoceptor blockade.

Secondly a suitable protocol was developed for the use of the best P_2-purinoceptor desensitising agent available, α,β-mATP. After desensitisation, its influence on nerve mediated responses was assessed.

Finally responses to ATP and mATP were studied and compared with the responses obtained by sympathetic stimulation.

These first two approaches have been used in the past but to limited success (Flavahan et al,1985),

mainly due to the ineffective dosage of mATP employed.

The P_2-purinoceptor desensitising agent mATP was believed to be a very stable analogue of ATP (Delbro et al,1985). It was on this assumption that investigations were carried out on responses obtained to sympathetic nerve stimulation after 'desensitisation' with a single dose of mATP. However the stability and effectiveness of mATP in vivo could be much less that reports from in vitro studies suggest (Milner-White & Rycroft, 1983) since they take into account only one of the enzyme reactions which degrade ATP and none of the uptake or dissipating mechanisms which occur in vivo. Therefore it seemed possible that the limited success of other workers was due to the ineffectiveness of mATP rather than because there was little or no purinergic involvement.

Based on this experience with vasopressor responses an examination was made of other systems, in which NANC components had been reported, in an attempt to identify any NANC components in nerve mediated depressor responses, vas deferens motor responses and in nerve mediated bronchodilator responses in the lung.

Stimulation of the spinal outflow between regions L_5 and S_2 produced depressor responses. These responses were unaffected by adrenergic, cholinergic or purinergic antagonists.

Several responses to nerve stimulation in the pelvic viscera show the pharmacological profile of NANC transmission. One such response is that to stimulation of motor responses from the rat vas deferens (Ambache & Zar, 1971; McGrath, 1978). By re-examining this in situ in the pithed rat it was possible to investigate the purinergic involvement in these motor responses. Blockade of the P_2-purinoceptors by mATP attenuated the motor response to the vas deferens by about 80%, therefore indicating a very large purinergic component at the stimulatory parameters used. Measured at the same time as the pressor responses to sympathetic nerve stimulation, the contraction of the vas deferens in situ provided an assay for the time course of the effectiveness of mATP in vivo.

In conditions of high resting airway smooth muscle tone, stimulation of the sectioned left vagal nerve bundle produced bronchodilations which were neither adrenergic nor cholinergic. Studies on the NANC inhibitory system in the cat lung have been both scarce and unproductive. Previous experimenters used non-specific drugs like quinidine for characterising and investigating any purinergic element in the response.

In an attempt to characterise the neurotransmitter associated with the respiratory NANC inhibitory system in the cat, the effects of two purinoceptor blockers,

mATP for P_2-purinoceptors, and 8-phenyltheophylline (8-PT) for P_1-purinoceptors were studied. This showed that while mATP had no effect on nerve mediated bronchodilations, 8PT attenuated these responses, therefore indicating P_1 rather than P_2-purinoceptor involvement in the responses.

INTRODUCTION: PART 1-

SYMPATHETIC NEUROCHEMICAL TRANSMISSION

Sympathetic Neurochemical Transmission

The concept that nervous impulses are transmitted across synapses and neuroeffector junctions by chemical mediators has been proposed since the turn of the twentieth century and superceded the previous theory of electrical transmission.

In 1901 Langley showed that the pressor response obtained on stimulation of sympathetic nerves was similar to the increase in blood pressure noted when adrenal extract was injected in vivo (Oliver & Schafer, 1894) and that this effect remained after denervation indicating a site of action on the effector system and not via the nerves.

The identity of the chemical responsible for the pressor action of the adrenal extract was termed adrenaline because of its close association with the adrenal medulla. It was not long after Oliver and Schafer's observation that this active substance adrenaline was isolated (Abel & Crawford, 1897; Takamine, 1901; Aldrich, 1901) and later synthesised (Stolz, 1904; Dakin, 1905; Abel & Taveau, 1905). However it was not until Elliot (1905) suggested that this active substance from the adrenal extract may also be liberated from sympathetic nerve endings that the possibility of chemical transmission took a step closer to reality.

The first conclusive evidence that chemical transmission occurs came from Loewi (1921). When two frog hearts were set up and perfused so that the perfusate flowed from the first to the second heart and the sympathetic nerve innervating the first heart was stimulated, it not only caused that heart to beat faster and stronger but this effect was also soon observed in the second heart, without the second heart being directly stimulated via its sympathetic nerves. Loewi concluded that a substance (which he termed 'acceleranstoff') was released from the sympatheic nerves, and that this substance was transported via the perfusate to cause the observed effects on the second heart. The striking similarity between the action of Loewi's 'acceleranstoff' and that of adrenaline on frog tissue led to investigations which eventually proved adrenaline to be the sympathetic transmitter in the frog.

However in mammals the sympathetic transmitter was shown to be noradrenaline rather than adrenaline. The evidence for this came as a result of a number of investigations.

The first evidence showing that noradrenaline mimicked responses to sympathetic nerve stimulation more closely than did adrenaline, appeared as early as 1910 (Barger & Dale, 1910) but was overlooked until 1921 when

Cannon & Uridil noted that the actions of an adrenaline-like substance, which was liberated on stimulation of sympathetic nerves, did not closely match those of adrenaline. He concluded that it was this adrenaline-like substance and not adrenaline that was released during sympathetic nerve transmission and named this substance 'sympathin' to indicate its association with sympathetic nerves and to distinguish it from adrenaline.

In a Loewi-type experiment, Finkleman in 1930 suspended one segment of rabbit intestine above another and allowed Locke's solution to flow from the first onto the second segment. Stimulation of sympathetic nerves to the upper tissue caused inhibition of its spontaneous movements and tissue relaxation, followed by a similar response in the second, lower preparation after a short delay. He showed that these effects were similar to those obtained by additions of adrenaline.

Clearly therefore the role of noradrenaline as the sympathetic neurotransmitter had still not been universally recognised. It was Von Euler in 1946 who showed that the main sympathetic transmitter was noradrenaline since very little adrenaline was present in sympathetically innervated tissue whereas the concentration of noradrenaline was very high by comparison. Furthermore, after sympathectomy of the tissues being studied, Von Euler showed a decrease in

noradrenaline levels indicating noradrenaline association with nerve rather than muscle and that the 'sympathin' of Cannon's experiments behaved like noradrenaline.

Adrenoceptors

Early in the history of the study of noradrenaline, it was appreciated that there were two main types of action (contraction or relaxation) which could be produced by noradrenaline or adrenaline.

Dale in 1906 showed that the pressor effect obtained by the addition of adrenaline in the cat in vivo, could be changed into a depressor response by pretreating with ergot. Hence it was shown that adrenaline has two effects, vasoconstriction (the dominant effect) and vasodilation (which can be unmasked on addition of ergot which blocks the vasoconstrictor effect).

Cannon & Rosenbleuth (1933) postulated that 'sympathin' (noradrenaline) or adrenaline had dual effects because 'sympathin 'and adrenaline could combine with either of two substances to produce either 'sympathin E' (resulting in excitation) or 'sympathin I' (resulting in inhibition).

This view was different to Langley's (1905) in that he envisaged two 'receptive substances' were fixed components of the effector cells and that a response (which depended on which one of the receptive substances was involved) occured on combination of transmitter with either of the receptive substance types. Hence the differences in responses were due to differences in the receptor sites on the effector cells and on the chain of events that occur after interaction of transmitter with these sites. Langley's view is essentially the current view since there is no evidence that noradrenaline (the sympathetic neurotransmitter) is modified in any way before initiating a response at the effector cells.

By comparing relative potencies of six sympathomimetics on a variety of tissues, Ahlquist (1948) noted that the order of potency differed between the smooth muscle types. For example, the order of potency for smooth muscle producing contraction was different to that producing relaxation, whilst the order of potency for stimulation of the heart was similar to that in smooth muscle that produced relaxation. This led to the postulation that two types of receptor ('alpha' and 'beta') existed. For muscle with 'alpha' receptor dominance adrenaline and noradrenaline were most sensitive whilst isoprenaline was practically insensitive. Those tissues with 'beta' receptors, were sensitive to isoprenaline but less sensitive to adrenaline and noradrenaline. This concept of alpha and

beta-adrenoceptors is now universally accepted and supported with the discovery of selective receptor antagonists.

So far the discussion has centered on receptors being located on the effector muscle. However the discovery of presynaptic alpha-adrenoceptors led to the pharmacological subdivision of alpha-adrenoceptors. This subdivision was stimulated by the multiple actions of the alpha-adrenoceptor blocking drug phenoxybenzamine (Brown & Gillespie, 1956). These receptors differed not only in location but also in effect, since these receptors did not produce contraction or relaxation like their post-junctional counterparts, but mediated inhibition of transmitter release from sympathetic nerve terminals in a negative feedback mechanism (mechanisms of which are detailed in reviews by Langer,1974; Starke,1977; Westfall, 1977; Vizi,1979).

The suggestion that pre and postsynaptic alpha-adrenoceptors differed came from observations made in the rabbit heart and cat spleen. At the presynaptic release-inhibiting receptors in the rabbit heart the relative potencies of phenylephrine, oxymetazoline and naphazoline differed with their relative potencies postsynaptically (Starke 1972), and in the cat spleen phenoxybenzamine was more potent postsynaptically than presynaptically (Langer,1973). As a result of these studies Langer suggested (1974) that postsynaptic

receptors should be referred to as $alpha_1$ and presynaptic receptors $alpha_2$.

However, there is now evidence for the occurence of both $alpha_1$ and $alpha_2$ postjunctional adrenoceptors and therefore subdivision on the basis of anatomical location is no longer a useful method of classification.

The well defined antagonism of prazosin on the contractile effect of noradrenaline in human visceral arteries was not observed in human digital arteries, which had a large prazosin resistant component (Moulds & Jauernig, 1977; Jauernig et al, 1978). Drew and Whiting (1979) extended this study by showing that prazosin strongly antagonised the pressor effect of phenylephrine in cats and rats but was not as effective against the pressor response to noradrenaline , whilst yohimbine was more potent against noradrenaline pressor responses than against phenylephrine. As observed by Moulds and Jauernig (1977), Drew and Whiting also showed that this blocking effect of prazosin against noradrenaline pressor responses was not consistent when comparing different vascular beds. The pressor responses to noradrenaline were relatively resistant to prazosin in the cat hindlimb and mesenteric vascular beds but not in the renal vasculature. The conclusion of this study explained that phenylephrine constricts blood vessels via the prazosin sensitive $alpha_1$ receptors whereas noradrenaline acts through both the $alpha_1$-adrenoceptor

and an adrenoceptor which is insensitive to prazosin.

This prazosin resistant site of action has now been classified as alpha$_2$. The pressor effects of selective alpha$_2$-adrenoceptor agonists in the pithed rat were unaffected by prazosin but were antagonised by the alpha$_2$-adrenoceptor antagonist yohimbine whilst the pressor response to phenylephrine was markedly attenuated by prazosin and only slightly affected by yohimbine (Docherty et al ,1979; Docherty and McGrath, 1980a). This observation has been repeated with a variety of alpha-adrenoceptor agonists all showing that the vasoconstriction they produce is at least partly resistant to alpha$_1$ blockade and that the resistant component can be blocked by alpha$_2$-adrenoceptor antagonists, indicating a role for alpha$_2$-adrenoceptors post-junctionally (Timmermans et al, 1979; Drew, 1980; Flavahan & McGrath, 1980; Kobinger & Pichler, 1980; Timmerman and van Zwieten, 1980; Madjar et al, 1980). The post-junctional alpha$_2$-adrenoceptor population may not be homogeneous and could vary in location (synaptic and extra-synaptic) (McGrath, 1982a) as well as between species (Alabaster et al, 1986).

Sympathetic Neurovascular Transmission

It has now also been proposed that in blood vessels, alpha-adrenoceptors- the proposed site of

action of the sympathetic neurotransmitter- are unlikely to be the only site of sympathetic mediation since many examples have been found where vascular responses to nerve stimulation remain partly resistant to alpha blockers. Recently two alternative proposals of alpha-mediated sympathetic transmission have been postulated inorder to explain experimental observations in isolated tissues.

Firstly the 'gamma-adrenoceptor' hypothesis (Hirst & Neild, 1980) suggests that noradrenaline is the transmitter but the junctional receptors are not alpha-adrenoceptors.

Secondly the 'co-transmitter' hypothesis puts forward the idea that another transmitter is released along with noradrenaline. Several candidates have been proposed as the neurotransmitter involved in the co-transmission including adenosine and the adenine nucleotides (Burnstock & Sneddon, 1985).

Preliminary investigations of the relative contributions to vascular neurotransmission from sub-types of alpha-adrenoceptor, unmasked an alpha-blocker-resistant component (Flavahan et al, 1985) in the vascular sympathetic response of the pithed rat. This non-alpha component of the pressor response could be slightly attenuated by the purinergic desensitising agent mATP, but only after alpha-adrenoceptor

antagonists had been added to block the alpha component of the response (Flavahan et al, 1985; Grant et al, 1985). This indicated a role, however minor, for purines in vascular sympathetic co-transmission.

INTRODUCTION: PART 2

PURINES

The Pharmacological and Physiological roles of Purines.

Drury & Szent-Gyorgi (1929) first investigated the actions of purines on the heart and cardiovascular system by showing that crude heart, brain, kidney and spleen extracts caused negative chronotropic effects on the heart and identified the active constituent as AMP. They further showed that adenosine prepared from yeast affected the heart in a similar way, as well as dilating arterial and coronary blood vessels, and inhibiting spontaneously active intestinal smooth muscle. The depressor effects of purines were also noted by Green & Stoner (1950).

Recent studies of cardiac hypoxia and hyperaemia have revealed a possible physiological role for adenosine (formed from the intracellular degradation of ATP) by indicating a link between myocardial adenosine levels and coronary blood flow (Berne, 1963; Berne, 1980; Mustafa,1980). The coronary vasodilatory effects of adenosine are blocked by the methylxanthines (Bunger et al, 1975). ATP has now been shown to be more potent than adenosine in inducing coronary vasodilation (Wolf & Berne, 1956; Moir & Downs, 1973) and is shown to be present in perfusates from hypoxic hearts (Paddle & Burnstock, 1974). These effects are distinct from the adenosine effects since they cannot be blocked by the

methylxanthines (Giles & Wilken, 1977).

This blood flow regulating role of adenosine and ATP has been extended from the coronary circulation and has now been proposed to play a role in other vascular beds including the skeletal muscle (Boyd & Forrester, 1968; Berne et al, 1975), renal (Haddy & Scott, 1968), adipose tissue (Sollevi & Fredholm, 1981) and cerebral (Rubio et al, 1975). The source of these purines, neuronal or non-neuronal, has yet to be determined (Burnstock, 1982).

The vascular effects of ATP were first linked to nerves when Holton and Holton (1954) proposed that ATP released from sensory nerves during antidromic nerve stimulation caused the observed vasodilation in the rabbit ear artery, and later showed that rabbit ear vessel dilation was accompanied by ATP release (Holton, 1959).

Purinergic receptors and their subclassification.

In 1978, Burnstock distinguished two types of peripheral purine receptors; P_1-purinoceptor and P_2-purinoceptor (Table 1). Adenosine and AMP were more potent than ATP at P_1-purinoceptors and were antagonised by methylxanthines like theophylline. P_1-purinoceptor activation leads to changes in cAMP via a modulation of adenylate cyclase activity.

Table 1

Two types of peripheral purinoceptor have been identified in various tissues: the P_1- and the P_2-purinoceptor. Both of these receptor types have now been further subdivided according to function, agonist potency, and preferential antagonist. Details of these subdivisions are given in the text.

RECEPTOR	SUBTYPE	AGONIST	ANTAGONIST	FUNCTION/LOCATION
P_1	all	Ad > ATP	according to subtype	-cAMP & adenylate cyclase modification
	P_{1a} ($=R_a=A_2$)	NECA > Ad > PIA	methylxanthines	-activates adenylate cyclase/ cAMP accumulation -receptor located on extracellular membrane surface (eg. airway smooth muscle)
	P_{1i} ($=R_i=A_1$)	PIA > Ad > NECA	methylxanthines	-inhibits adenylate cyclase/ cAMP accumulation -receptor located on extracellular membrane surface
	P	2',5'-5' dioxyadenosine	methylxanthine resistant	-inhibits cAMP accumulation -receptor located intracellularly
P_2	all	ATP > Ad	according to subtype	-Ca^{2+} / K^+ / Na^+ movement -prostaglandin formation
	P_{2x}	αβ mATP > βγ mATP > ATP >> 2-methylthio ATP	$ANAPP_3$ αβ mATP	-smooth muscle contraction (eg. constricts rabbit mesenteric artery)
	P_{2y}	2-methylthioATP > ATP > βγ mATP >> αβ mATP	reactive blue 2	-smooth muscle relaxation (eg. relaxes rabbit mesenteric artery)

However at P_2-purinoceptors, ATP and ADP were more potent than adenosine, and they were not antagonised by methylxanthines. It was later shown that responses mediated via these receptors could be specifically antagonised by arylazido amino propionyl ATP (ANAPP3) (Hogaboom et al, 1980) or desensitised by α,β- methylene ATP (Kasakov & Burnstock, 1983) thus further substantiating the P_1/P_2 hypothesis.

The P_1 receptors

Adenosine receptor subtypes were originally classified by a biochemical approach. This led to some confusion in the nomenclature as the various groups of workers adopted different nomenclature according to the system studied, when comparing and contrasting the receptor subtypes.

Using the criterion of receptor location, the adenosine receptors were first subdivided into 'R' and 'P' sites (Londos & Wolff, 1977), where 'R' designated requirement for an intact ribose ring for receptor interaction to mediate changes in adenylate cyclase activity. These 'R' sites are located extracellularly.'P' sites require intact purine ring; these sites are thought to have intracellular location and are not thought to contribute to the actions of exogenously administered adenosine in vivo (Braun &

Levitski, 1979).

The extracellular receptors are further subdivided into two classes of cell membrane adenosine receptors. This subdivision has been based on two types of observation.

Firstly, adenosine receptor activation can have either stimulatory or inhibitory effects on adenylate cyclase activity in isolated cells. The corresponding receptor subtypes are designated Ra and Ri respectively (Londos et al, 1980). Similarly, Van Calkner and co-workers (1979) made a subdivision of the adenosine receptor but this time on the basis of how receptor activation affected cAMP accumulation: A_2 receptors increasing cAMP accumulation; A_1 recptors inhibiting cAMP accumulation.

Secondly, a series of adenosine analogues exhibit two different and distinct orders of potency on a range of both biochemical and pharmacological preparations. The Ra/A_2 receptor is more susceptible to 5' - N-ethylcarboxamide adenosine (NECA) and less responsive to L-N^6-phenyl isopropyladenosine (L-PIA) whereas the reverse is true for the Ri/A_2 receptor (Bruns et al, 1980).

Simplifying these above classifications of extracellular adenosine receptors, it is possible to

combine them such that P_{1a} receptors are equivalent to Ra & A_2 whereas P_{1i} receptors are equivalent to Ri & A_1 (Brown & Burnstock, 1981).

The P_2 receptors

From potency studies of the structural analogues of ATP, the P_2-purinoceptor was further divided into P_{2x} & P_{2y} subclasses. At the P_{2y}-purinoceptor, 2-methylthio-ATP was more potent than ATP, and α,βm ATP was less potent than ATP in producing a response, whereas at the P_{2x}-purinoceptor, phosphate modified analogues of ATP (like α,βm ATP) were more potent than ATP in producing a response (Burnstock & Kennedy, 1985).

The P_{2x}-purinoceptors, whose activation causes contraction of vascular smooth muscle (Burnstock & Warland, 1987a) may be antagonised by ANAPP3 (Hogaboom et al, 1980) or desensitised by α,βm ATP (Kasakov & Burnstock, 1983; Delbro et al, 1985). The P_{2y}-purinoceptor, whose activation causes relaxation of vascular smooth muscle (Burnstock & Warland, 1987b) may be antagonised by Reactive Blue 2 (Kerr & Krantis, 1979; Burnstock & Warland, 1987b).

ATP: Principle neurotransmitter, co-transmitter or neuromodulator?

ATP: A Principle Neurotransmitter.

Despite earlier reports of 'atropine-resistant' parasympathetic nerve mediated responses occuring in the gastrointestinal tract and bladder (Langley & Anderson, 1895a; Langley, 1898; Ambache, 1951) it was not until the 1960's that autonomic nerves other than adrenergic and cholinergic were suggested (Paton & Vane, 1963; Burnstock et al, 1963; 1964; 1966). These are the non-adrenergic non-cholinergic (or NANC) nerves.

Some of the substances associated with NANC transmission have now been identified and established as neurotransmitters. Evidence suggests that ATP, which fits all the necessary criteria to be identified as a neurotransmitter, could be the transmitter associated with some of the NANC responses (Burnstock et al, 1970; 1972).

Firstly, systems for the synthesis and storage of ATP were shown to be present in nerve terminals. One problem in suggesting ATP as a neurotransmitter was not in its rarity but rather in its abundance, since it is present in cells as a high energy metabolite in both neural and non-neural tissue. It was not until specific

storage compartments for ATP were suggested (Su et al,1971) and finally observed as 'large opaque vesicles' (Robinson et al, 1971; Burnstock & Iwayama, 1971) that ATP became a serious contender.

Secondly, ATP was shown to be released on stimulation of nerves from the taeni coli and the bladder, by using the sensitive ATP-specific firefly luciferin-luciferinase assay (Burnstock et al, 1978). However most excitable tissues can release ATP including muscle cells thus making interpretation of results more complex as well as providing a major stumbling block to the purinergic hypothesis.

Thirdly, exogenous administration of ATP mimicked the response to nerve stimulation by acting on post-synaptic receptors for ATP. For example in the intestine, ATP causes a relaxation of a type which exactly mimics the NANC responses (Ambache & Zar, 1970; Dean & Downie, 1978) and in the bladder ATP produces a contractile response which wanes rapidly in a way which mimics that to NANC nerve stimulation (Burnstock et al, 1972).

Fourthly, mechanisms for terminating the action of released ATP exist, either by enzymic degradation or by uptake mechanisms. These mechanisms not only exist, but are very efficient as observed by the very short duration of action of both exogenously applied ATP and

of NANC nerve stimulation. Until recently it was thought that only adenosine underwent re-uptake but in 1970 Chaudry & Gould showed the existence of an uptake mechanism for ATP as well. The action of dipyridamole is to block the uptake of adenosine. This is shown by a decreased uptake of ^{3}H-adenosine (Satchell et al, 1973; Satchell & Burnstock, 1975; Macguire & Satchell, 1979). The enzymes responsible for the degradation of ATP are the ATPases which are located on cell plasma membranes (Manery & Dryden, 1979) and can almost completely degrade ATP to AMP on a single passage through the rat heart and lung (Baer & Drummond, 1968). 5'-adenylate kinase, nucleoside diphosphatase and 5'- nucleotidases all play a part in the degradation of ADP and AMP to adenosine (Macguire & Satchell, 1981). Adenosine itself can undergo deamination by adenosine deaminase (which can be inhibited by erthro-9-2-hydroxy-3-nonyl adenine (EHNA) (Macguire & Satchell, 1979)) to inosine or can be taken up into the nerves or smooth muscle.

Finally, drugs which modify the response to nerve stimulation have a similar effect on the response to exogenously applied ATP. The lack of specific antagonists of ATP delayed the acceptance of the 'purinergic nerve' hypothesis for some time. Initial examinations using high concentrations of non-specific blockers of the ATP response like quinidine (Burnstock et al, 1970) phentolamine and similar imidazolines (Satchell et al, 1973; Tomita & Watanabe, 1973)and 2,2'-

pyridylisatogen (Hooper et al, 1974) yielded some promising results but no conclusive evidence.

Apamin, a bee neurotoxin, inhibits the relaxant effects and inhibitory junction potentials (i.j.p.s) to both nerve stimulation and ATP in the guinea-pig taenia coli (Vladmirova & Shuba, 1978) but this compound once again is non-specific and acts by blocking K^+ channels rather than as a specific competitive blocker of purinergic receptors (Banks et al, 1979). The development of $ANAPP_3$ (Hogaboom et al, 1981), an ATP analogue and specific ATP receptor antagonist brought about more conclusive evidence. $ANAPP_3$ specifically blocks responses to nerve stimulation and to ATP in the guinea-pig bladder (Westfall et al, 1980) cat bladder (Theobald, 1983) and in the guinea-pig vas deferens (Fedan et al, 1981). However in some preparations, like the guinea-pig stomach fundus (Frew & Lundi, 1982) and rabbit anococcygeus (Sneddon et al, 1982), responses to both ATP and nerve stimulation remain unaffected by $ANAPP_3$. Discrepancies also exist in the guinea-pig taenia coli where ATP relaxations but not NANC nerve mediated relaxations are blocked by $ANAPP_3$ (Westfall et al, 1981; 1982). The slowly degradable analogue of ATP (α,βm ATP) (Milner-White & Rycroft, 1983) is identical to ATP in structure except that it possess a methylene group instead of an oxygen atom between the α and β phosphorous atoms (Figure 1). This analogue can activate P_2-purinoceptors in the same way as does ATP, but unlike

Figure 1

The phosphorous atoms of the triphosphate moiety of ATP are designated α, β, γ. The replacement of these indicated oxygen atoms by methylene residues yields ATP analogues like α,β-methylene ATP and β,γ-methylene ATP.

HO
OH
P
O
α
OH
P
O
β
OH
P
O
γ
O
CH2
OH
OH
O
H
C
N
N
C
NH2
C
C
N
N
CH

ATP it does not initiate synthesis of prostaglandins. Kasakov and Burnstock (1983) used mATP as a desensitising agent for P_2-purinoceptors to show the presence of a purinergic component in nerve mediated responses of the guinea pig urinary bladder.

Purines in Co-transmission

Burn and Rand (1965) were the first to propose that more than one chemical neurotransmitter could be present in a nerve, by suggesting that acetylcholine was present in adrenergic neurones and was involved in the release of NA from the nerve terminal. Until then the view was held that nerve cells synthesized and stored only one transmitter. This view, known as 'Dale's Principle' (Eccles, 1976) stated that " all branches of the neurone should have the same chemical transmitter" (Nothnagal lecture, 1934). This concept has now been re-examined by applying the term 'transmitter' to any substance that is synthesised and stored in nerve cells, is released during nerve activity and whose interaction with specific receptors on the post-synaptic effector leads to changes in post-synaptic activity. This has led to a variety of substances being identified as neurotransmitters including ATP hence leading to a

breakdown of the original idea of neurotransmission being identified as either adrenergic (releasing NA) or cholinergic (releasing ACh). Advancing from this plurality of transmission, it is also possible to see how more than one substance may be released from a nerve by considering that other substances may be released together with the principle transmitter by exocytosis of vesicle contents at the nerve terminals. For example, in adrenergic nerves, NA is stored with chromogranin A, dopamine B-hydroxylase and ATP (Smith, 1972).

With the development of immunocytochemistry, several biologically active peptides have now also been considered as co-transmitters alongside the more established neurotransmitters like ACh, NA and 5-HT (Burnstock, 1982).

However in the main it has been ATP that has attracted the most attention as a possible co-transmitter. ATP has been shown to be both stored and released from adrenal chromaffin cells (Douglas & Poisner, 1966; Douglas, 1968; Stevens et al, 1972) and indications are that it is released alongside NA in adrenergic neurones (Su et al, 1971). Recent studies agree with these preliminary findings by showing a release of [^{3}H]-adenosine and NA caused by K^+-induced stimulation of rabbit aorta and portal vein (Su, 1975; 1978). Release and storage of purines has also been detected from adrenergic nerves in guinea-pig vas

deferens (Westfall et al, 1978; Fedan et al, 1981).

However these neuronally released purines are not found in some sympathetic nerves like in the spleen (Sjarne et al, 1970; Langercrantz, 1976) and those purines that have been detected in other areas have been found to be exclusively of post-synaptic origin (Fredholm, 1976; Fredholm & Hedquist, 1978; Fredholm & Hjemdahl, 1979; Luchelli-Fortis et al, 1979).

The direct pressor response to stimulation of the sympathetic outflow in the pithed rat can only be partially blocked by reserpine or alpha-adrenoceptor antagonists (Grant et al, 1985) suggesting that NA is not the only transmitter involved in this response. However it can be completely blocked by pre-treatment with 6-OHDA suggesting that only nerves possessing uptake and accumulation sites for phenylethanolamines are involved (Flavahan et al, 1985). This would indicate that the entire response arises from adrenergic nerves but part of it does not require NA, indicating a possible role for a co-transmitter. This study also indicated that ATP may be involved in the transmission process since the P_2-purinoceptor desensitising agent, mATP, slightly attenuates the response but only in the presence of alpha-adrenoceptor antagonists.

Langer and Pinto (1976) suggested that the large non-adrenergic non-cholinergic (NANC) response in the

cat nictitating membrane which persisted after reserpine pre-treatment may be due to release of ATP remaining in the adrenergic nerves. In the vas deferens, the first phase of the bi-phasic response to nerve stimulation is resistant to alpha-adrenergic blockade and to pre-treatment by reserpine (Ambache & Zar, 1971; Gillespie & McGrath, 1974; Brown et al, 1983), but was greatly reduced by guanethidine (Burnstock & Holman, 1964) and 6-OHDA (Fedan et al, 1981). The initial component has now also been shown to be antagonised by the specific ATP antagonist $ANAPP_3$ (Fedan et al, 1981) and by the desensitising agent mATP (Meldrum, 1984; Cunnane & McGrath, unpublished work).

Purinergic Neuromodulation

Much controversy surrounds the actual definition of 'neurotransmitter', therefore whether ATP and related purines are classed as principal neurotransmitters, co-transmitters or neuromodulators depends on this definition. If a neurotransmitter is a substance that is synthesized and stored in nerves, released during nerve activity, and if its interaction with specific receptors in the post-synaptic membrane leads to changes in post-synaptic activity, then ATP is a neurotransmitter. However if that substance is present in very low concentrations and plays a minor 'modulating' role, that substance has been termed neuromodulator rather than neurotransmitter.

A neuromodulator can act on either prejunctional receptors to alter transmitter release, or can act postjunctionally to modify the action of the principle neurotransmitter on the effector cell. The concept of prejunctional neuromodulation by activation of presynaptic receptors has been widely reviewed (see Starke, 1972; Langer, 1974; Westfall, 1977; Vizi, 1979; and Gillespie, 1980). Among the substances suggested as neuromodulators are the purines (Vizi, 1979; Clanachan, 1979; Stone, 1981; Burnstock, 1982a). Modulatory actions of purines reported so far include that on ACh release from the skeletal neuromuscular junction (Ginsborg & Hirst, 1972; Riberio & Walker, 1975) and from autonomic nerve endings (Vizi & Knoll, 1976) as well as on NA release in a variety of tissues (Paton, 1981). These presynaptic effects are thought to be mediated via P_1-purinoceptors on the basis of higher adenosine potency and preferential antagonism by theophylline (Clanachan et al, 1977; Vizi & Knoll, 1976; Ribero & Dominquez, 1978).

Suggested mechanisms of action of purinergic modulation include altering the inflow of Ca^{2+} to the effector cell (Holck & Marks, 1978; Ribero, 1979; Dowdle & Maske, 1980) or by changing receptor sensitivity to the principal neurotransmitter whether it be NA or ACh (Buchthal & Kahlson, 1944; Ewald, 1976a,b; Nakanishi & Takeda, 1973; Hedquist & Fredholm, 1976; Holck & Marks,

1978; Kazic & Milosavljevic, 1981; Krishnamurty & Kadowitz, 1982).

INTRODUCTION: PART 3-

NANC Transmission in the Lung

NANC innervation has now been reported in a wide variety of tissues and organ systems including the respiratory tract. The smooth muscle of the lungs and airways is innervated by excitatory cholinergic and inhibitory adrenergic nerves as well as having a large NANC inhibitory component. The first example of NANC nerves associated with the airways was reported in the guinea-pig trachea (Coburn & Tomita, 1973; Coleman & Levy, 1974). This showed that while a small part of the relaxatory response obtained by stimulating the trachea by field stimulation was attenuated by adrenergic blockade, the major neural inhibitory pathway was neither adrenergic nor cholinergic. Subsequent reports of NANC innervation of airways of other species followed. In man these are of particular importance since there is no direct adrenergic innervation of airway smooth muscle in humans (Barnes, 1984) unlike other species such as the guinea-pig (Coburn & Tomita, 1973).

The neurotransmitter associated with the lung NANC inhibitory system has yet to be identified but several candidates have been proposed. It was thought that the existence of purinergic nerves in the airways was unlikely since antagonists used to block purine effects did not block the NANC inhibitory effects (Irvin et al, 1982). However due to the lack of specific purinergic antagonists used in these studies, it was still not

possible to totally rule out purinergic involvement.

Since these preliminary investigations were negative towards the purinergic proposal of neurotransmission, other possible neurotransmitters were investigated. Neuropeptides were presented as convincing candidates for neurotransmitters since they can be localised to nerves in the respiratory tract (Polak & Bloom, 1986). The forerunner in this category is vasoactive intestinal peptide (VIP). As well as airways having a rich VIPergic innervation (Polak & Bloom, 1986; Laitinen et al, 1985), VIP is the most potent relaxant of human bronchi in vitro (Palmer et al, 1986) even although there are very little or no peripheral effects. Other evidence against the possibility of VIP being the neurotransmitter associated with the lung NANC inhibitory system came with the reports that NANC inhibitory effects still occur after exposure to maximally effective concentrations of VIP (Karlsson & Persson, 1984).

In addition to an inhibitory NANC system experimental investigations also indicate an excitatory system in the tracheobronchial tree, which is neither cholinergic nor adrenergic (Grundstrom & Andersson, 1983). Peptides such as substance P are thought to be the neurotransmitters of these nerves (Lundberg et al, 1984).

Therefore to simply say that the lungs and airways have an adrenergic, a cholinergic and a third NANC system is perhaps an oversimplification. It is likely that several different types of nerves and neurotransmitters (or co-transmitters) are involved.

As more specific antagonists become available the nature of a neurotransmitter is further elucidated. For example, we now have available, 8-phenyl theophylline (8-PT) for P_1-purinoceptors (Griffith et al, 1981) and $\alpha\beta$-methylene ATP (mATP) for desensitising P_2-purinoceptors (Delbro et al, 1985), thus enabling further investigation of possible purinergic involvement in the lung NANC system to be carried out.

Aims of the present study

The aims of this study were to isolate and identify non-adrenergic non-cholinergic (NANC) responses in different organ systems and in particular to study the NANC component in sympathetic vasopressor responses.

In studying the sympathetic nerve mediated vasopressor response it is necessary to characterise all the components of the response, both alpha-adrenergic as well as NANC components. After identification of the components of the vasopressor response, conditions for studying the NANC nerve mediated component can be optimised.

The study of the alpha-adrenergic component of pressor responses to sympathetic nerve stimulation requires an investigation of pressor responses to alpha-adrenergic agonists in vivo. In so doing a comparison between species can be made. This is dealt with in the first section of this thesis.

The second section investigates the fractions of alpha versus non-alpha components of vasopressor responses as assessed in the pithed rat preparation: a preparation which gives the advantages of working in vivo without the disadvantages associated with anaesthetised animals such as the involvement of autonomic reflexes which would complicate interpretation

of results. The pithed preparation is particularly useful in allowing selective stimulation from the spinal column.

By moving the pithing rod electrode up and down the spinal column it is possible to find optimum areas for stimulation of individual organ systems. This allows study of contractile responses to stimulation of the sympathetic supply to the vas deferens in the rat as well as of the pressor and depressor responses. A study of the depressor responses is covered in Section 3 of this thesis.

The final section examines the NANC component present in the respiratory system. The innervation of the lungs and airways consists of three neuronal components: the cholinergic, the noradrenergic , and the NANC inhibitory component. In the cat as well as in man, this NANC system appears to provide the major inhibitory neural input to airway smooth muscle. For this reason the experimental investigations were performed in the cat. The neurotransmitter associated with the lung NANC inhibitory system has yet to be identified, but several candidates have been proposed including adenosine and adenine nucleotides. In an attempt to characterise this, this study employed two purinoceptor blockers, mATP for P_2-purinoceptors, and 8-phenyltheophylline (8-PT) for P_1-purinoceptors.

METHODS

The Pithed Rabbit Preparation

Pithed rats and rabbits were used to examine and compare the alpha$_2$-adrenoceptor-mediated pressor responses between two species. This study served to extend previously documented in vitro comparative studies between these species. The protocol followed for the pithed rabbit was similar to that first used by McGrath and MacKenzie (1977).

Adult male New Zealand white rabbits of between 3 and 4 kg were pretreated with atropine (1mg/kg i.p.) and anaesthesia was induced by introducing althesin (2ml/kg) into the marginal ear vein. The body temperature of the rabbit was maintained at 38^{o} C throughout the experiment by placing the animal on a homeothermic blanket regulated by a rectal probe thermistor.

The anaesthesia induced by althesin lasted approximately 5 minutes and allowed a mask supplying halothane to be placed on the animal without causing distress, which can lead to breath holding. Another advantage in inducing anaesthesia by this method is that it allowed the potentially irritant gaseous anaesthetic halothane to be given in a much lower concentration than would be possible without pretreating with althesin. Anaesthesia is continued with 2% halothane in a mixture of 1 volume oxygen to 2 volumes nitrous oxide.

Tracheal intubation was performed on disappearance of withdrawal reflexes and a double vagotomy carried out thereafter. The left carotid artery was cannulated for administration of propranolol and the right carotid artery cannulated for continuous recording of blood pressure via an Elcomatic pressure transducer (EM 750/752) and monitoring of heart rate with a devices instantaneous rate meter (model 4522). The right jugular vein was also cannulated for administration of all other drugs.

After the animal was turned to the prone position and the head secured in a restrainer, the animal was connected to a respiration pump and anaesthesia was continued at 2% halothane in 100% oxygen. Hyperventilation at 50 breaths per minute reduced arterial PCO_2, which leads to a reduction in brain volume and also depresses spontaneous breathing allowing uncomplicated mechanical ventilation. The anaesthetic levels were adjusted to give a steady blood pressure recording of approximately 30mmHg (diastolic) and 60mmHg (systolic). This low B.P. level minimises bleeding on decerebration but is sufficiently high to allow for the large drop in blood pressure which follows pithing.

A trephine hole (1.5cm) was drilled through the skull and enlarged by bone nibblers allowing

decerebration by suction to be carried out. Decerebration was performed whilst holding the vertebral arteries, so reducing the amount of blood flow to the brain. Following decerebration the skull cavity was lined with oxycell and packed with swabs and sterospon to reduce bleeding and blood pressure was allowed to stabilise at 50mmHg (diastolic) and 100mmHg (systolic) by removing anaesthetic and ventilating with 100% oxygen (25 b.p.m./ 50ml).

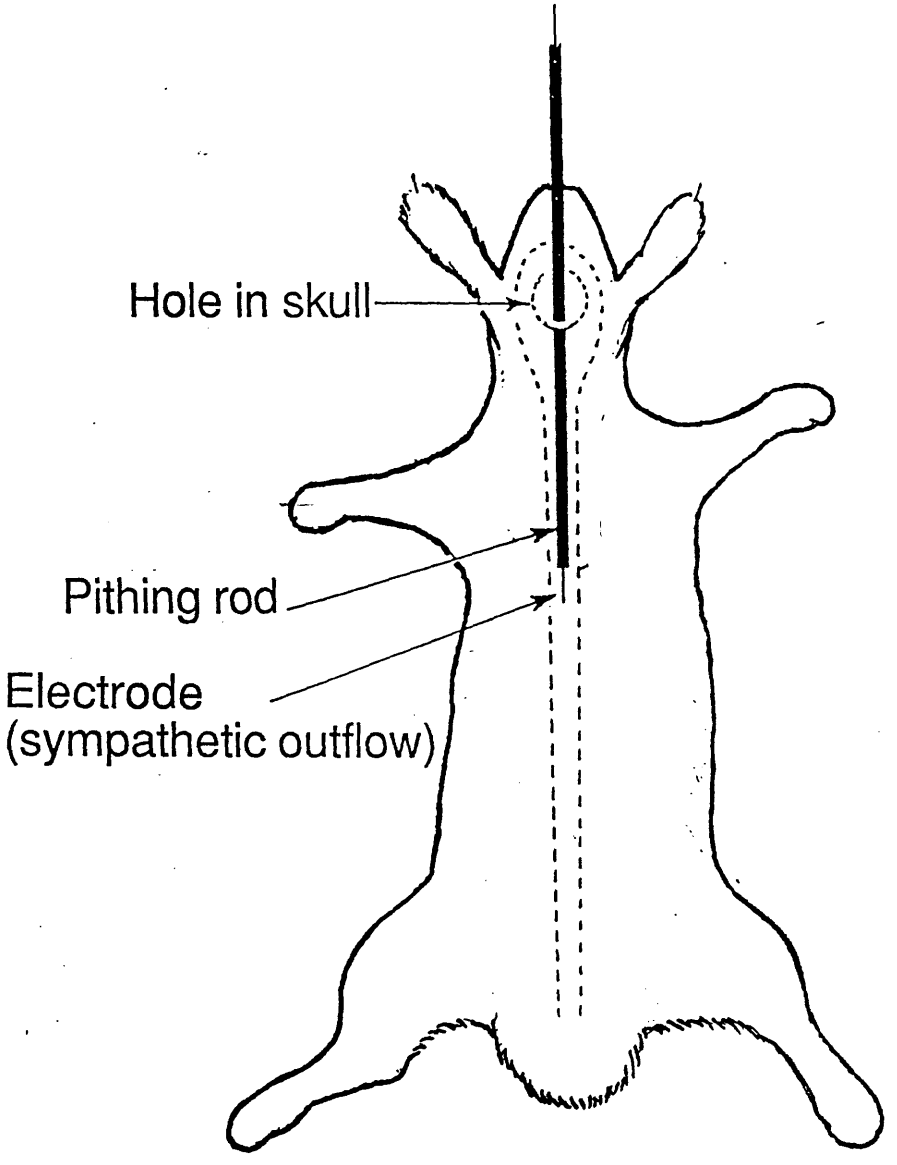
Gallamine (10mg/kg) was administered intravenously to prevent skeletal twitching during pithing. After removal of the swabs the rabbit was pithed through the trephine hole and the skull was repacked with swabs (Figure 2). The pithing rod used was a stainless steel rod of 2mm in diameter.

Since blood pressure measurements were recorded throughout the experiment it was possible to observe the effects of pithing on arterial blood pressure. The effects were two-fold: a large increase in blood pressure due to release of catecholamines from the sympathetic outflow (106$\pm$11mmHg diastolic, 160$\pm$13mmHg systolic); and a secondary drop in blood pressure due to removal of sympathetic tone on the vasculature. The blood pressure remained at this low level (17.0$\pm$8.2mmHg diastolic, 50$\pm$4.5mmHg systolic) (n=10).

Artificial ventilation was continued and parameters

Figure 2

Stimulation of the sympathetic outflow in the pithed rabbit preparation is via the pithing rod electrode, which is inserted through a trephine hole. For selective stimulation of the outflows the pithing rod is partly covered in teflon for insulation.


Hole in skull
Pithing rod
Electrode
(sympathetic outflow)

changed to 30 breaths per minute, stroke volume 50ml. Monitoring blood gas tensions from blood samples taken from the preparation showed that these ventilatory parameters were sufficient to maintain normal blood gas values.

Administration of noradrenaline (NA) (up to 5 i.v. boluses of 1µg/kg) immediately after completion of pithing improved the viability of the preparation, as well as serving as a sensitisation schedule for NA, before starting the agonist dose response curves.

The Pithed Rat Preparation

Male Wistar rats (240-260g) were initally anaesthetised in a box with 4% halothane in a mixture of 1 volume oxygen to 2 volumes nitrous oxide which was reduced to 2% halothane and fed to the rat through a mask in the same volumes of oxygen and nitrous oxide on disappearance of withdrawal reflexes. After tracheal intubation the rats were pithed by the method first employed by Gillespie and Muir (1967) and further developed by Gillespie, MacLaren and Pollok (1970). This involved the insertion of the pithing rod assembly (Figure 3 & 4) consisting of a short steel tube (13 s.w.g.) or trochar which was introduced through the orbit and advanced through the foramen magnum to lie at the sixth cervical vertebra. Through this was pushed a

Figure 3

Rats were pithed via the orbit, the pithing rod then served as an electrode for selective stimulation of the spinal outflow. Blood pressure and heart rate was monitored from the left carotid artery and bolus injections of drugs were introduced via the left jugular vein.

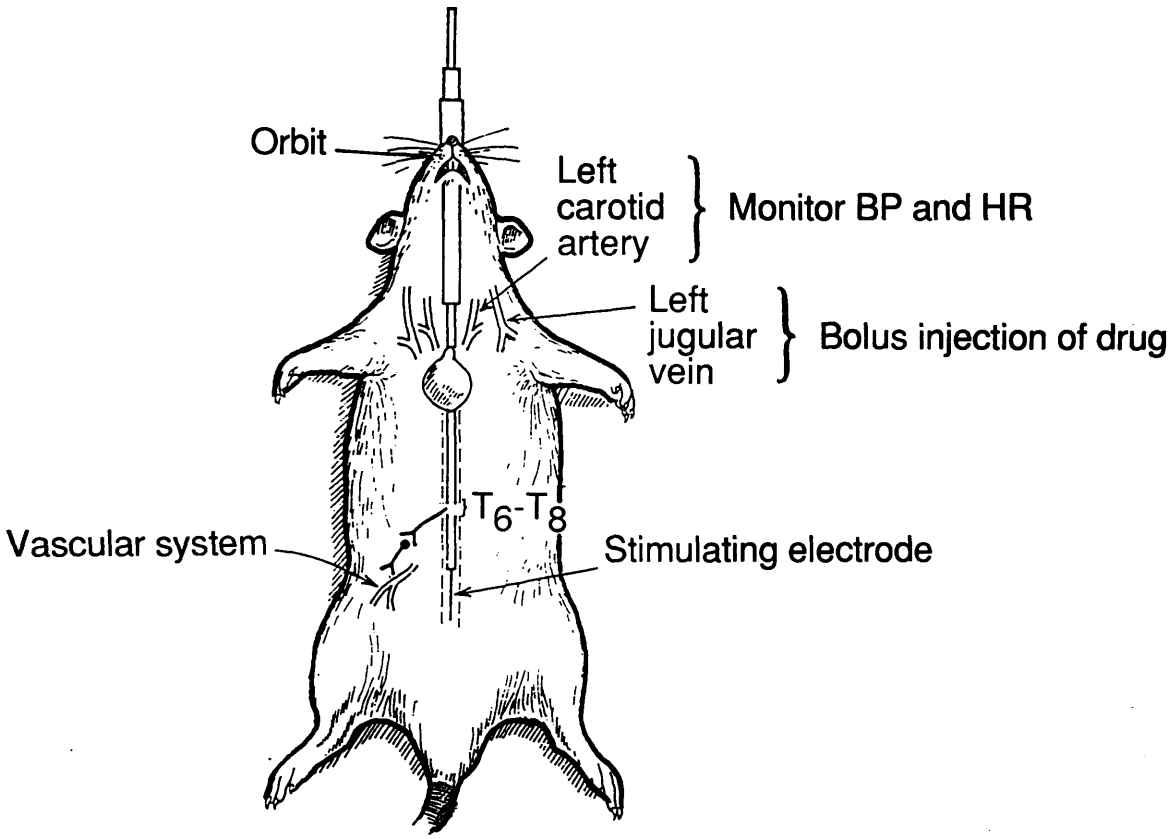
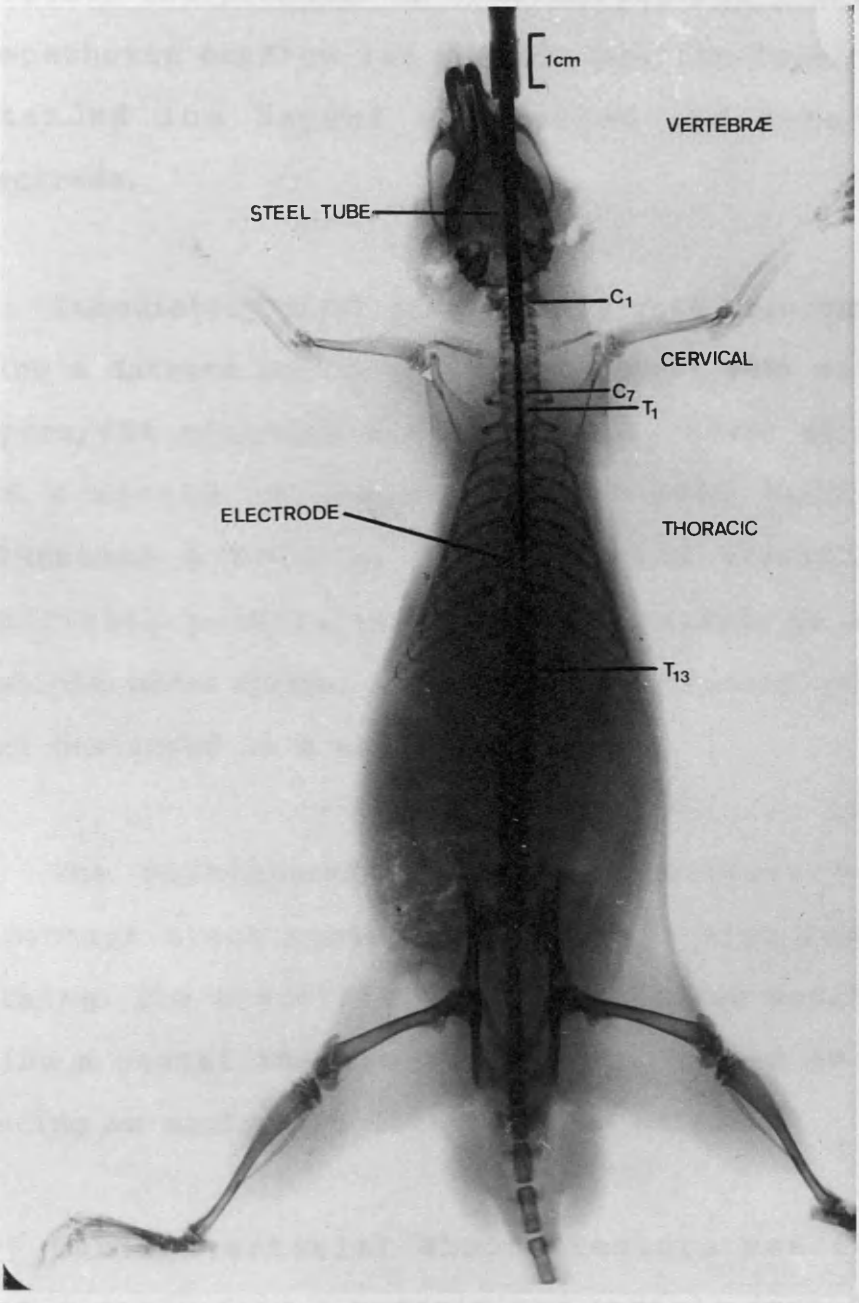

Orbit
Left carotid artery
Monitor BP and HR
Left jugular vein
Bolus injection of drug
T_6-T_8
Vascular system
Stimulating electrode

Figure 4

X-ray of a pithed rat showing the pithing rod electrode and position of outflows C_1, C_7, T_1 and T_{13}.


1cm
VERTEBRÆ
STEEL TUBE
C1
CERVICAL
C7
T1
ELECTRODE
THORACIC
T13

narrow steel rod, coated in teflon tubing, down the spinal column as far as the lower lumbar region to complete the pithing. In experiments where the spinal sympathetic outflow was stimulated, the inner rod was extended 1cm beyond the teflon and acted as an electrode.

Immediately after pithing, the rats were ventilated using a Harvard rodent ventilator (model 680) with a 40% oxygen/60% nitrogen mixture (Grant, 1985) at 60b.p.m. and a stroke volume of 1ml per 100g body weight (Clanachan & McGrath, 1976). It was essential that artificial ventilation should commence as soon as possible after pithing since the respiratory centre had been destroyed as a result of pithing.

The maintenance of body temperature was also important since control of this was also lost after pithing. The temperature of the rats was monitored by using a rectal thermometer and maintained at 37^{o}C by placing an anglepoise lamp over the animal.

Carotid arterial blood pressure was recorded continuously from a heparinised cannula inserted into the carotid artery by an Elcomatic pressure transducer (EM 750/752). Heart rate was recorded with a Devices instantaneous rate meter (model 4522) triggered by the arterial pulse. Drugs were administered via a cannula inserted into a jugular vein at fixed volumes of 1ml/kg

washed in by the same volume of 0.9% saline.

The recording device used to display the blood pressure and heart rate was a U.V. oscillograph (SE 3006 DL).

Sympathetic Nerve Stimulation from the Spinal Outflow -Stimulation of vasopressor nerves.

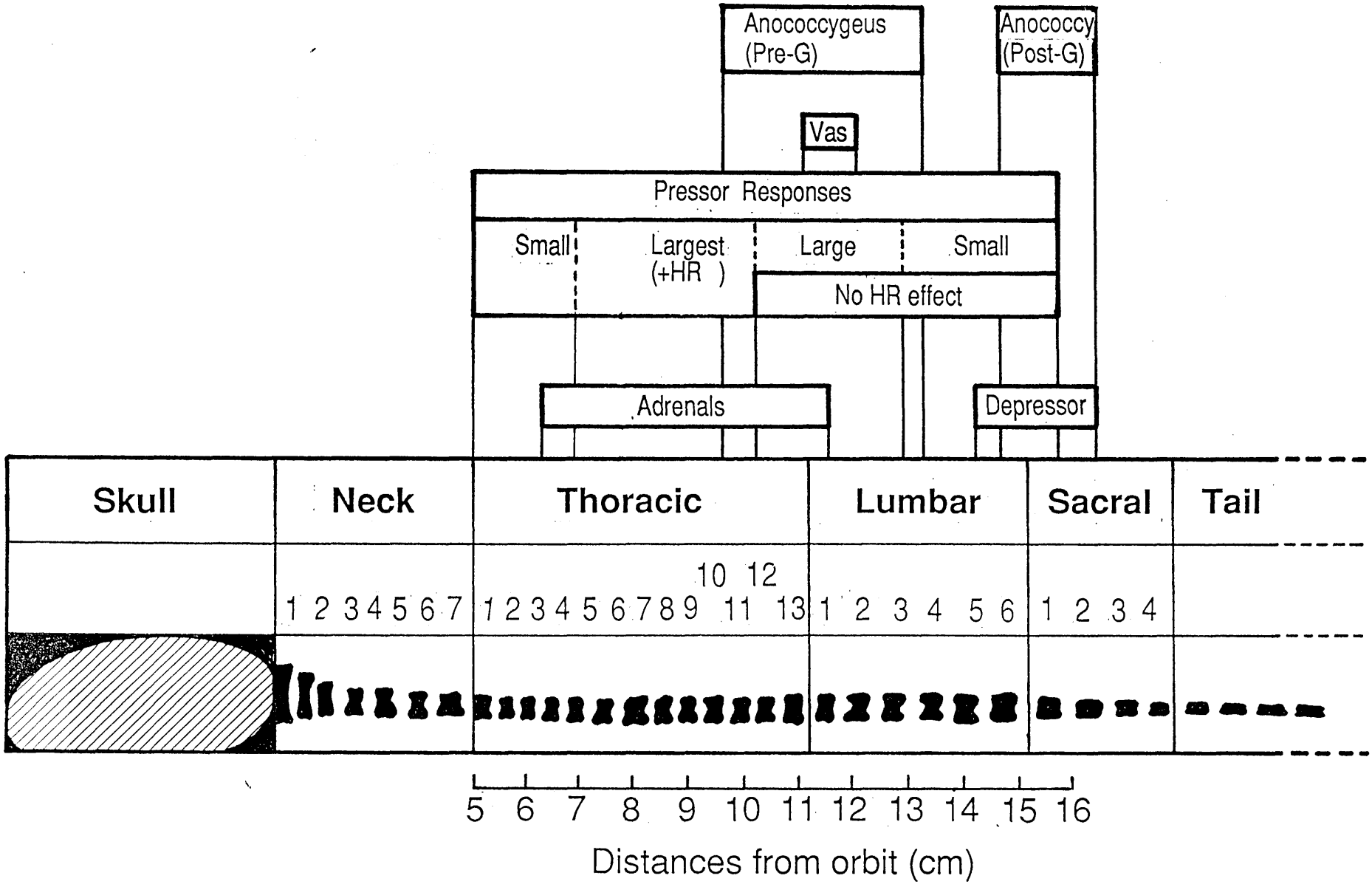
In experiments where the spinal sympathetic outflow was stimulated, the 1cm exposed tip of the pithing rod was used as an electrode. The arrangement of the pithing rod is outlined in Figure 3. It is possible to move the pithing rod with its 1cm exposed tip to positions up and down the spinal column in order to selectively stimulate various organ systems (Figure 5). For example the vas deferens can be selectively stimulated at T_{13} - L_1 (McGrath, 1973), anococcygeus at T_{11} - L_3 and L_6 - S_2 (McGrath,1973), and vasopressor nerves at most points in the region C_6 - S_2.

An indifferent electrode (silver plate 15cm by 6mm) was placed subcutaneously parallel to and extending the full length of the spinal column to limit current spread.

The sympathetic outlow was stimulated using a Grass S88 stimulator or a Square One Instruments stimulator at

Figure 5

This representation of a pithed rat shows the optimum positions for stimulation of different organ systems including the vas deferens, anococcygeus, adrenals and vasculature.


Anococcygeus (Pre-G)
Anococcy (Post-G)
Vas
Pressor Responses
Small
Largest (+HR)
Large
Small
No HR effect
Adrenals
Depressor
Skull
Neck
Thoracic
Lumbar
Sacral
Tail
1 2 3 4 5 6 7
1 2 3 4 5 6 7 8 9 10 11 12 13
1 2 3 4 5 6
1 2 3 4
5 6 7 8 9 10 11 12 13 14 15 16
Distances from orbit (cm)

a supramaximal voltage of 40v and pulse width 0.05ms for 1sec and at a range of frequencies of 1 -20 Hz. Although the pulse width employed produced minimal skeletal muscle twitching, gallamine was added in some experiments to abolish any interference on the recordings which were due to experimental artefact.

The pithing rod electrode was placed at different regions in the spinal column and vasopressor responses were assessed by diastolic pressure changes since systolic measurements are affected by cardiac sympathetic responses, in particular when the electrode was placed C_6 - T_2 where maximal cardioaccelerator responses are obtained (Docherty & McGrath, 1979).

The effects of various antagonists ($alpha_1$, $alpha_2$ and beta-adrenoceptor antagonists as well as purinoceptor antagonists) on the diastolic pressure changes at various areas of sympathetic spinal outflow and at different frequencies were examined. This study was also extended to examine the depressor responses obtained on stimulating the spinal outflow at regions L_5 - S_2.

Sympathetic Nerve Stimulation from the Spinal Outflow -Stimulation of the vas deferens.

Changes in longitudinal isometric tension of the vas deferens to nerve stimulation from the spinal outflow and by field stimulation were measured in some experiments.

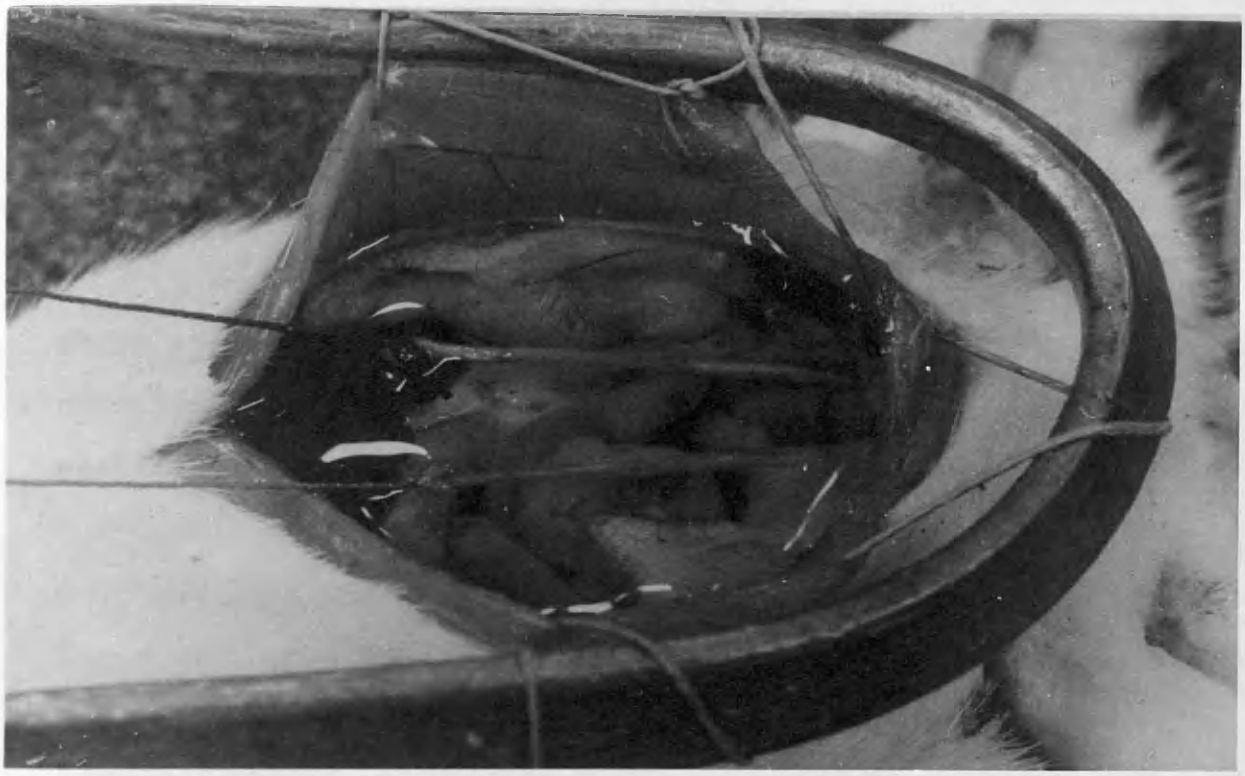
In these experiments, after pithing and carrying out cannulations as described earlier, the abdomen of the rat was opened by a mid-line incision from the sternum to the penis. A bath was then formed by stitching the skin to a brass ring, and was filled with liquid paraffin which kept tissues moist throughout the experiment as well as limiting current spread (Figure 6).

Two parallel ties were placed round the epididymal ends of the vasa deferentia which were cleared of connective tissue before severing the link between them and the epididymis by making an incision between the two ties. These freed epididymal ends of the vasa deferentia were then tied to isometric tension transducers (Grass FT03c) and an initial tension of 1g was applied. The linkage was chosen to be broken at the epididymal ends of the vasa deferentia rather than at the prostatic ends as the nerve and blood supply enter and leave at the prostatic end (Sjostrand, 1965).

Figure 6

Photograph of the vasa deferentia in situ, set up to measure changes in longitudinal isometric tension. The tissues are kept moist in a bath of liquid paraffin.

(From McGrath, 1974).



In the experiments where stimulation was from the spinal sympathetic outflow, the pithing rod electrode was placed at the region T_{13} - L_1 the optimum regions for vas stimulation (McGrath 1973).

It was also possible to place bipolar electrodes on each vasa deferentia allowing responses to post-junctional to be measured and compared to those obtained pre-junctionally by stimulation from the spinal outflow.

The stimulation parameters used in this study were 5Hz / 40v (supramaximal)/ 0.5ms pulse width/ for 1 sec. With this larger pulse width of 0.5ms employed in these experiments, compared to that used in experiments measuring vasopressor nerve mediated responses, it was necessary to administer gallamine (10mg/kg) to prevent skeletal muscle twitching.

The preparation measuring the changes in tension of the vas deferens in vivo proved to be a useful assay for the action of the P_2-purinoceptor antagonist mATP on sympathetic nerve stimulation since it allowed a comparison of its actions on vasopressor mediated responses and on the responses of the vas deferens using the same time course.

The Anaesthetised Cat Preparation

Cats (2.7 - 3.1 kg) starved for 18 hours were anaesthetised initially with a 1:2 O_2/N_2O mixture and 5% halothane and were then given α-chloralose (100mg/kg i.v.) via a vygon tube inserted into the left femoral vein. This dose of αchloralose maintained anaesthesia for the duration of the experiment. Sagatal (1mg/kg i.v.) was administered after surgical preparation in order to prevent spontaneous breathing during respiratory measurements.

Arterial blood pressure was recorded continuously from the right femoral artery with a Bell and Howell pressure transducer. Heart rate was recorded with a Grass tachograph triggered by the arterial pulse. Drugs were administered via a cannula inserted in the right jugular vein and advanced to the entrance of the right atrium to ensure their rapid distribution to the pulmonary circulation. 5HT infusions, where performed, were administered by continuous infusion into the cannulated left femoral vein.

The animals were tracheostomized and artificially ventilated on room air at a constant frequency (30 breaths per minute) and constant volume (20ml/kg) with a Palmer Ideal respiration pump (model 16/24). The left

vagus nerve bundle was isolated, ligated and sectioned before being placed on a bipolar platinum electrode. The cut vagus nerve was kept moist in a saline pool throughout the experiment and was stimulated by a Scientific Research Instruments stimulator at the optimal parameters for stimulation of 25v, 0.5ms pulse width at a frequency of 15hz for 30 seconds to initiate either bronchoconstriction or bronchodilation according to the experimental protocol followed. Body temperature was monitored with a rectal thermometer and maintained at 36° to 39° C by placing the animal on a thermorequlated operating table.

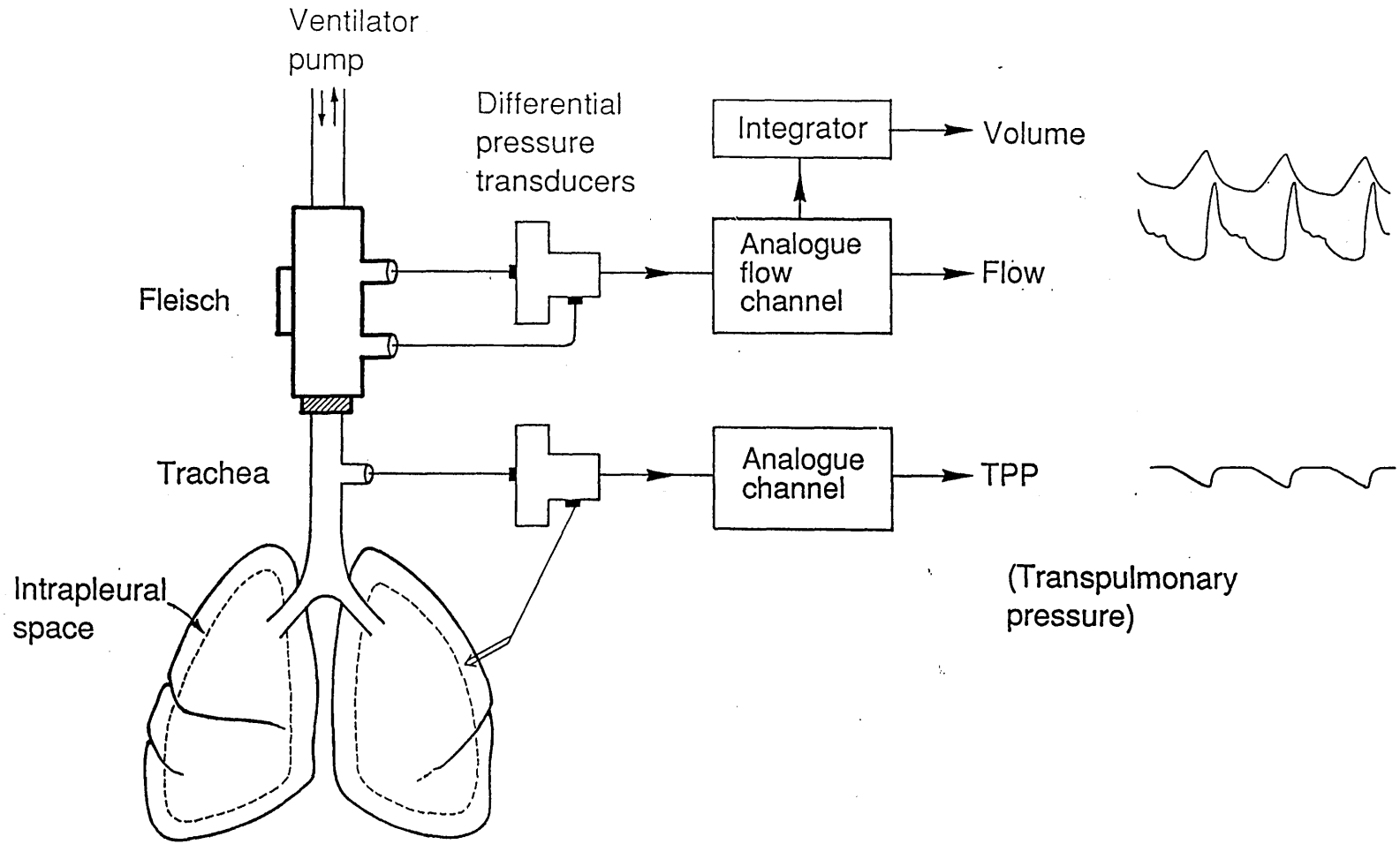
The right vagus remained intact throughout the experiment.

Airflow was determined by a pneumotachograph (Fleish 0) connected to the tracheal cannula and measuring the pressure drop across the device with a differential pressure transducer (Pioden Type). Tidal volume was determined by electrically integrating the airflow signal. Transpulmonary pressure was measured with another differential pressure transducer (Pioden Type) analysing the pressure difference between the tracheal pressure (from a side arm of the tracheal cannula) and intrapleural pressure (from a metal cannula inserted into the intrapleural space at the position of the left fifth intercostal space), as show in Figure 7.

Dynamic lung compliance and airway resistance were computed according to the method of Amdur and Mead (1958). The pressure tending to inflate the lung is the

Figure 7

This representation of the cat lung preparation in situ, illustrates how airflow is monitored from a differential pressure tranducer, which measures the difference in air pressure across the Fleisch tube. Tidal volume is determined by electrically integrating the airflow signal. Transpulmonary pressure (TPP) is monitored via another differential pressure transducer and measures the difference in pressure taken from a side arm of the tracheal cannula and from a needle inserted into the intrapleural space. The resulting traces of signals taken from a Grass Polygraph recorder are displayed.


Ventilator
pump
Differential
pressure
transducers
Integrator
Volume
Analogue
flow
channel
Flow
Fleisch
Trachea
Analogue
channel
TPP
Intrapleural
space
(Transpulmonary
pressure)

transpulmonary pressure (TPP) and comprises an elastic component (which relates to the elastic recoil of the lungs) and a flow- resistive component (which relates to resistance to flow of gas in the lungs and airways and to viscous resistance of the lung tissue).

The elastic and flow-resistive components can be evaluated separately using specific points during the respiratory cycle (Figure 4:2). At points of equal volume in the respiratory cycle, the elastic forces should be approximately equal, therefore the pressure change observed at this point must be related to resistance to flow in the lung and airways. Resistance can thus be calculated.

$$\text{Resistance (cm } H_2O/l/\text{sec)} = \frac{\text{change in pressure}}{\text{change in flow}}$$

Any changes in resistance usually reflect changes in the upper airways (ie. constriction of larger airways makes a bigger difference to the flow signal than constriction in the smaller lower airways).

Bronchoconstriction is usually reflected by an increase in resistance and a decrease in compliance. If

no increase in resistance is observed then we can say that the agent causing the bronchoconstriction must be acting in the lower peripheral airways.

To calculate compliance (a measure of elasticity) (Figure 4:2), pressure is measured at points at the beginning and at the end of inspiration when the flow-resistive component drops out (ie. no air being moved in or out), then at this point

$$\text{Dynamic lung compliance (mls/cm } H_2O) = \frac{\text{Tidal volume}}{\text{Change in pressure}}$$

Changes in compliance relate to changes in lower airways, alveoli and in pulmonary circulation etc. Hence compounds such as 5HT, that on bronchoconstriction cause an increase in resistance and a decrease in compliance, we can then say that it affects both lower and upper airways.

Blood Gas Analysis

Arterial blood samples (0.2ml) were extracted from the carotid arterial cannula into a heparinised syringe, kept airtight and directly inserted into the blood gas analyser IL 213 (for the rabbits and rats) and Radiometer ABL3 (for the cats) to measure blood gas

tensions. Blood was sampled both at the beginning and at the end of most experiments (except in the cases of the cat and rabbit experiments, where samples were taken approximately every 20 minutes) to see if the arterial blood gas tension had altered during the experiment. For the pithed animals no difference was noted but in the anaesthetised cats which were bronchoconstricted by infusing 5HT, constant monitoring was required and corrections were made to HCO_3- levels when the preparation tended towards acidosis.

Physiological arterial blood gas tensions (values of approximately pH 7.38 / Pa CO_2 40 mmHg/ Pa 100 mmHg) were obtained in the pithed rat by ventilating at a rate of 60 strokes per min. and at a volume of 1ml per 100g body weight per stroke (or 600ml/kg/min) (Clanachan & McGrath, 1976). The ventilatory parameters used in the pithed rabbit to attain these values were 30 strokes per min and a volume of 50ml (approximately 15ml/kg) per stroke (or 450ml/kg/min). For the anaesthetised cat the parameters used were 30 strokes per min and a volume of 20ml/kg per stroke (or 600ml/kg/min). When used in the cat with low resting airway smooth muscle tone (i.e. before 5HT infusions) these parameters produced physiological blood gas tensions within the normal range.

Statistics

The means and standard error of the means for the groups of experiments contained in this study, were calculated and the means were compared using a paired Student's t-test (MacIntosh software 'Statswork'). P values of 0.05 or less were considered to be significant and marked on a scale comprising * to *** , where,

* = $P < 0.05$

** = $0.001 < P < 0.01$

*** = $P < 0.001$

n is the number of preparations refered to in a particular study.

Drugs

The following drugs were used in this study :

Acetylcholine bromide (Sigma), Adenosine 5'-triphosphate (ATP) (Sigma), Althesin (Glaxo), Angiotensin I (Sigma), Angiotensin II (hypertensin) (Ciba), Atropine sulphate (B.D.H.), Captopril (Squibb), 2-Chloroadenosine (2-ChlAd) (Sigma), Dipyridamole (Boeringer Ingelheim), Enalapril (MK-421) (Merck Sharp & Dohme), Flurbiprofen (Boots), Gallamine Triethiodide (Flaxedil) (May &

Baker), Halothane (Fluothane) (ICI), 5-hydroxytryptamine creatine sulphate (5-HT) (Sigma), α,β-Methylene adenosine 5'-triphosphate (m ATP) (Sigma), Nifedipine (Bayer), Noradrenaline (L-arterenol bitartrate) (NA) (Sigma), Phentolamine mesylate (Ciba), 8-Phenyltheophylline (8-PT) (Calbiochem), Prazosin HCl (Pfizer), Propranolol HCl (Sigma), Rauwolscine (Roth), Saralasin ([sar^1,ala^8]-angiotensin II) (Sigma), Teprotide (SQ 20,881) (Squibb), UK 14304 tartrate (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline) (Pfizer), Wy 26703 (N-methyl-N-(1,3,4,6,7,11b -hexahydro-2H-benzo-[a]-quinolizin-2 -yl)-i-butanesulphonamide HCl (Wyeth).

All drugs were dissolved in 0.9% saline except flurbiprofen, mATP, prazosin, and rauwolscine which were all dissolved in distilled water. Nifedipine was dissolved in PEG and ethanol (1:4) and 8-PT in PEG and Na OH, before being diluted down in distilled water.

All drug concentrations are expressed in terms of the salt.

SECTION I

THE RELATIVE CONTRIBUTION OF THE ALPHA-ADRENOCEPTOR SUBTYPES TO PRESSOR RESPONSES TO SYMPATHOMIMETC AGONISTS: A COMPARISON OF THE POTENCIES OF ALPHA$_2$-ADRENOCEPTOR AGONISTS AND ANTAGONISTS BETWEEN THE PITHED RABBIT AND RAT.

Introduction

Recently it has been suggested that the alpha$_2$-adrenoceptor sub-type may have different pharmacological properties in different species (Nahorski *et al*, 1985; Waterfall *et al*, 1985; Alabaster *et al*, 1985, 1986). For example the pre-junctional alpha$_2$-adrenoceptors in vas deferens of rat and rabbit had a different antagonist potency series, yohimbine being more potent than Wy 26703 in the rabbit but less potent in the rat. It was subsequently found that that the potency of each antagonist at post-junctional alpha$_2$-adrenoceptors in the rabbit saphenous vein was similar to that found at the pre-junctional alpha$_2$-adrenoceptors in rabbit vas deferens (Alabaster *et al*, 1986).

A comparison of the post-junctional alpha$_2$-adrenoceptors which mediate pressor responses was carried out in the pithed rat and rabbit since this type of preparation has been used extensively in the basic classification and investigation of alpha-adrenoceptor sub-types (Drew & Whiting, 1979; Docherty & McGrath, 1980). In order to keep conditions as similar as possible between the two species, the physiological agonist noradrenaline was employed in the presence of the alpha$_1$-adrenoceptor antagonist prazosin. The effects of rauwolscine and Wy 26703 were then compared.

When comparing properties of alpha$_2$-adrenoceptors in the vasculature of rat and rabbit, dose/diastolic carotid arterial pressor response curves to NA were constructed. The alpha$_2$-adrenoceptor mediated response was isolated by the addition of prazosin (1 mg/kg) which blocked the alpha$_1$-adrenoceptor mediated response of NA. The effects of 2 alpha$_2$-adrenoceptor antagonists, rauwolscine and Wy 26703, on the alpha$_2$-adrenoceptor mediated response, were tested in each species.

Results

Control experiments

(i) Rabbit. In an earlier study of the pithed rabbit (McGrath & Mackenzie, 1977) it was demonstrated that the cardiac output, heart rate, peripheral resistance and arterial blood pressure remained constant over a 6 hour period during which reproducible responses to a variety of reversible agonists and to sympathetic nerve stimulation were obtained. In the present study a short series of three experiments was carried out in which dose-response curves to noradrenaline were repeated 8 to 10 times over a 5 to 6 hour period and it was found that responses were reproducible. In the protocol for the first series of experiments (results in figures 1:1 and 1:2) four consecutive dose/response curves to noradrenaline were carried out to ensure

viability of the preparation and reproducibility before testing the effects of antagonists. In the second set of experiments (results in figures 1:3 and 1:4) a single control curve was obtained before proceeding to examine the effects of antagonists.

(ii) Rat. In the pithed rat, four consecutive dose/response curves to noradrenaline were obtained. In contrast to the rabbit, in which the relatively small additions to plasma volume had no effect on the resting cardiovascular system, in the rat the diastolic blood pressure remained relatively constant but the pulse pressure increased throughout the experiment. This was presumably due to an increase in plasma volume and hence an increase in cardiac output. However, this had no significant effect on the pressor responses to noradrenaline measured as increases in diastolic pressure.

On the basis of these control experiments it is reasonable to assume that shifts in dose/response curves produced by antagonists reflect the action of the antagonists and not time-related decreases in sensitivity of the preparations.

Pharmacological properties of vascular alpha-receptors in the pithed rabbit-
Effects of antagonists on the responses to alpha-adrenoceptor agonists and to sympathetic nerve stimulation

Noradrenaline in the range 10ng/kg-10µg/kg produced dose related pressor responses in the pithed rabbit (Figure 1:1a&b & Figure 1:3c&d).

Prazosin (10µg/kg-1mg/kg), given on its own, produced a dose-related rightward shift in the NA dose/response curve. Figure 1:1a shows this for prazosin (1 mg/kg). This shift was not parallel and resulted in a shallower gradient for the NA dose/response curve, i.e. prazosin was more effective at higher doses of NA than at the lower dose range (Figure 1:1a&b).

Rauwolscine (100µg/kg-1mg/kg) (Figure 1:1a&b) or imiloxan (10mg/kg) (Fig.1:2a&b) given on their own, produced small rightward shifts of the NA dose/response curve. Rauwolscine was more potent than imiloxan and shifted the curve further. Given after prazosin (1mg/kg) each of these alpha$_2$-adrenoceptor antagonists produced a further rightward shift, greater than in the absence of prazosin. Combined alpha$_1$- and alpha$_2$- adrenoceptor blockade caused a parallel rightward shift in the dose-response curve.

FIGURE 1:1

The effects of sequential administration of antagonists on pressor responses in the pithed rabbit to a) noradrenaline b) noradrenaline on an expanded scale c) phenylephrine and d) sympathetic nerve stimulation (T8, 20 pulses, via the pithing rod) .

Propranolol 1mg/kg was present throughout. Control responses (▽) were obtained and repeated after prazosin then 3 increasing doses of rauwolscine (left panels) or rauwolscine then prazosin (right panels). Symbols indicate the last drug administered.

Prazosin 1mg/kg ▲

Rauwolscine 100μg/kg ○

Rauwolscine 300μg/kg □

Rauwolscine 1mg/kg △

Responses are shown as mean ± standard error of the mean. I-bars are omitted where these are smaller than the symbols or, in (b), for clarity. n=6

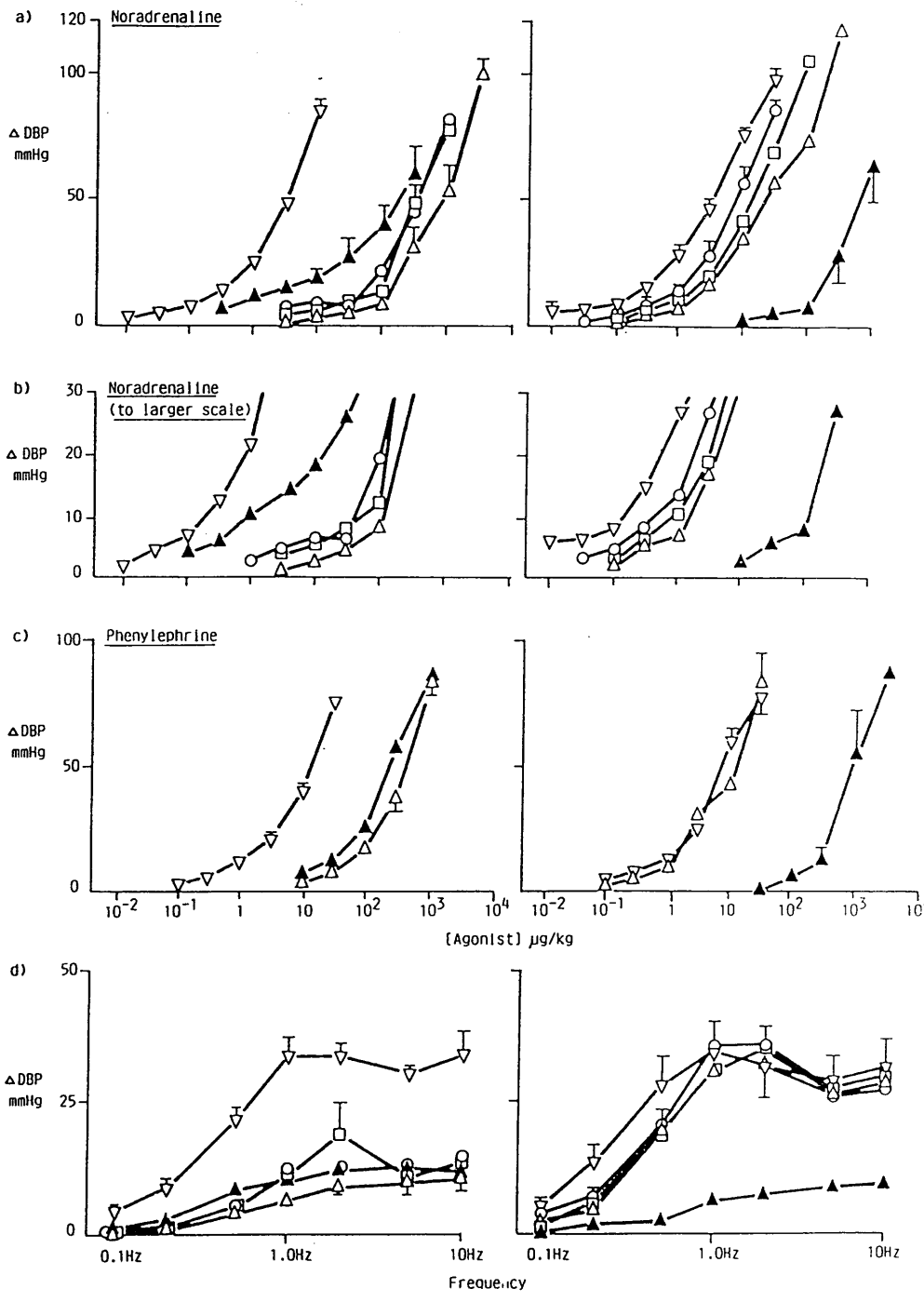

a)
Noradrenaline
120
100
50
0
Δ DBP
mmHg
b)
Noradrenaline
(to larger scale)
30
20
10
0
Δ DBP
mmHg
c)
Phenylephrine
100
50
0
Δ DBP
mmHg
10⁻²
10⁻¹
1
10
10²
10³
10⁴
[Agonist] μg/kg
d)
50
25
0
Δ DBP
mmHg
0.1Hz
1.0Hz
10Hz
Frequency

FIGURE 1:2

The effects of sequential administration of antagonists on pressor responses in the pithed rabbit to a) noradrenaline b) noradrenaline on an expanded scale and c) sympathetic nerve stimulation via the pithing rod (1cm electrode, T8, 20 pulses).

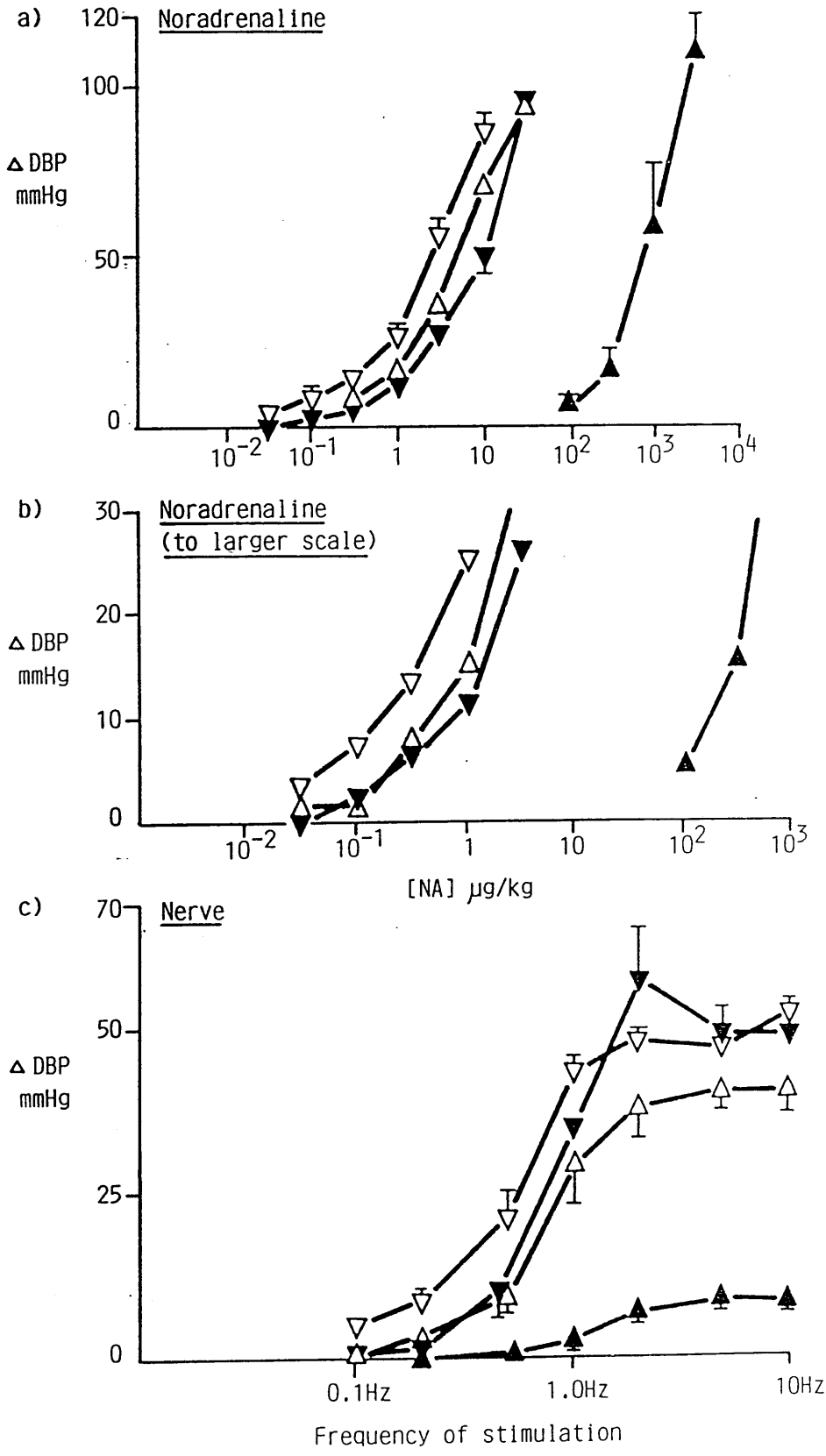
Propranolol 1mg/kg was present throughout. Control responses (▽) were obtained and repeated after a sequence of 3 increasing doses of imiloxan at concentrations of 1mg/kg (not shown), 3mg/kg (not shown) and finally 10mg/kg (as shown).

Prazosin 1mg/kg ▲

Rauwolscine 1mg/kg ▼

Imiloxan 10mg/kg △

Responses are shown as mean ± standard error of the mean. I-bars are omitted where these are smaller than the symbols or, in (b), for clarity. n=6


a)
Noradrenaline
120
100
50
0
Δ DBP
mmHg
10-2
10-1
1
10
102
103
104
b)
Noradrenaline
(to larger scale)
30
20
10
0
Δ DBP
mmHg
10-2
10-1
1
10
102
103
[NA] µg/kg
c)
Nerve
70
50
25
0
Δ DBP
mmHg
0.1Hz
1.0Hz
10Hz
Frequency of stimulation

Phenylephrine produced dose-related pressor responses in the range 100ng/kg-30µg/kg (Figure 1:1c). Prazosin shifted the dose-response curve to phenylephrine to the right. Subsequent addition of rauwolscine (0.1 and 0.3 mg/kg) had no effect and rauwolscine (1 mg/kg) produced only a further small shift to the right. Rauwolscine given on its own had no effect on the phenylephrine dose-response curve and had no influence on the effectiveness of prazosin.

Guanabenz (1µg/kg-300µg/kg) produced dose-related pressor responses (Figure 1:5a). Rauwolscine significantly reduced responses to guanabenz but tachyphylaxis to guanabenz made accurate assessment of blockade impossible.

Pressor nerve stimulation (T8, 20 pulses, 0.1-10Hz) produced frequency related responses which reached a plateau at 1Hz (Figures 1:1d & 1:2c). Prazosin, rauwolscine and imiloxan were all effective antagonists at some point on this frequency-response curve but to different degrees at different frequencies. When given first, rauwolscine and imiloxan were more effective against low frequency nerve stimulation but did not reduce responses to higher frequencies. Prazosin was effective in reducing responses at both low and high frequencies, but between 1Hz and 10Hz a residual component remained. The combination of prazosin and

rauwolscine, or, imiloxan with rauwolscine and prazosin (each at 1mg/kg), produced a greater inhibition than did prazosin alone but did not produce a complete block.

Angiotensin 11 (AII) and Angiotensin 1 (AI) at 1µg/kg and 10µg/kg produced dose related pressor responses in the rabbit. To investigate whether endogenous AII is necessary for the production of the response mediated by $alpha_2$-adrenoceptors, the effects of angiotensin converting enzyme inhibitors (ACE-inhibitors) on the pressor responses to agonists in the pithed rabbit were also studied in a small number of preparations. Results with enalopril are shown in Figure 1:7. The ACE-inhibitors teprotide, captopril and enalapril (each at 0.1mg/kg) all reduced the pressor response to AI (1µg/kg) by approximately 80%. Peak responses to NA (100ng/kg-10µg/kg), the $alpha_1$-adrenoceptor agonist phenylephrine (100ng/kg-30µg/kg) and to the $alpha_2$-adrenoceptor agonist UK14304 (1µg/kg-30µg/kg) were unaffected by any of the ACE-inhibitors, as were the late phases of these responses. In the pithed rat, peak responses to NA also were unaffected at this dose of teprotide. However the late phase of the NA response was significantly reduced (Grant & McGrath, 1984).

Comparison of alpha$_2$-adrenoceptor-mediated pressor responses to NA in the rat and rabbit.

Prazosin (1mg/kg) in the rat produced a parallel rightward shift in the NA dose/response curve (Figure 1:3 a & b). In the rabbit, prazosin (1mg/kg) produced a greater inhibition against higher doses of NA than against lower doses, thus leaving a shallower dose/response curve to NA (Figure 1:3c & d).

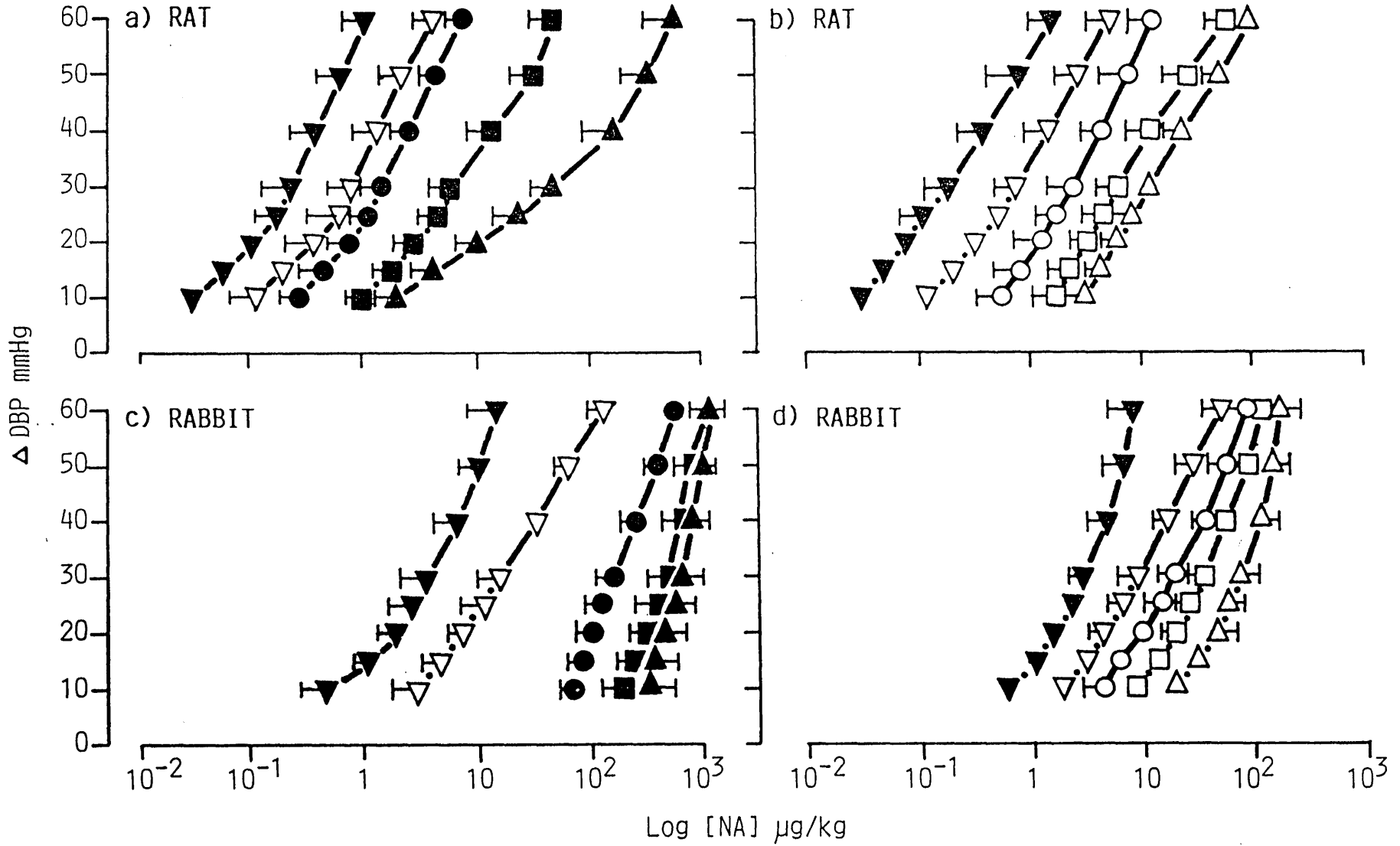
In either species (Figure 1:3) given after prazosin, rauwolscine and Wy 26703 produced a further rightward shift. For Wy 26703 the shifts were approximately parallel. In the rabbit, rauwolscine produced a greater inhibition against lower doses of NA than against higher doses. In the rat, the higher doses of rauwolscine (3mg/kg) tended to reduce the slope of the curve indicating a non-specific action of rauwolscine. At these doses, no significant pressor responses were produced by the antagonists.

These shifts were expressed as dose ratios (the dose of NA required to produce a standard response in the presence of the alpha$_2$-adrenoceptor antagonist as well as prazosin, divided by the dose of NA required to produce the same response in the presence of prazosin only). Dose ratios were measured at changes in diastolic blood pressure (DBP) of between 10 and 60 mmHg. For the illustration of alpha$_2$-adrenoceptor

FIGURE 1:3

The effects of sequential administration of blocking agents on the NA pressor/response curve in the pithed rat (a&b) and pithed rabbit (c&d). For this figure log dose-response curves were drawn as in Figures 1:1 and 1:2 for individual rabbits and the doses to produce responses of various sizes from 10 to 60mmHg were interpolated. The means of the doses for each response were then plotted with their s.e.m.s. I-bars are omitted where these are smaller than the symbols. $n=5$

Order of administration	Key
1) Propranolol 1mg/kg	▼
2) Prazosin 1mg/kg	▽
3) Rauwolscine 300µg/kg	●
or Wy 26703 300µg/kg	○
4) Rauwolscine 1mg/kg	■
or Wy 26703 1mg/kg	□
5) Rauwolscine 3mg/kg	▲
or Wy 26703 3mg/kg	△


a) RAT
b) RAT
c) RABBIT
d) RABBIT
Δ DBP mmHg
60
50
40
30
20
10
0
10^{-2}
10^{-1}
1
10
10^{2}
10^{3}
Log [NA] µg/kg

antagonist potency the graph of dose ratio against alpha$_2$-adrenoceptor antagonist concentration at a change in diastolic blood pressure of 10 mmHg showed the greatest shift, indicating that at these lower pressor changes the alpha$_2$-adrenoceptor-mediated component dominates.

At a change in DBP of 10 mmHg (Figure 1:4a) absolute antagonist potency and order of potency were found to differ between the species. In the rat rauwolscine and Wy 26703 were equipotent. In the rabbit rauwolscine was more potent than Wy 26703. Rauwolscine was more potent in the rabbit than in the rat. Wy 26703 was more potent in the rat than in the rabbit (Results were analysed by Student's paired t-test and results with $p>0.05$ were considered to be non-significant).

At 20 mmHg change in DBP (Figure 1:4b) rauwolscine and Wy 26703 were still equipotent in the rat. In the rabbit rauwolscine was again more potent than Wy 26703. The order of potency of antagonists was the same as that found at a change in DBP of 10 mmHg.

At 30 mmHg change in DBP (Figure 1:4c) rauwolscine was more potent than Wy 26703 in both species. At lower antagonist concentrations rauwolscine was more potent in the rabbit than in the rat, but at higher concentrations rauwolscine was more potent in the rat. Wy 26703 was more potent in the rat than in the rabbit.

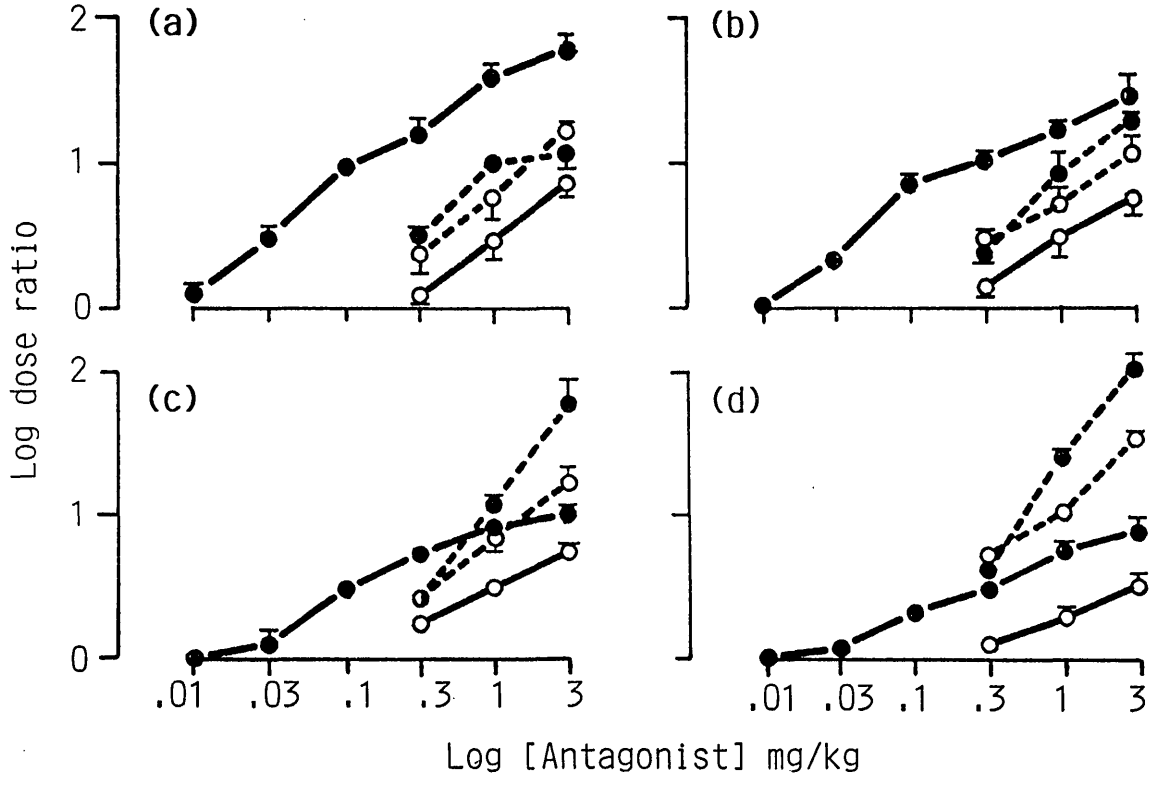
FIGURE 1:4

Log. dose ratio against log.[antagonist] illustrating antagonist potency at changes in DBP of a) 10mmHg b) 20mmHg c) 30mmHg and d) 60mmHg.

Rauwolscine in rat--●--or rabbit- -●-

Wy 26703 in rat---○-or rabbit- -○-

n=5-6


(a)
(b)
(c)
(d)
2
1
0
Log dose ratio
.01
.03
.1
.3
1
3
Log [Antagonist] mg/kg

At 60 mmHg change in DBP (Figure 1:4d) rauwolscine again, was more potent than Wy 26703 in both species. Both antagonists were more potent in the rat than in the rabbit. It seems that in the rabbit high doses of NA produce responses characterised by a relatively low dose-ratio to alpha$_2$-adrenoceptor antagonists.

Comparing pressor responses mediated by alpha$_1$-adrenoceptors and alpha$_2$-adrenoceptors to selective agonists in two species.

Alpha 1-adrenoceptors

In the rabbit, the alpha$_1$-adrenoceptor agonist phenylephrine (Figure 1:1c) in the range 100ng/kg-30µg/kg produced dose-related but short lived pressor responses giving peak responses comparable to those found with NA as the agonist (Figure 1:1a), although the NA response was longer lasting.

In the rat (Figure 1:5), the pressor effects of phenylephrine were found to be very short lived in comparison with those of NA, but were of a comparable height. In both species prazosin (1mg/kg) produced a large shift in the phenylephrine dose/response curve while the alpha$_2$-adrenoceptor antagonist, rauwolscine

FIGURE 1:5

Pressor responses to a) guanabenz and b) phenylephrine in the pithed rat and rabbit in the presence of propranolol (1mg/kg). n=5

a) ○ - guanabenz in the rat; ◇ - guanabenz in the rabbit

b) △ - phenylephrine in the rat (with rauwolscine 1mg/kg ▲)

□ - phenylephrine in the rabbit (with rauwolscine 1mg/kg ■)

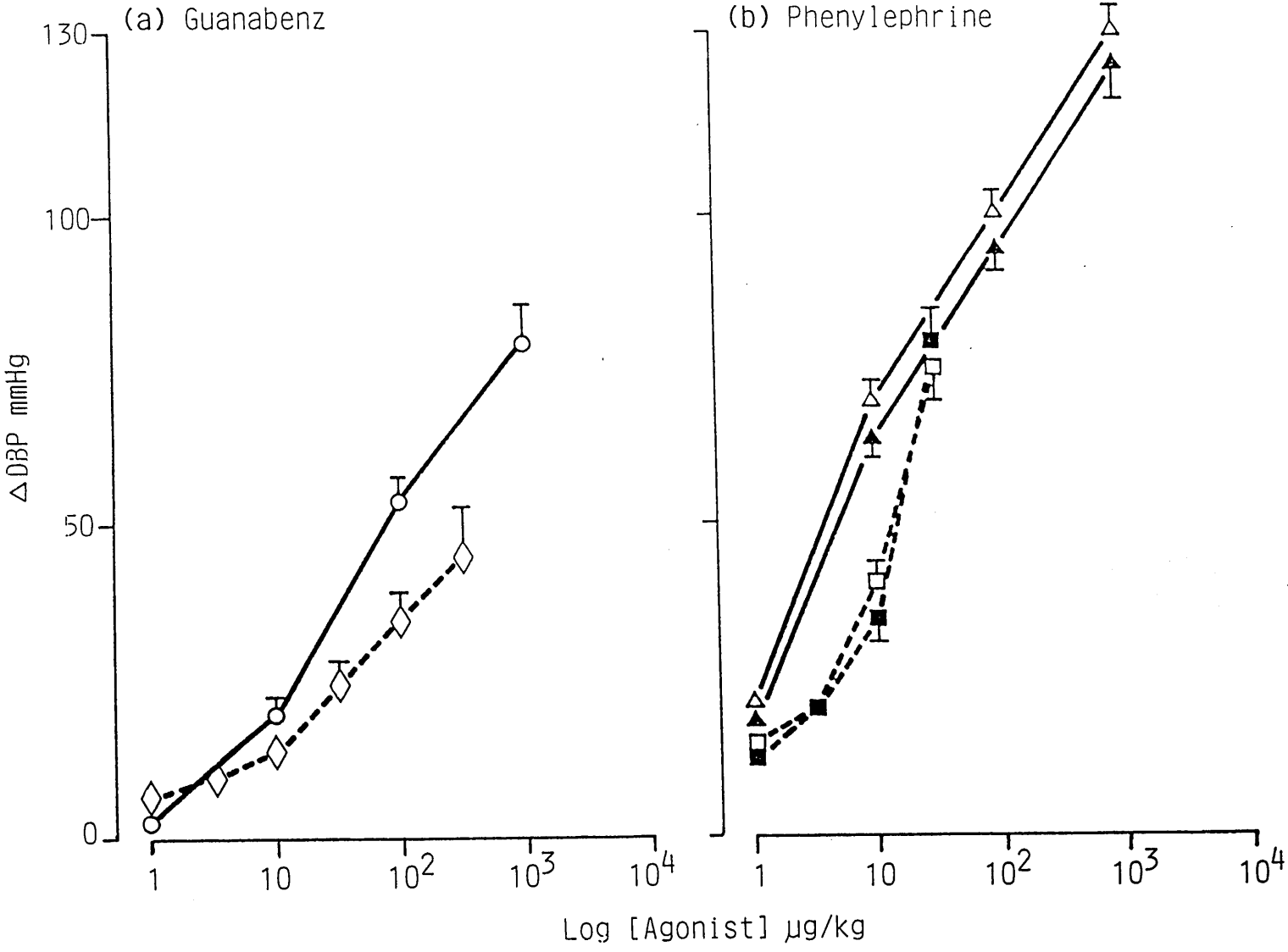
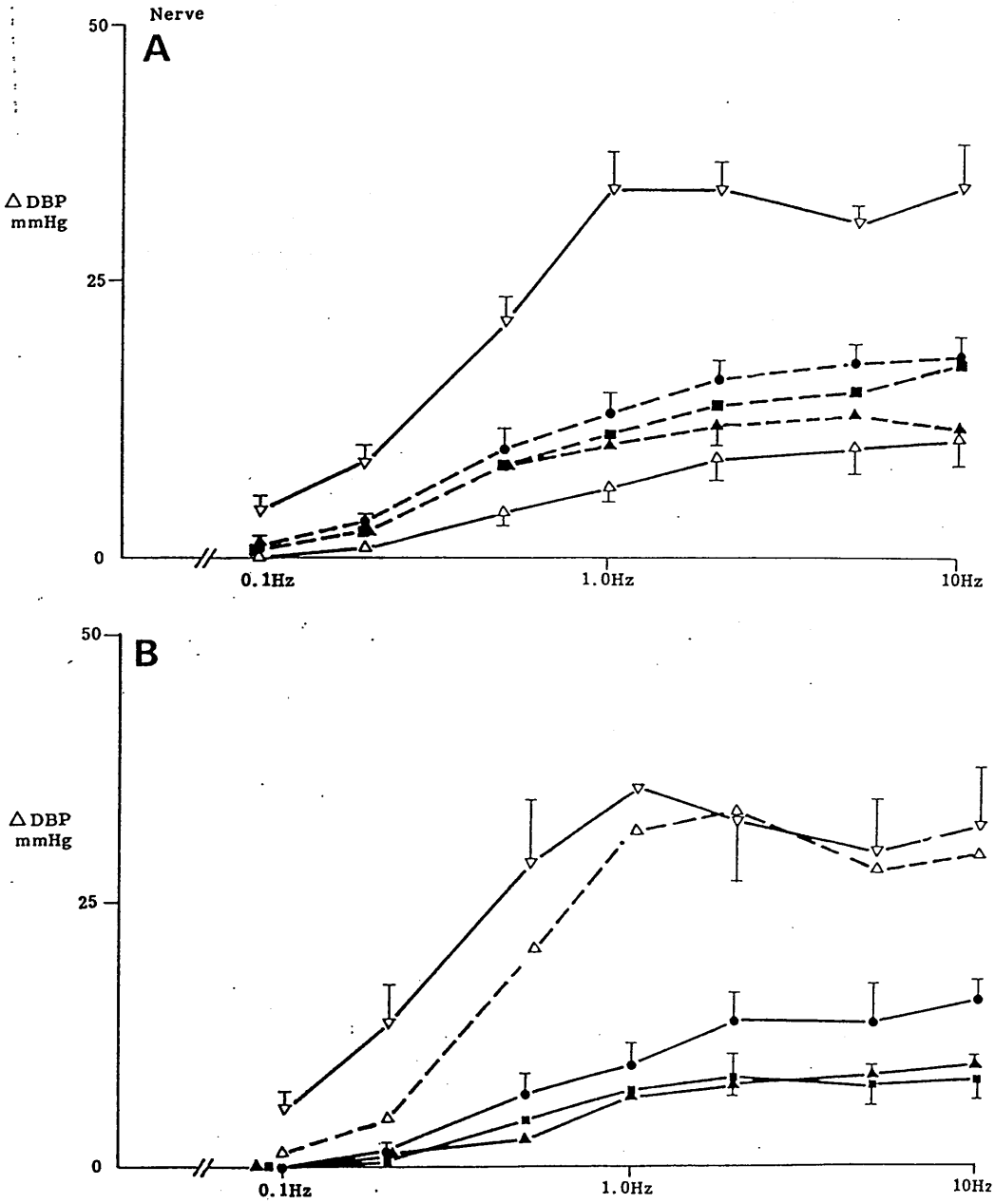

(a) Guanabenz
(b) Phenylephrine
130
100
50
0
ΔDBP mmHg
1
10
10²
10³
10⁴
Log [Agonist] µg/kg

FIGURE 1:6

The effects of sequential administration of antagonists on pressor responses in the pithed rabbit to sympathetic nerve stimulation (T8, 20 pulses, via the pithing rod).
Propranolol 1mg/kg was present throughout. Control responses (▽) were obtained and repeated after 3 increasing doses of prazosin followed by rauwolscine (panel A) or rauwolscine then 3 increasing doses of prazosin (Panel b). Symbols indicate the last drug administered.

Prazosin 100µg/kg ●
Prazosin 300µg/kg ■
Prazosin 1mg/kg ▲
Rauwolscine 1mg/kg △

Responses are shown as mean ± standard error or the mean. I-bars are omitted where these are smaller than the symbols. n=6


Nerve
A
50
25
0
△DBP
mmHg
0.1Hz
1.0Hz
10Hz
B
50
25
0
△DBP
mmHg
0.1Hz
1.0Hz
10Hz

(1mg/kg) left the responses virtually unaltered.

Phenylephrine was 3 times more potent in the rat than in the rabbit when measuring control peak pressor responses. This ratio is similar to that of NA after $alpha_2$-adrenoceptor blockade (Table 1:1).

Alpha 2-adrenoceptors

In the rabbit, the $alpha_2$-adrenoceptor agonist guanabenz (Figure 1:5a) in the range 1µg/kg-300µg/kg produced dose-related diastolic pressor responses. Another $alpha_2$-adrenoceptor agonist, UK14304, produced dose related pressor effects which reached a similar plateau of 60mmHg at 100µg/kg (Figure 1:7).

In the rat, guanabenz (Figure 1:5) within the range 100ng/kg-100µg/kgproduced dose-related pressor responses. Guanabenz was twice as potent in the rat than in the rabbit (Table 1:1).

The $alpha_2$-adrenoceptor potency of NA is assessed as responses after 1mg/kg prazosin, as illustrated in Table 1:1, and was 25 times more potent in the rat.

Figure 1:7

The effect of the ACE-inhibitor enalapril (0.1mg/kg) and sequentially administered alpha$_1$-antagonist prazosin (1mg/kg), on vasopressor responses produced by: Ang I (1µg/kg); Ang II (1-10µg/kg); NA (1-10µg/kg); phenylephrine (1-10µg/kg); and UK 14304 (3-100µg/kg) in the pithed rabbit.

(n=3)

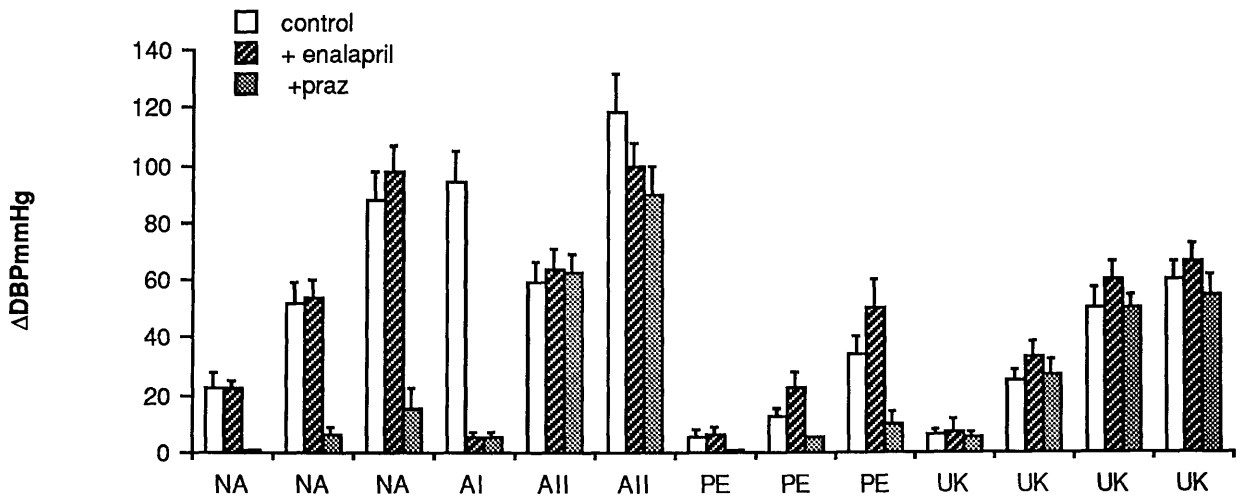

control
+ enalapril
+praz
ΔDBPmmHg
140
120
100
80
60
40
20
0
NA
NA
NA
AI
AII
AII
PE
PE
PE
UK
UK
UK
UK

Table 1:1

	RAT	RABBIT	RATIO RABBIT/RAT
ED_{20} NA (α_1)	0.3 µg/kg	7.0 µg/kg	23.8
ED_{20} PE	1.0 µg/kg	3.0 µg/kg	3.0
Molar ratio of ED_{20} NA/PE	0.47	3.6	
ED_{20} NA (α_2)	0.3 µg/kg	7.0 µg/kg	25.0
ED_{20} Guanabenz	10.0 µg/kg	20.0 µg/kg	2.0
Molar ratio of ED_{20} NA/G	0.04	0.5	
Molar ratio of ED_{20} NA (α_1)/NA (α_2)	1.0	1.0	

The potency of alpha-adrenergic agonists (phenylephrine (PE), guanabenz, NA in the presence of either prazosin (NA (α_2)) or rauwolscine (NA (α_1))) in the pithed rat and rabbit measured in the presence of propranolol (1mg/kg). ED_{20} values are the doses of the agonist required to produce changes in diastolic blood pressure of 20 mmHg. Figures are given in µg/kg.

Table 1:2

	RAT	RABBIT	RATIO RABBIT/RAT
Rauw.	0.3	0.03	0.1
Wy26703	0.4	1.2	3
RATIO Wy/Rauw.	1.3	40	

The potency of the alpha$_2$ antagonists rauwolscine and Wy 26703 measured at a change in diastolic blood pressure of 10mmHg, where potency is taken as the dose of antagonist (mg/kg) required to give a 5 fold shift in the NA dose-response curve. In the rabbit rauwolscine is 10 times more potent than in the rat, whereas in the rat Wy 26703 is 3 times more potent than in the rabbit.

SECTION II

AN INVESTIGATION OF PURINERGIC CO-TRANSMISSION IN SYMPATHETIC VASCULAR CONTROL AND IN THE VAS DEFERENS MOTOR RESPONSE.

It has been proposed that cotransmission involving adrenergic and purinergic elements may occur in some vascular smooth muscle (Burnstock and Sneddon,1984) and further evidence for this phenomenon has since been reported in vitro (Byrne & Large, 1986). There is evidence suggesting that this occurs also in vivo. In the pithed rat, the P_2-purinoceptor desensitising agent, mATP attenuated pressor responses to sympathetic nerve stimulation after blockade of alpha receptors but not in the absence of alpha-blockade (Flavahan et al, 1985;Grant et al, 1985). This seemed to suggest a relatively minor role for purinergic co-transmission in rat vasculature.

In the present study the rats were pithed under halothane anaesthesia, were artificially ventilated with 40% oxygen and 60% nitrogen and were given gallamine (10mg/kg,i.v.) to stop skeletal muscle twitching and propranolol (1mg/kg, i.v.) to eliminate vasodilation due to catecholamines released from the adrenal medulla ('endogenous adrenaline reversal'). Drugs were administered via the right external jugular vein. Right carotid arterial blood pressure and heart rate, derived electronically from this signal, were monitored continuously. Diastolic pressor responses to sympathetic nerve stimulation via the pithing rod (1cm electrode,T6-T8,1sec,1-20Hz) were measured.

A COMPARISON OF AGONIST-INDUCED AND NERVE-MEDIATED RESPONSES.

Nerve mediated pressor responses

Stimulation of the spinal sympathetic outflow (T_1 - L_4) via the pithing rod electrode (1cm tip exposure) evoked pressor responses. The size and shape of these responses depended on position of stimulation. The largest and most reproducible vasopressor responses with minimal cardioaccelerator effects were obtained when the pithing rod electrode tip was positioned between T_6 and T_8 (Figure 1:5 & 2:1) but stimulation of any area within the region T_1- L_4 produced pressor responses. The vasopressor responses were frequency dependent (Figure 2:2). Flavahan et al (1985) showed that when the stimulating electrode is placed between regions T_3 and L_4, the resulting pressor response is biphasic and comprises a direct pressor component and a delayed pressor component due to stimulation of the adrenals.

Noradrenaline

The intravenous administration of NA (10ng/kg - 1mg/kg) resulted in biphasic pressor responses (Figure 2:3). The pressor responses were dose dependent (Figure

Figure 2:1

Vasopressor responses obtained to stimulation at different spinal positions at 20Hz frequency, 0.05ms pulse width, 40v, 1 cm electrode, for 1sec.

n = 4-8

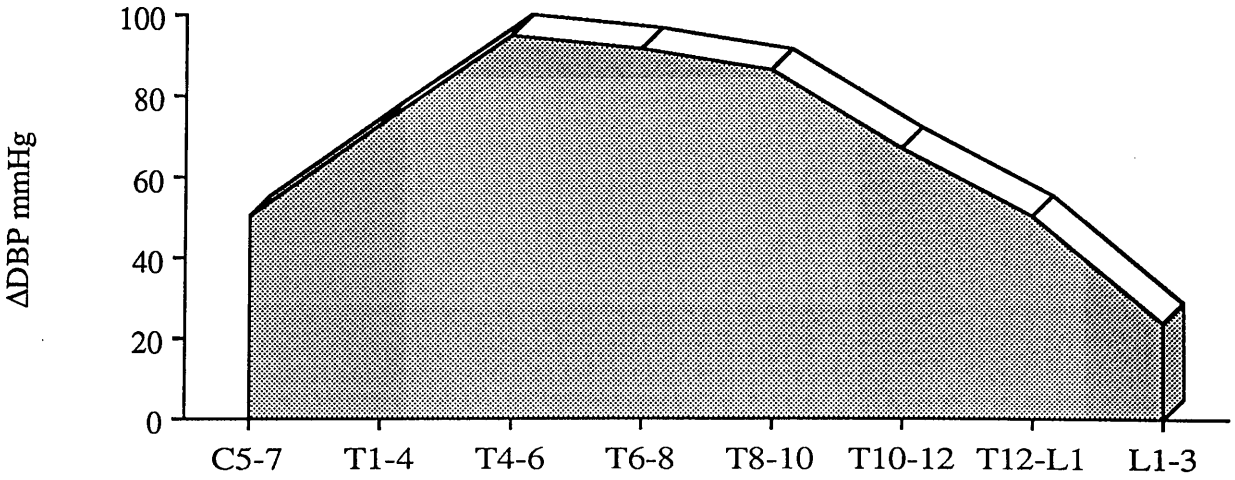

ΔDBP mmHg
100
80
60
40
20
0
C5-7
T1-4
T4-6
T6-8
T8-10
T10-12
T12-L1
L1-3

Figure 2:2

Responses (changes in diastolic blood pressure) to stimulation of the sympathetic spinal outflow between regions T_6 - T_8 at frequencies 1 - 20Hz, for 1 sec, were measured in: control conditions (propranolol (1mg/kg) & gallamine (10mg/kg)(□)); in the presence of prazosin (0.1mg/kg(◆), 0.3mg/kg(◘),and 1mg/kg(◈)) and in the presence of rauwolscine (1mg/kg(■)). The antagonists were added sequentially. n=4.

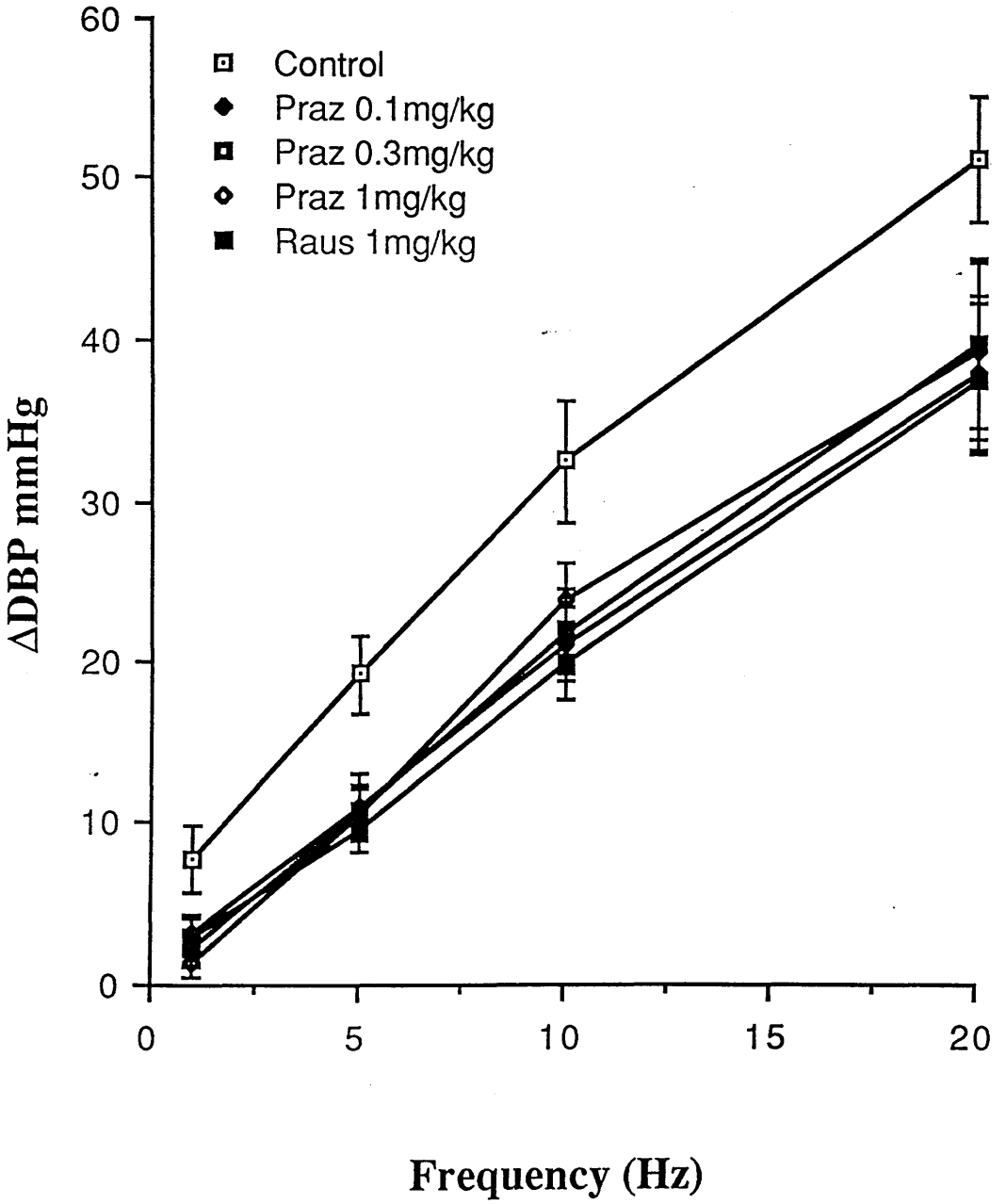
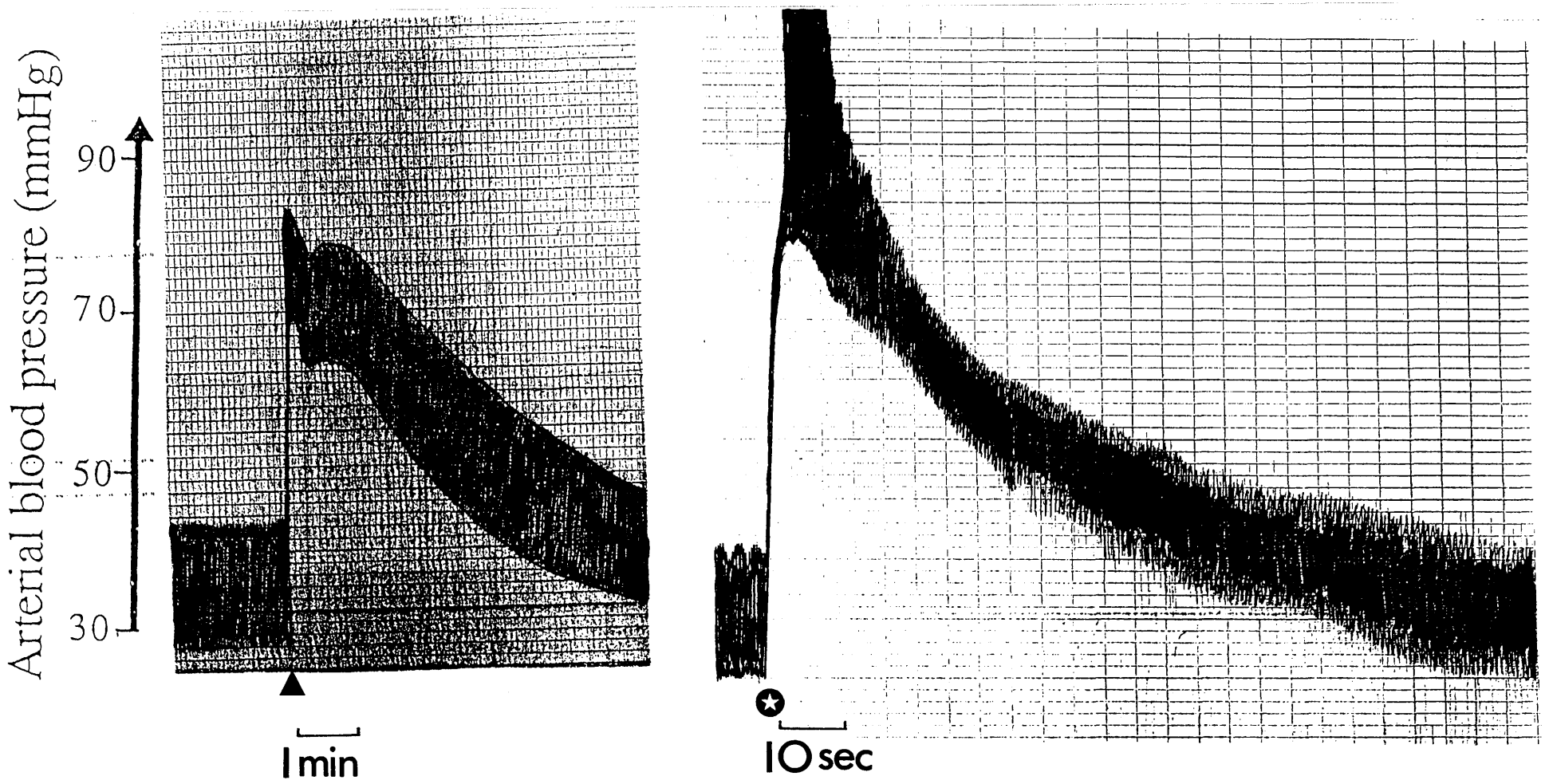

60
50
40
30
20
10
0
ΔDBP mmHg
Control
Praz 0.1mg/kg
Praz 0.3mg/kg
Praz 1mg/kg
Raus 1mg/kg
0
5
10
15
20
Frequency (Hz)

Figure 2:3

Arterial blood pressure recording from a pithed rat showing pressor responses obtained to intravenous administration of:

A) NA (0.3ug/kg) ▲

B) α,β-methylene ATP (0.05mg/kg) ✪


Arterial blood pressure (mmHg)
90
70
50
30
1 min
10 sec

1:3) were antagonised by prazosin, rauwolscine or Wy 26703, and were abolished by a combination of $alpha_1$ and $alpha_2$-adrenoceptor antagonists.

Adenosine and ATP

The intravenous administration of adenosine (100µg/kg - 10mg/kg) or ATP (10µg/kg - 1mg/kg) produced depressor responses (Figure 2:4). Responses to 100µg/kg adenosine and 10ug/kg ATP were blocked by the P_1-purinoceptor antagonist 8PT (10mg/kg).

In one out of four preparations the intravenous administration of ATP produced a biphasic response: the first component was small, pressor and subject to tachyphylaxis. This was similar to the response observed on intra-arterial administration of ATP. The second component was depressor and not subject to tachyphylaxis.

Alpha, beta-methylene ATP

Unlike adenosine or ATP, the intravenous or intra-arterial administration of mATP (0.01mg/kg - 0.5mg/kg) produced large but short lived pressor responses (Figure 2:3). The first few additions produced pressor responses that were biphasic and hence similar in appearance to both NA and nerve mediated pressor responses. mATP administration always resulted in pressor responses even

Figure 2:4

Arterial blood pressure recording from a pithed rat showing responses obtained to intravenous administration of ATP (1µg/kg) and adenosine (1mg/kg).

Arterial blood pressure
(mmHg)
0
20
40
60
80
100
35:22
40
60
80
100
ATP (1μg/kg)
39:22
40
60
80
100
Adenosine (1mg/kg
43:22
40
60
80
100

when vascular tone was raised by infusing NA. Since mATP responses were subject to tachyphylaxis, it was not possible to construct a dose/response curve in a single preparation. Instead a comparison of pressor responses to first additions of mATP was made between animals (Figure 2:5). Prazosin did not appear to alter these responses, whereas pretreatment with nifedipine (0.3mg/kg) resulted in pressor responses to mATP being smaller than in controls, although neither antagonist altered the biphasic nature of the mATP response.

Effects of antagonists on pressor responses to stimulation of spinal sympathetic outflow within the region T_6 - T_8.

Responses to sympathetic nerve stimulation were only partially blocked by the administration of the alpha-adrenoceptor antagonists prazosin and rauwolscine together (each 1mg/kg) (Figure 2:6); approximately 60% of the response remained when stimulating the T_8 region for 1 sec at 20Hz. On their own either prazosin or rauwolscine were capable of attenuating both the direct component and the secondary indirect pressor component at this frequency but each of these antagonists affected the time course of the nerve mediated pressor response differently. This is shown in figures 2:7 and 2:9.

Figure 2:5

Changes in diastolic blood pressure produced by intravenous administration of 0.001mg/kg mATP; 0.01mg/kg mATP; 0.01mg/kg mATP in the presence of prazosin (1mg/kg); and 0.01mg/kg mATP in the presence of nifedipine (0.3mg/kg). All data were obtained from different animals to overcome problems of tachyphylaxis. n=4 for each column.

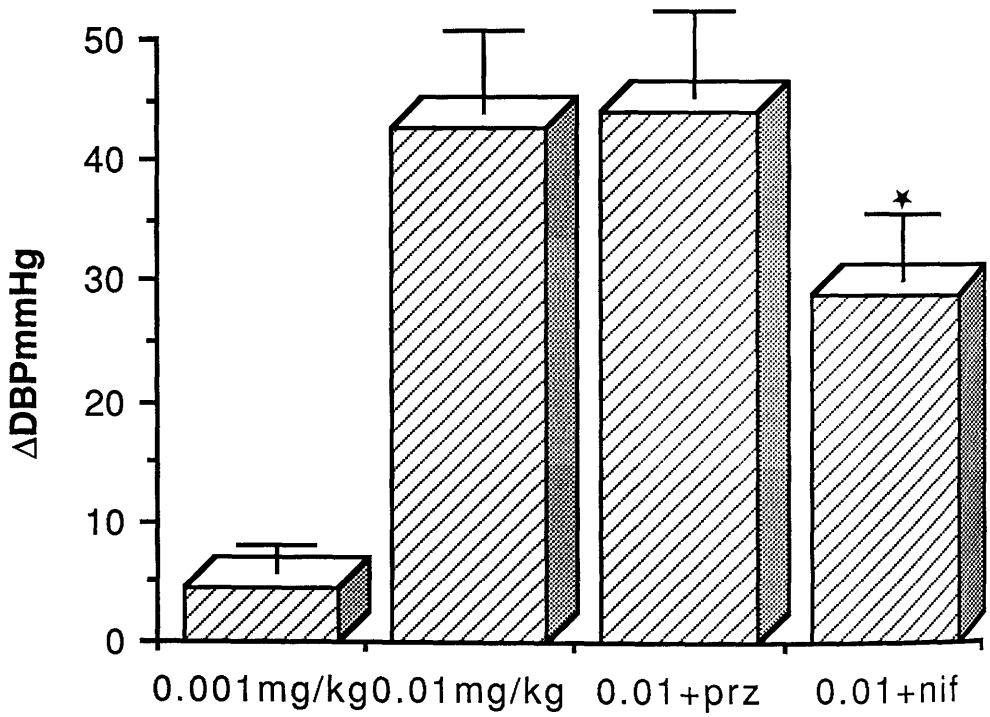

ΔDBPmmHg
50
40
30
20
10
0
0.001mg/kg
0.01mg/kg
0.01+prz
0.01+nif

Figure 2:6

Peak vasopressor responses to sympathetic nerve stimulation (T6-T8) for 1sec at 20Hz were measured as changes in diastolic blood pressure (DBP) in control conditions (in the presence of propranolol (1mg/kg) and gallamine (10mg/kg)) and after the sequential administration of antagonists; prazosin (1mg/kg), rauwolscine (1mg/kg), and a desensitising dose of mATP.

n=6

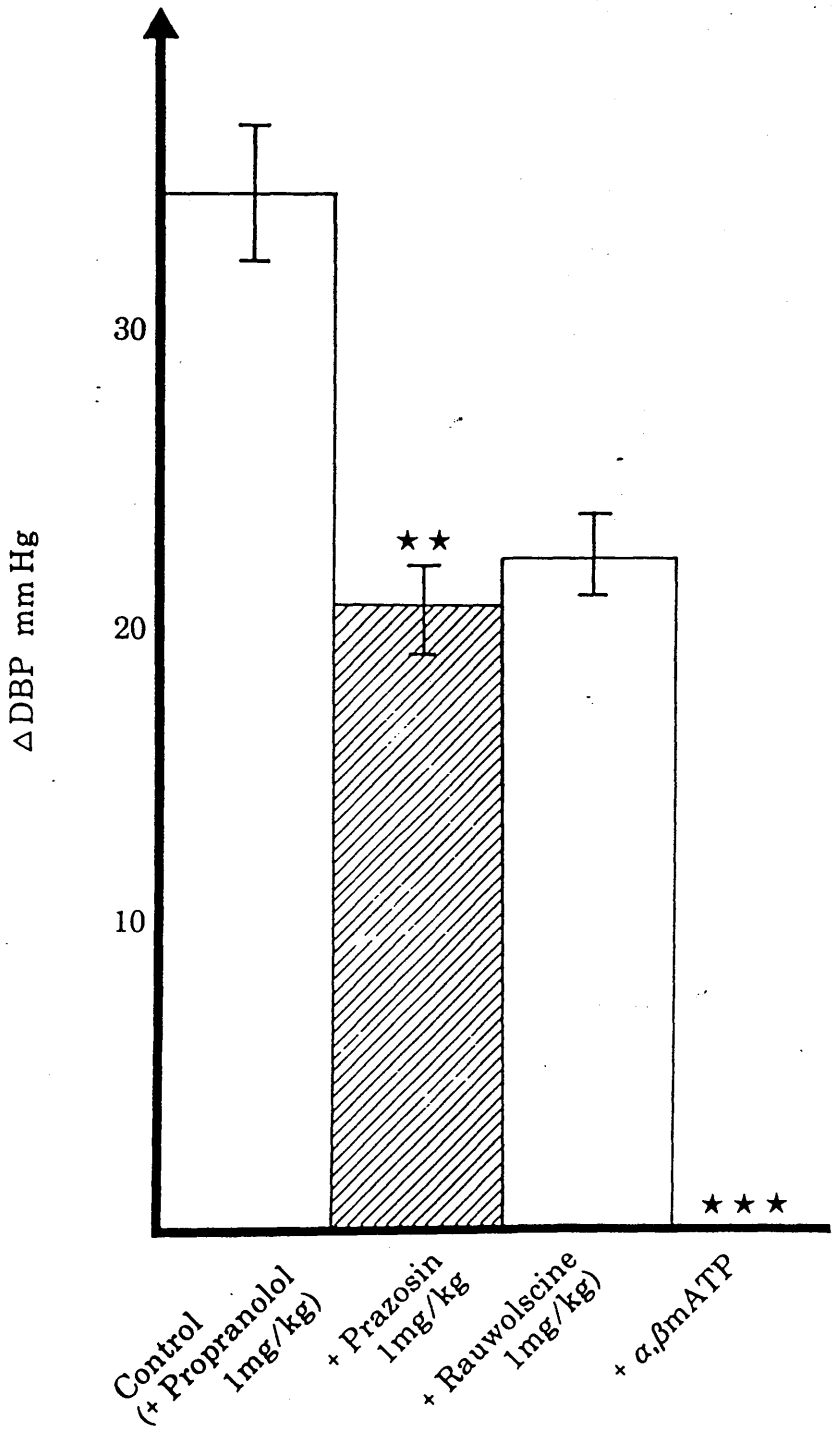
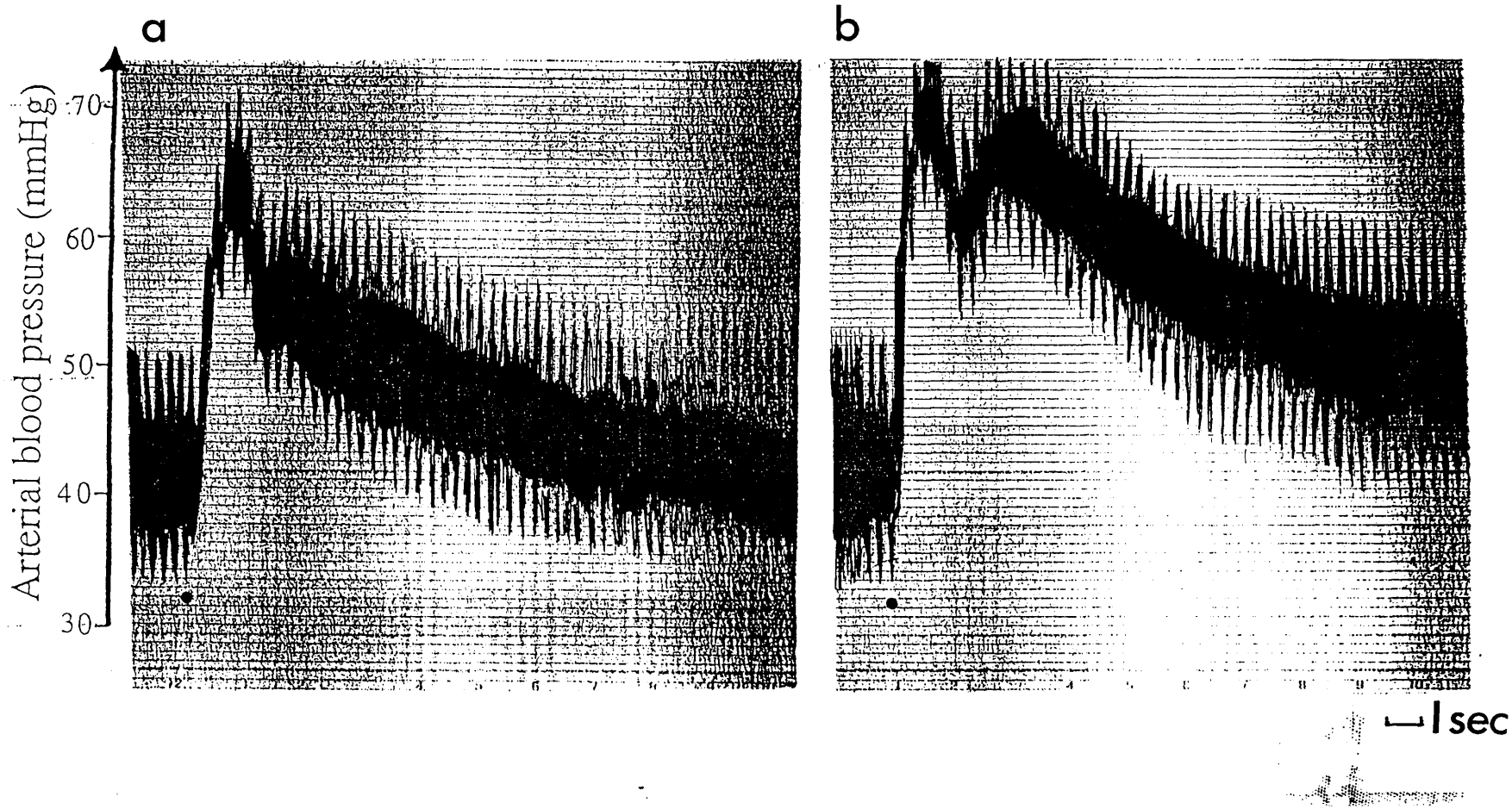

ΔDBP mm Hg
30
20
10
★★
★★★
Control
(+ Propranolol
1mg/kg)
+ Prazosin
1mg/kg
+ Rauwolscine
1mg/kg)
+ α,βmATP

Figure 2:7

Arterial blood pressure recording from a pithed rat showing pressor responses obtained to stimulation of the sympathetic spinal outflow●(T_6 - T_8, 1cm electrode, 0.05ms pulse width, 1sec, 20Hz) in: a) the absence of any agents; b)after the addition of propranolol(1mg/kg); c) after gallamine (10mg/kg); and d) after rauwolscine (1mg/kg).


a
b
Arterial blood pressure (mmHg)
70
60
50
40
30
1 sec

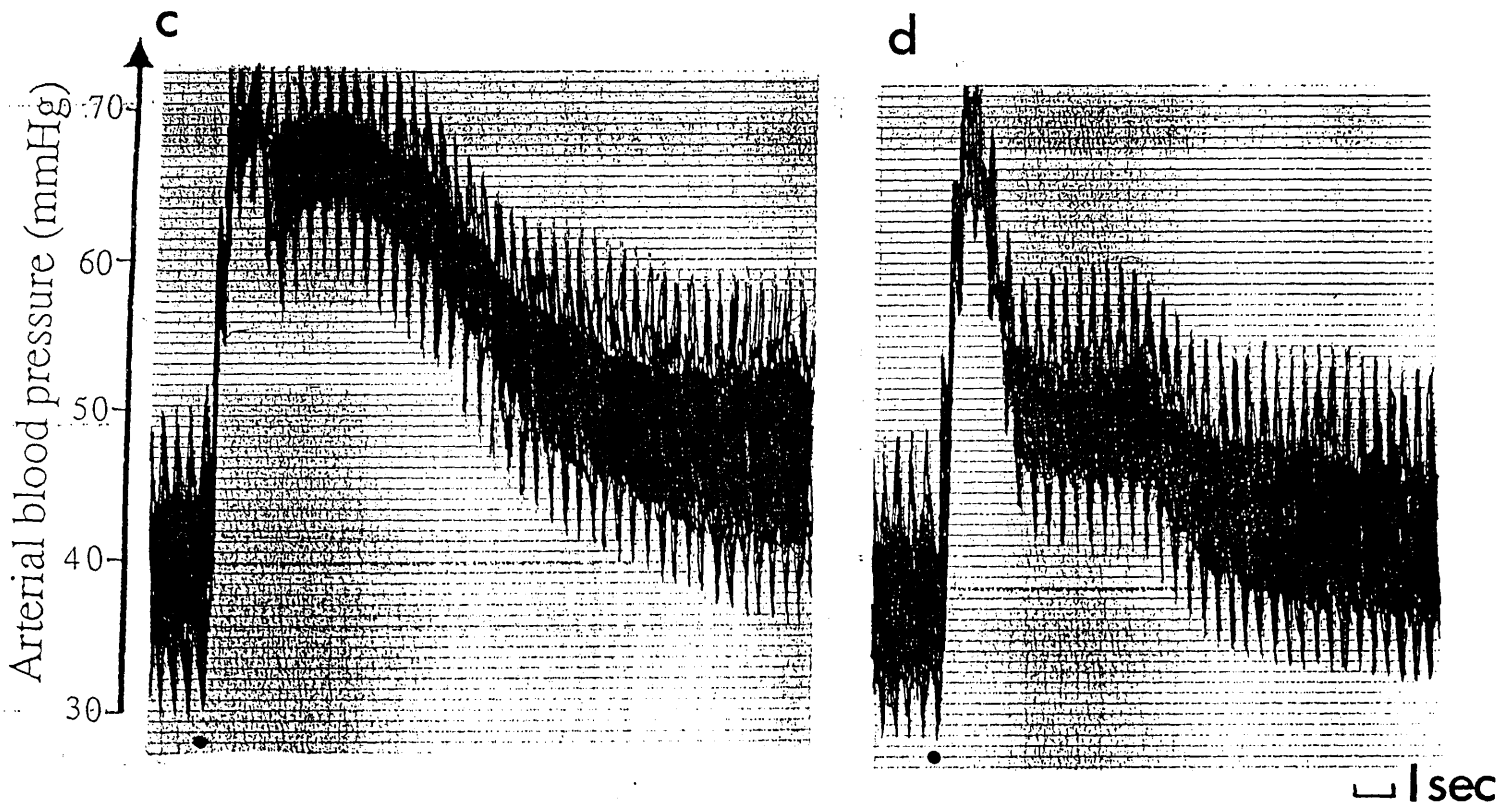

c
d
Arterial blood pressure (mmHg)
70
60
50
40
30
1 sec

Figure 2:8

Arterial blood pressure recording from a pithed rat showing pressor responses obtained to stimulation of the sympathetic spinal outflow●(T_6 - T_8, 1cm electrode, 0.05ms pulse width, 1sec, 20Hz) after a) guanethidine (10mg/kg), b)prazosin (1mg/kg), and c) rauwolscine (1mg/kg).

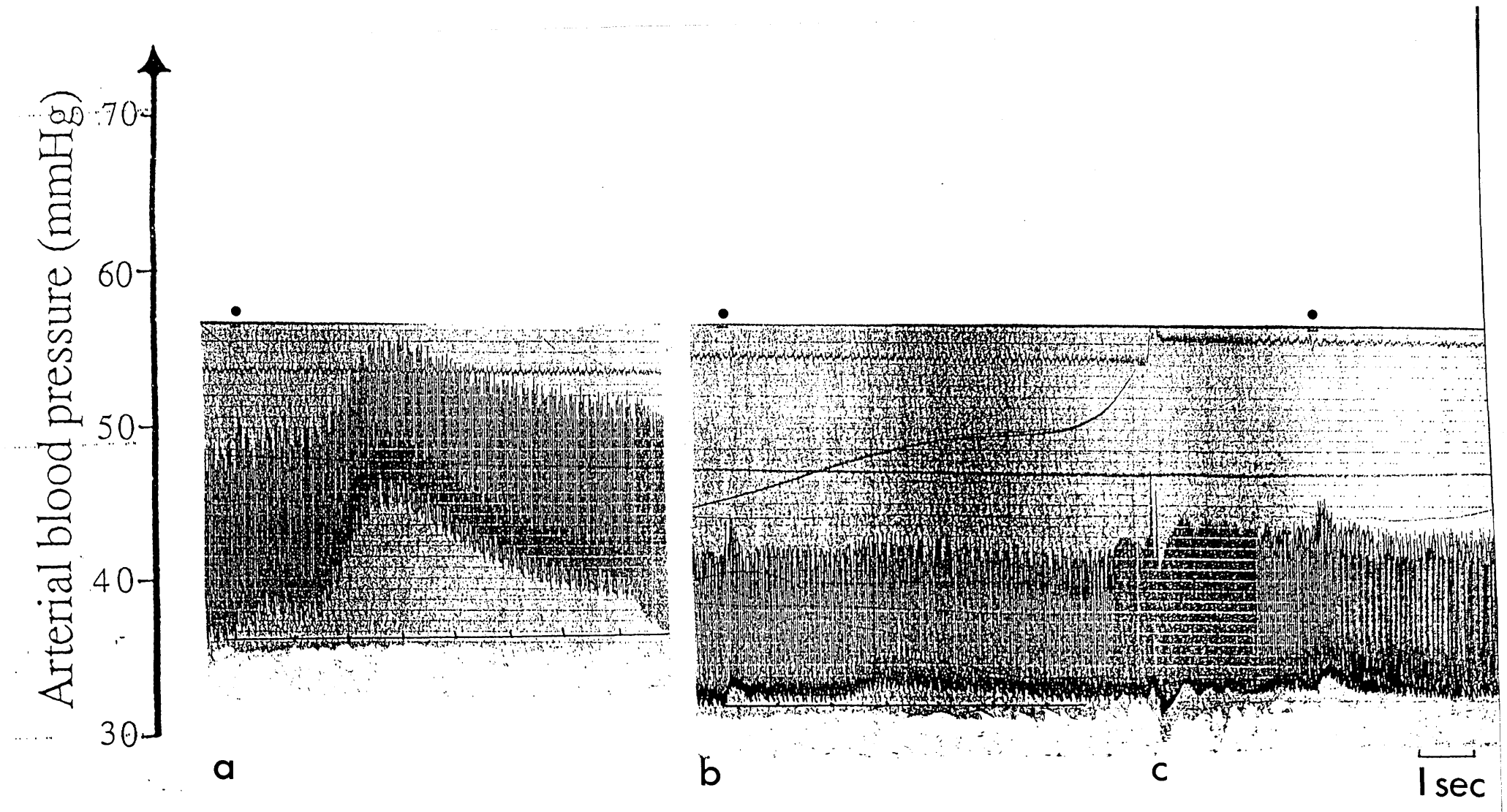
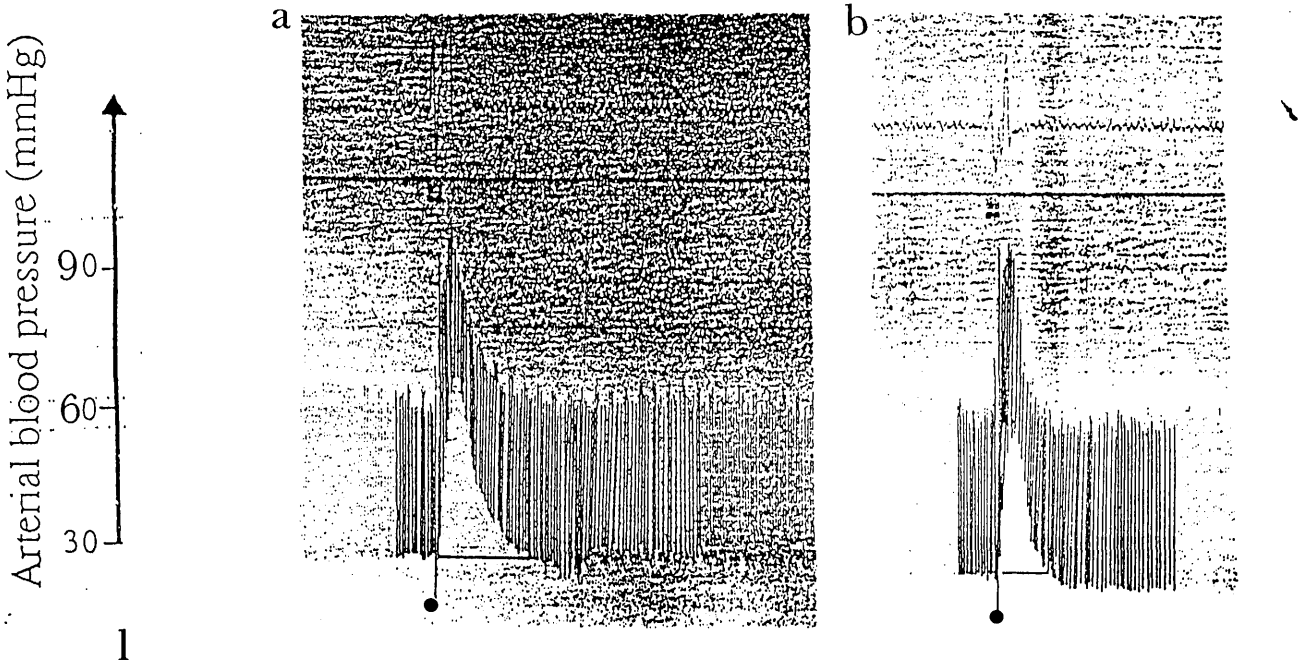
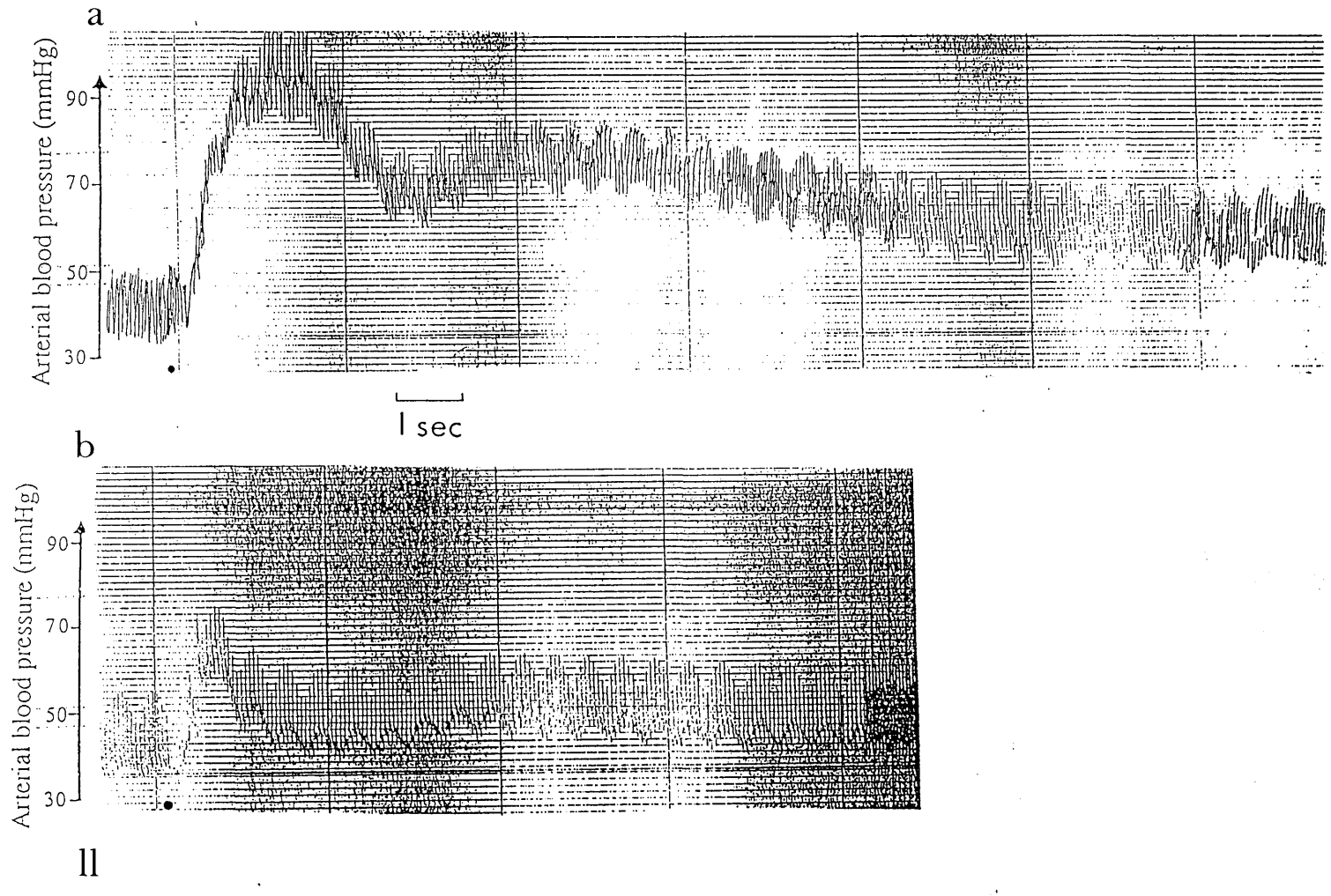

Arterial blood pressure (mmHg)
70
60
50
40
30
a
b
c
1 sec

Figure 2:9

Arterial blood pressure recordings from two pithed rat preparations (I & II) showing pressor responses obtained to stimulation of the sympathetic spinal outflow (T_6 - T_8, 1cm electrode, 0.05ms pulse width, 1sec, 20Hz) : a) in the presence of propranolol (1mg/kg) and gallamine (10mg/kg); and b) after prazosin (1mg/kg). Trace II shows a large and unexpected attenuation by prazosin (1mg/kg). Trace I shows the more typical degree of attenuation produced by prazosin (1mg/kg).



1


a
Arterial blood pressure (mmHg)
90
70
50
30
1 sec
b
Arterial blood pressure (mmHg)
90
70
50
30

ll

Propranolol

Following propranolol (1mg/kg), both the direct component and the adrenal component of the pressor response were increased (Figure 2:7a). The time course of the responses also altered, becoming more prolonged. In subsequent experiments, all preparations were pretreated with propranolol (1mg/kg) to block beta-adrenoceptor mediated effects of the released catecholamines.

Gallamine

Gallamine (10mg/kg) was administered to the preparations after pithing to stop skeletal muscle twitching. The intravenous administration of gallamine often produced pressor responses. In a few preparations the effects of gallamine on the nerve-induced pressor responses were monitored. Gallamine did not attenuate the vasopressor responses and in some preparations increased them slightly (Figure 2:7b) as well as smoothing out any experimental artifacts caused by electrically induced twitching.

Guanethidine

The addition of guanethidine (10mg/kg) almost completely blocked the direct component of the nerve-

induced pressor response, whilst leaving the secondary indirect (adrenal) component intact. Subsequent administration of prazosin and rauwolscine blocked the adrenal component (Figure 2:8).

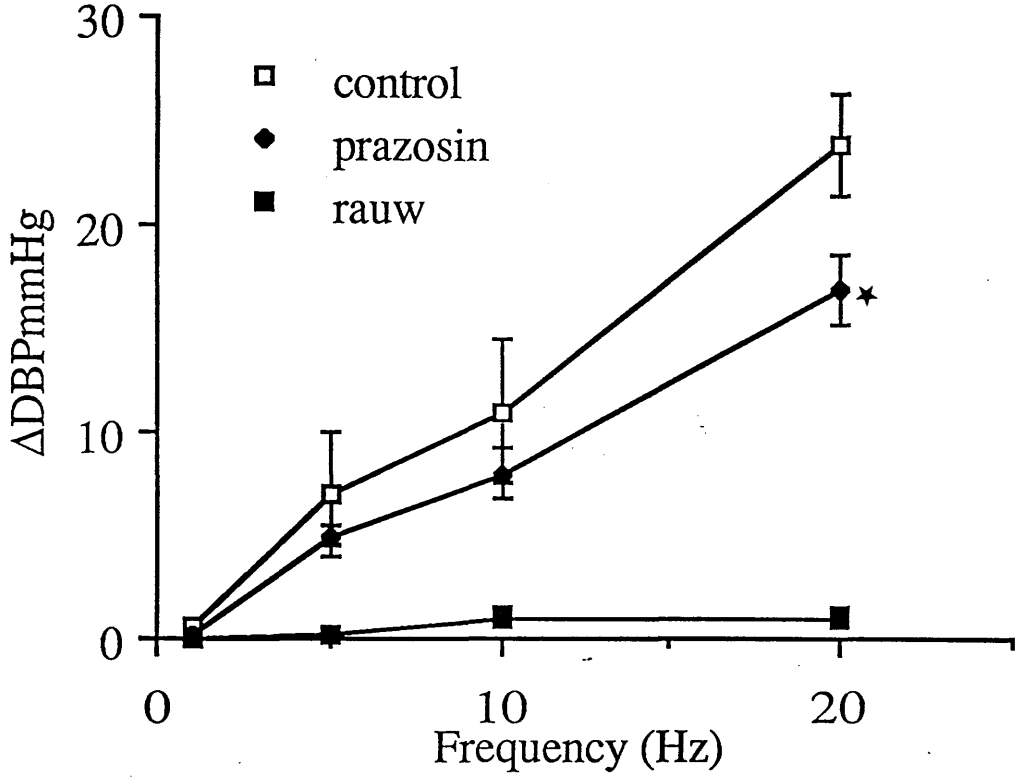
Prazosin

The $alpha_1$-adrenoceptor antagonist prazosin, attenuated both the direct and indirect components of the pressor response (Figure 2:9a&b). The effects of prazosin were tested in animals pretreated with propranolol and gallamine. Prazosin at a dose level of 0.1mg/kg, significantly reduced both of the components of the pressor response at each frequency tested (1 - 20 Hz). Higher doses of prazosin at 0.3mg/kg and 1mg/kg produced no further significant decrease in the pressor response (Figure 2:2). These prazosin-resistant components could be further antagonised by the addition of the $alpha_2$-adrenoceptor antagonist rauwolscine (1mg/kg).

The prazosin-resistant component of the adrenal response was abolished by the addition of rauwolscine (1mg/kg) (Figure 2:10). However the prazosin-resistant component of the direct response was only partially attenuated and was sometimes increased after the addition of rauwolscine. This post-junctional antagonism was frequency dependent, being more effective at the lower frequencies of 1 & 5 Hz. At these lower

Figure 2:10

The indirect (adrenal) component of the vasopressor responses to sympathetic nerve stimulation (T6-T8) for 1sec at 20Hz was measured as changes in diastolic blood pressure ($\triangle$ DBP) in control conditions (in the presence of propranolol (1mg/kg) and gallamine (10mg/kg)) and after the sequential administration of antagonists; prazosin (1mg/kg), rauwolscine (1mg/kg). n=5


30
20
10
0
ΔDBPmmHg
control
prazosin
rauw
0
10
20
Frequency (Hz)

frequencies, rauwolscine reduced the height of the pressor response as well as the time course, whereas at higher frequencies of 10 & 20 Hz, the height of the response was quite often increased even although the time course of the response was reduced (Figure 2:7b). After the addition of prazosin and rauwolscine an alpha-blocker resistant component persisted (Figure 2:6).

Rauwolscine

Following propranolol (1mg/kg), the administration of rauwolscine (1mg/kg) produced significant attenuation of the adrenal component of the vasopressor response evoked by sympathetic nerve stimulation from the spinal outflow (Figure 2:11). The rauwolscine-resistant component was abolished by the addition of prazosin (1mg/kg).

The effect of rauwolscine, on its own, on the direct component was frequency-dependent (Figure 2:12). At the lower frequencies of 1 & 5 Hz, rauwolscine reduced the response both in height and in duration, whereas at higher frequencies the effect was seen as a shortening of the time course of the direct component (returning blood pressure to its previous resting value quicker) but only slightly reducing the height of the response in some preparations (Figure 2:7b) and increasing it in others. The subsequent administration of prazosin significantly attenuated the rauwolscine-

Figure 2:11

The indirect (adrenal) component of the vasopressor responses to sympathetic nerve stimulation (T6-T8) for 1sec at 20Hz was measured as changes in diastolic blood pressure ($\triangle$DBP) in control conditions (in the presence of propranolol (1mg/kg) and gallamine (10mg/kg)) and after the sequential administration of antagonists; rauwolscine (1mg/kg) then prazosin (1mg/kg). n=5

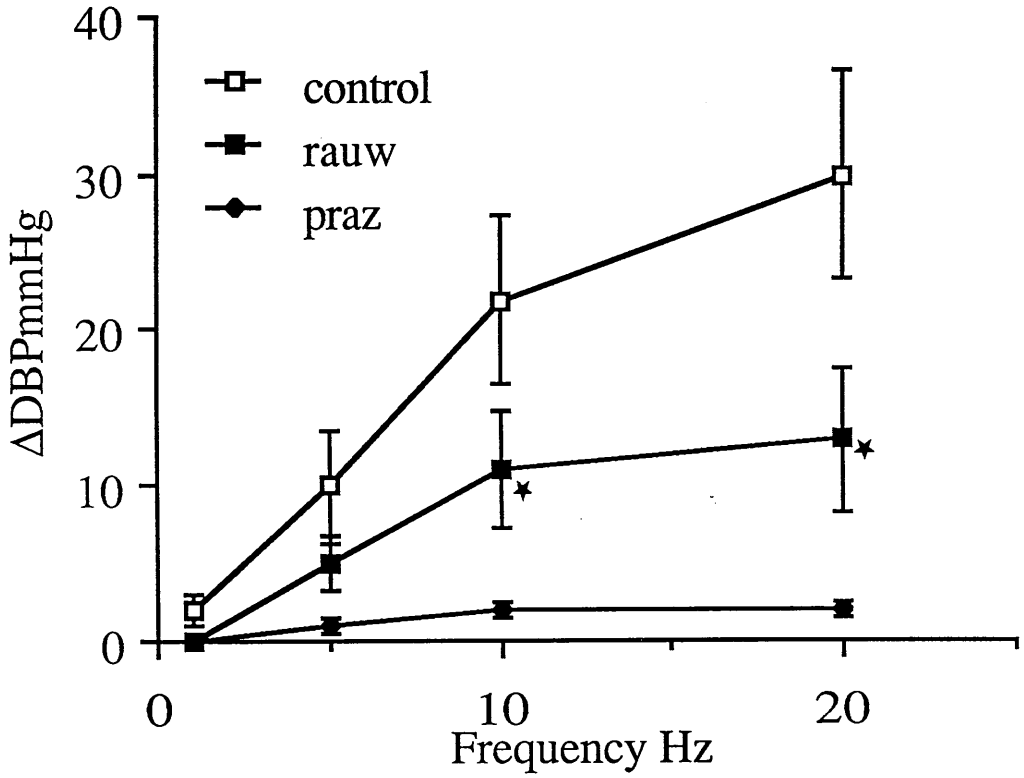
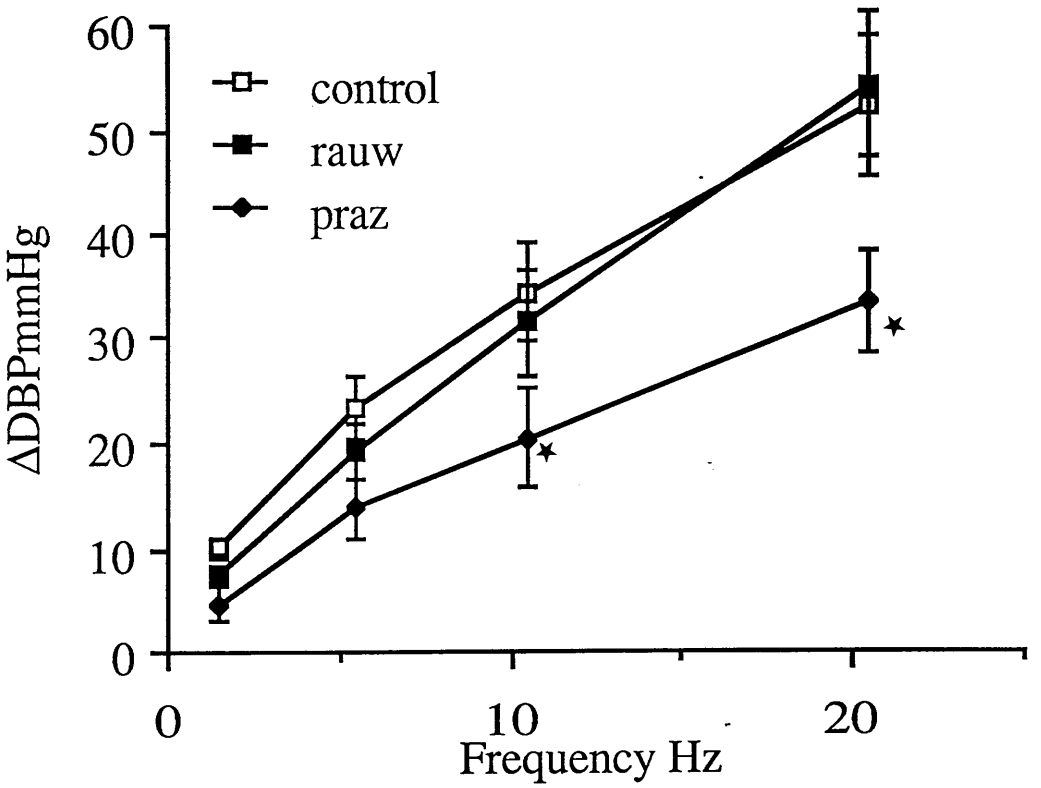

40
30
20
10
0
ΔDBPmmHg
control
rauw
praz
0
10
20
Frequency Hz

Figure 2:12

The direct component of the vasopressor response to sympathetic nerve stimulation (T6-T8) for 1sec at 20Hz was measured as changes in diastolic blood pressure ($\triangle$DBP) in control conditions (in the presence of propranolol (1mg/kg) and gallamine (10mg/kg)) and after the sequential administration of antagonists; rauwolscine (1mg/kg) then prazosin (1mg/kg). $n=5$


60
50
40
30
20
10
0
ΔDBPmmHg
control
rauw
praz
0
10
20
Frequency Hz

resistant component but did not completely abolish it. An alpha-blocker resistant component could again be uncovered.

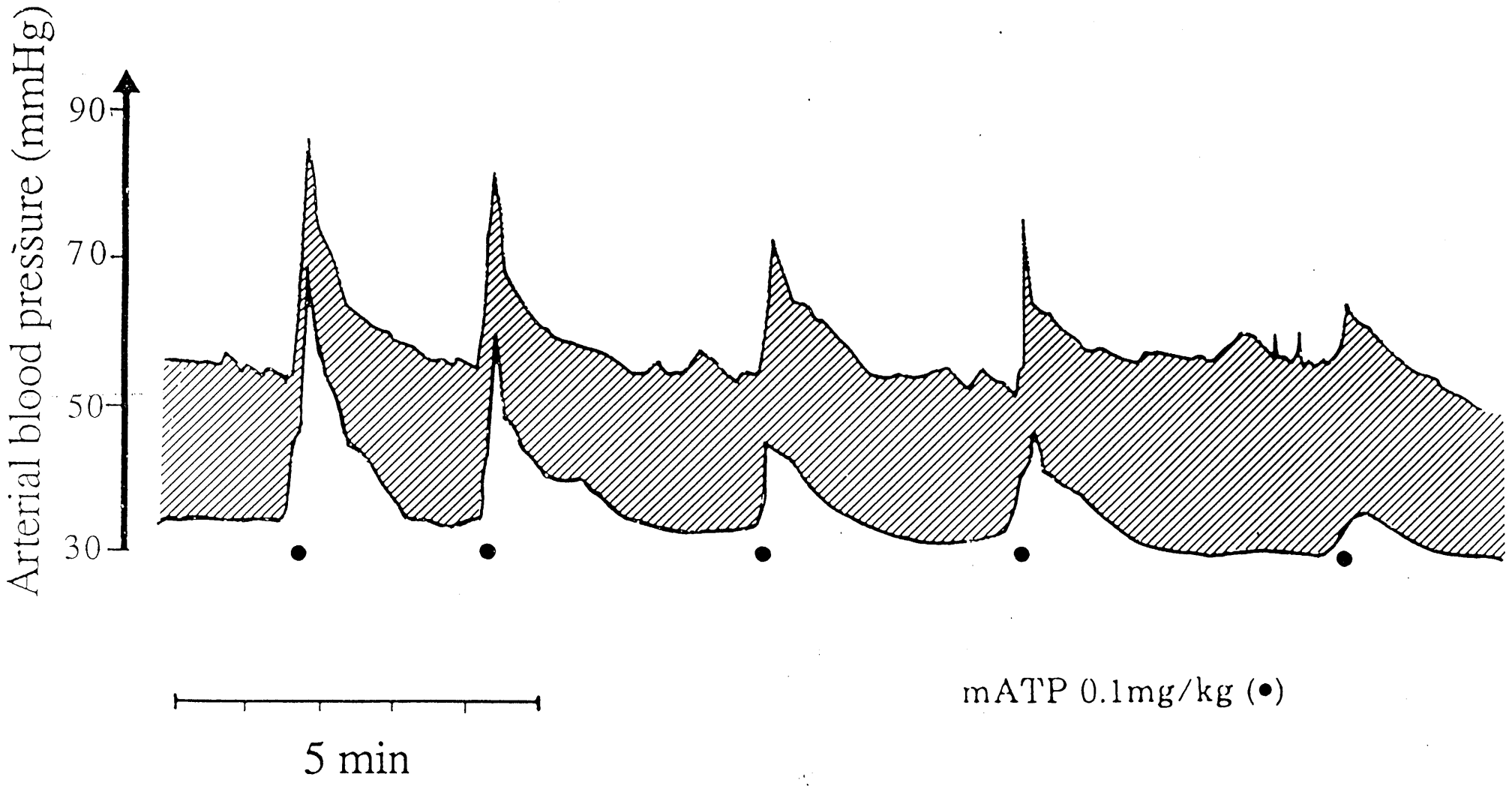
mATP

By adopting a novel desensitisation schedule for mATP and taking into account the short life of mATP in vivo, pressor responses due to sympathetic nerve stimulation can be shown to be more sensitive to mATP than was previously suggested (Grant et al, 1985).

The i.v. administration of mATP (0.01-0.5mg/kg) produced a short-lived pressor response which was subject to tachyphylaxis (Figure 2:13). The pressor response to the first dose of mATP given in each experiment, irrespective of dose within the range tested, differed from the subsequent pressor responses to mATP. The first response was biphasic having an immediate transient short-lived pressor effect and a second longer lasting pressor phase. It was also accompanied by an increase in heart rate. Subsequent doses produced a monophasic pressor response equivalent to the second phase and did not alter heart rate. After desensitisation the pressor responses were not significantly larger than responses to the equivalent volume of 0.9% saline.

Figure 2:13

This is a recording of arterial blood pressure monitored from the carotid artery and shows the effect of intravenous administration of boluses of mATP (0.1 mg/kg). The first bolus of mATP produces a large pressor response but subsequent administrations of the same dose showed tachyphylaxis.


Arterial blood pressure (mmHg)
90
70
50
30
5 min
mATP 0.1mg/kg (•)

The desensitisation procedure for P_2-purinoceptors in vivo:

An initial single intravenous bolus injection of 0.5mg/kg mATP caused heart failure in the rats used in four preliminary experiments. Therefore in subsequent studies requring the presence of high concentrations of mATP, an initial low dose of mATP of 0.05mg/kg was introduced to desensitise the heart to this effect of mATP. A final concentration of mATP of approximately 2.5mg/kg was gradually introduced to the rat. The first 2 boluses of mATP were given at a dose level of 0.05mg/kg. This was followed by 5 separate boluses of mATP at a dose level of 0.5mg/kg approximately every 60 seconds. This desensitisation procedure spanned a 7 minute period.

Following completion of the desensitisation schedule for mATP, attenuation of nerve-mediated pressor responses occurred in a time dependent manner: maximum blockade occurred 1 min after the addition of mATP but faded within 10 min to a maintained desensitisation (Figure 2:14).

In the absence of alpha-adrenoceptor antagonists, the desensitising doses of mATP reduced pressor responses to sympathetic nerve stimulation to approximately 40% of control levels (Figure 2:15).

In the presence of alpha-adrenoceptor antagonists,

Figure 2:14

This recording of carotid arterial blood pressure shows the effect of stimulation (↑) of the spinal sympathetic outflow (T6-T8) for 1 sec at 20Hz, in: a) control conditions (in the presence of propranolol (1mg/kg) and gallamine (10mg/kg) only); and b) after additions of mATP to produce desensitisation of the P_2-purinoceptors.

The final addition of mATP (0.1 mg/kg) in the desensitising schedule is also indicated on the trace (●).

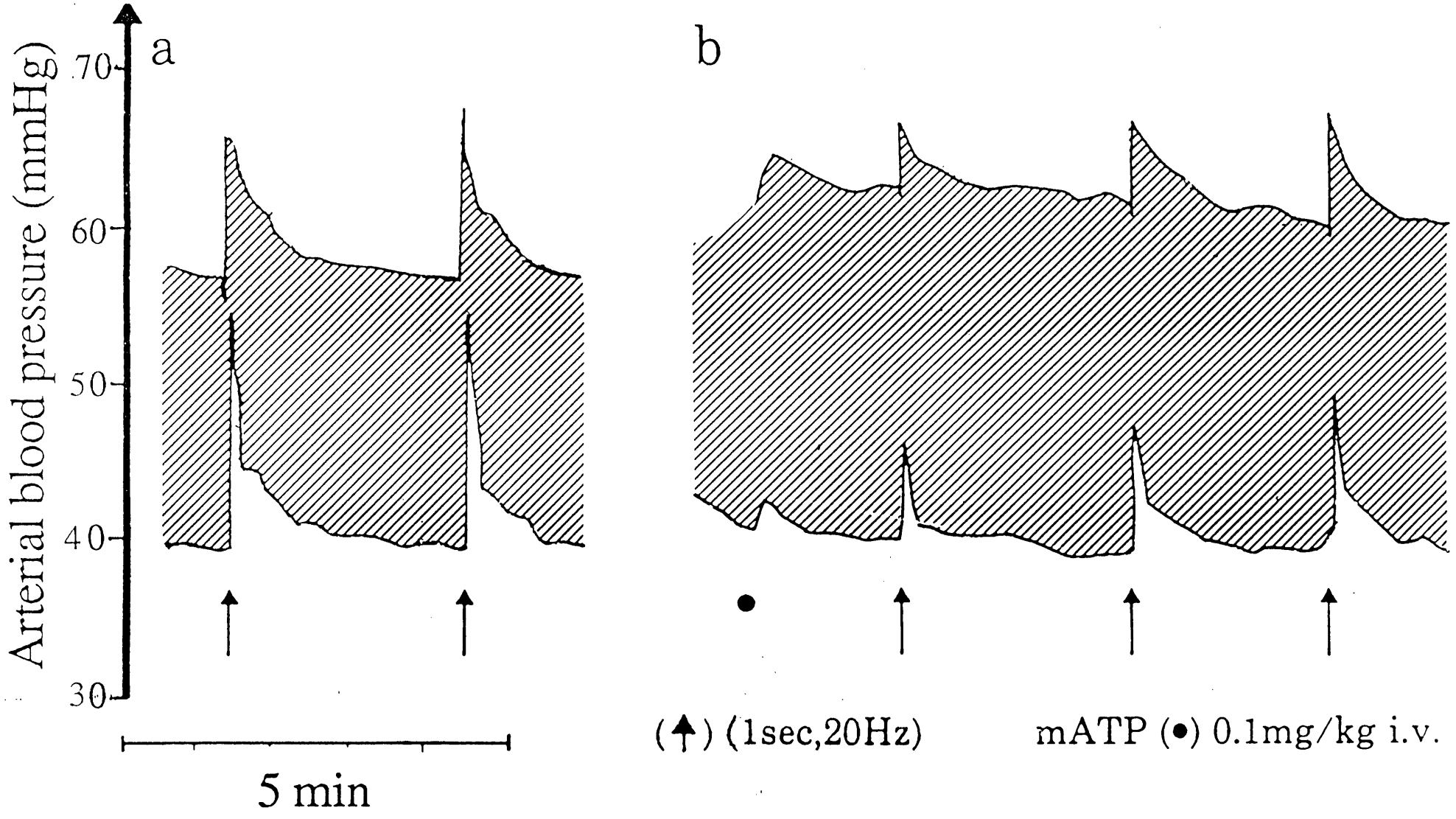
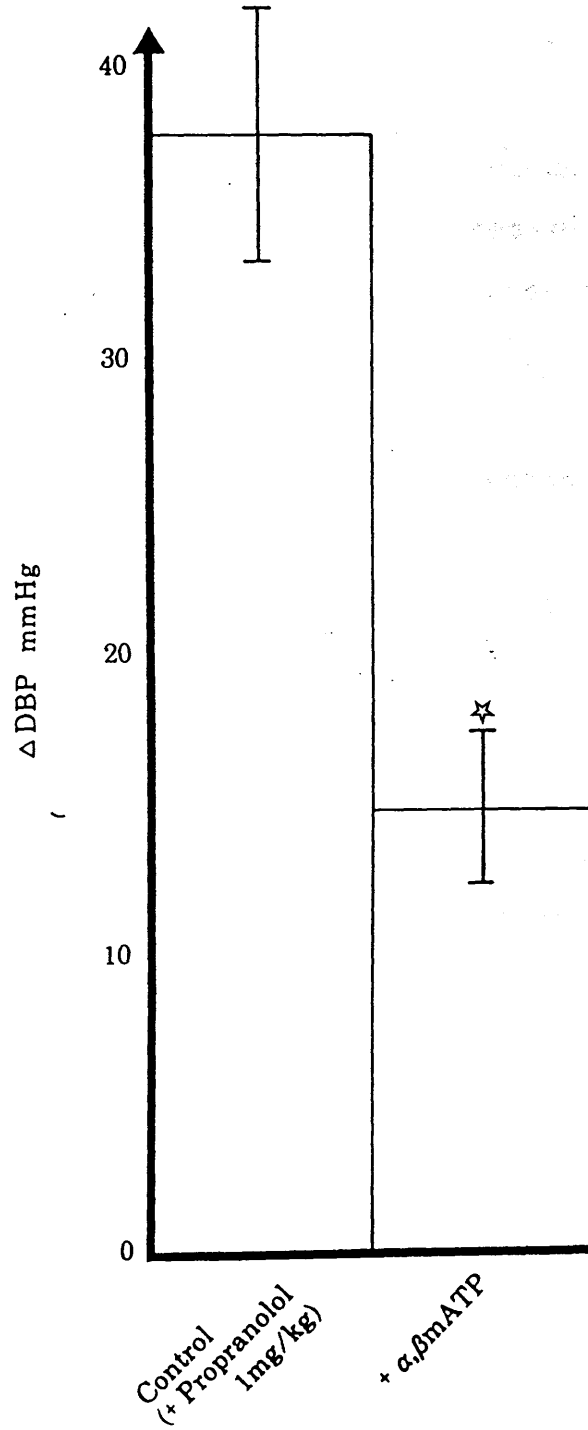

a
b
Arterial blood pressure (mmHg)
70
60
50
40
30
5 min
(↑) (1sec,20Hz)
mATP (●) 0.1mg/kg i.v.

Figure 2:15

Peak vasopressor responses to sympathetic nerve stimulation (T6-T8) for 1 sec at 20Hz were measured as changes in diastolic blood pressure (DBP) (in the absence of alpha-adrenoceptor antagonists); in control conditions (propranolol 1mg/kg and gallamine 10mg/kg) and after P_2-purinoceptor desensitising doses of mATP.
(n=5)


40
30
20
10
0
△DBP mmHg
Control
(+ Propranolol
1mg/kg)
+ α,βmATP

subsequent additions of mATP which gave a final concentration of 2.5mg/kg completely blocked the pressor responses (Figure 2:6).

Nifedipine

The effect of the slow Ca^{2+} channel blocking agent, nifedipine (0.3mg/kg i.a.) on the vasopressor response to stimulation of the spinal outflow between regions T6 - T8 was also studied.

At 20Hz frequency, after the administration of the alpha-adrenoceptor antagonists prazosin (1mg/kg) and rauwolscine (1mg/kg), the intra-arterial administration of nifedipine (0.3mg/kg) further attenuated the direct component of the pressor response by up to 50%, leaving a small pressor response which was approximately 15% of control level (Figure 2:16). This was frequency dependent with nifedipine having its largest effect at higher frequencies. The subsequent administration of mATP sufficient for desensitising (P_{2x}-purinoceptors) completely blocked the remaining pressor response at frequencies of 1 - 10 Hz and left a vasopressor response of less than 5mmHg at 20Hz (approximately 6% of control response).

The blocking effect of nifedipine was also seen before the addition of the alpha-adrenoceptor antagonists (Figure 2:17a&b, 2:18). Subsequent

Figure 2:16

The effect of nifedipine on alpha-blocked vasopressor responses to stimulation of the sympathetic outflow (T6-T8, for 1 sec at 1 - 20Hz) are measured as changes in diastolic blood pressure. Responses were measured after propranolol (1mg/kg) (□), then prazosin (1mg/kg) (◆), rauwolscine (1mg/kg) (◘), nifedipine (0.3mg/kg i.a.) (◆) and finally mATP (■). n=5

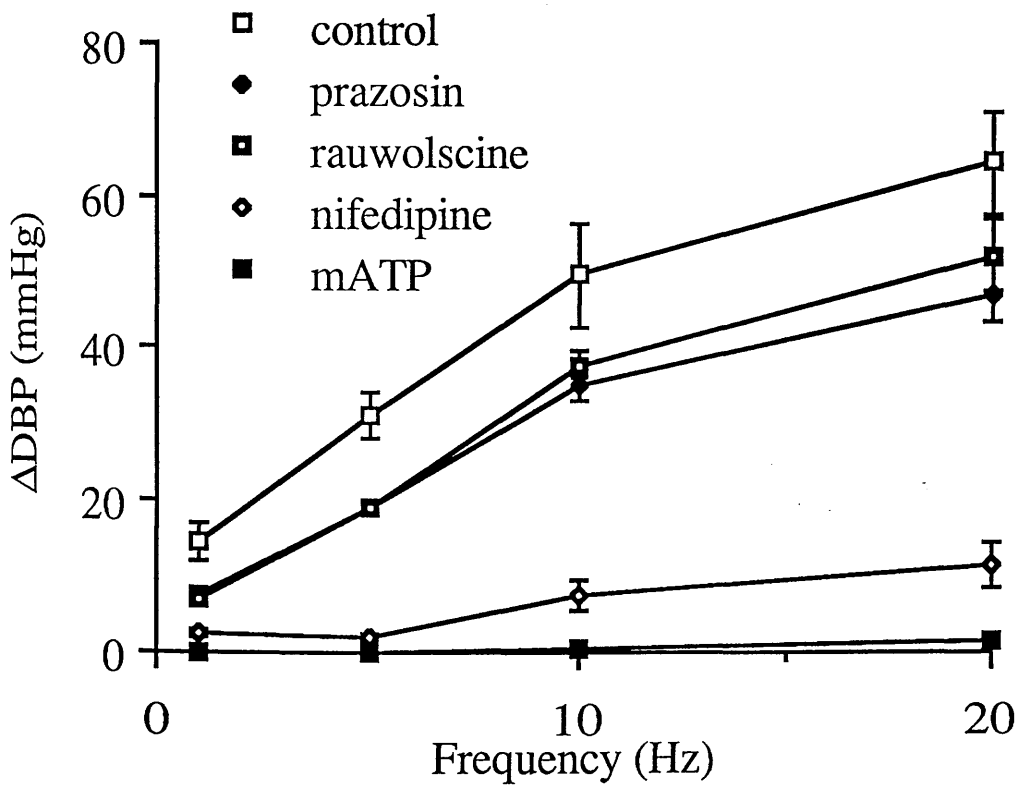

control
prazosin
rauwolscine
nifedipine
mATP
80
60
40
20
0
ΔDBP (mmHg)
0
10
20
Frequency (Hz)

Figure 2:17

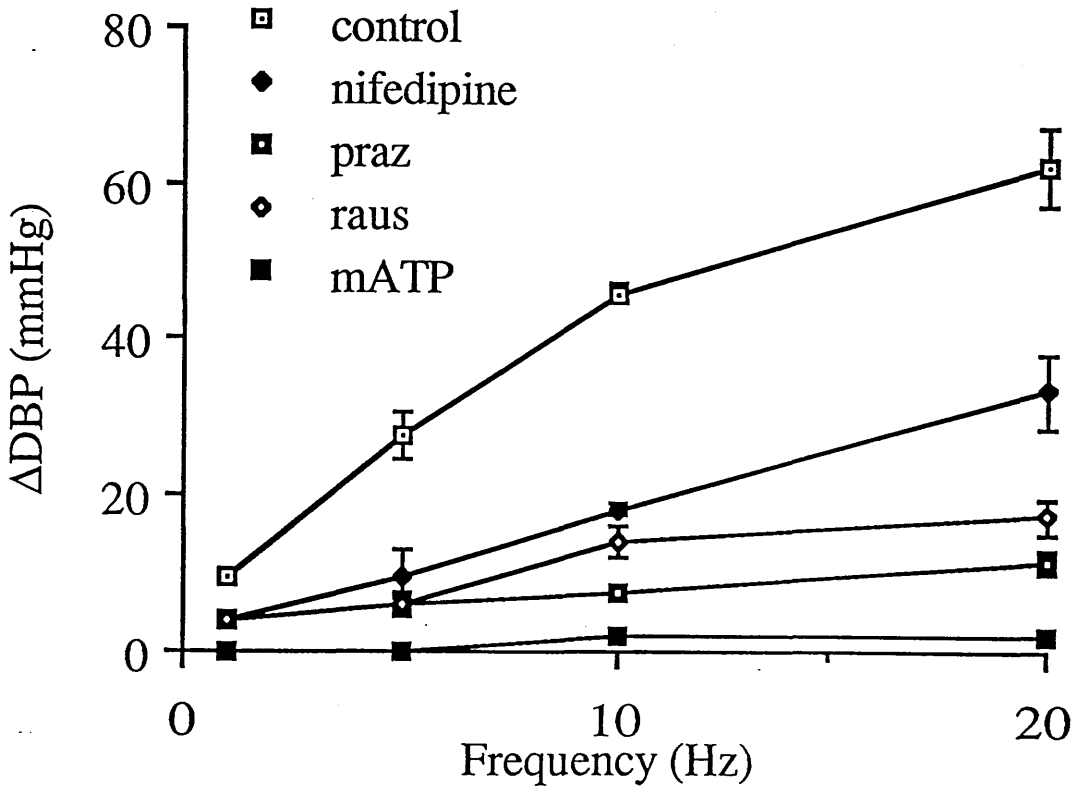
Arterial blood pressure recording from a pithed rat showing pressor responses obtained to stimulation of the sympathetic spinal outflow (T_6 - T_8, 1cm electrode, 0.05ms pulse width, 1sec, 20Hz) : a) in the presence of propranolol (1mg/kg) and gallamine (10mg/kg); and b) after nifedipine (↑) (1mg/kg).

Arterial blood pressure (mmHg)
30
50
70
90
A

Arterial blood pressure (mmHg)
30
50
70
90
B

Figure 2:18

The effect of nifedipine on unblocked vasopressor responses to stimulation of the sympathetic outflow (T6-T8, for 1 sec at 1 - 20Hz) are measured as changes in diastolic blood pressure. Responses were measured after propranolol (1mg/kg) (⊡), then nifedipine (0.3mg/kg i.a.) (◆), prazosin (1mg/kg) (▪), rauwolscine (1mg/kg) (◈), and finally mATP (■). n=5


80
60
40
20
0
ΔDBP (mmHg)
control
nifedipine
praz
raus
mATP
0
10
20
Frequency (Hz)

administration of prazosin attenuated the response by about 60%, indicating that a large alpha-adrenoceptor component remains after nifedipine treatment. Using a different sequence of administration of antagonists, it can be shown that nifedipine has very little effect on the vasopressor response after the administration of a P_{2x}-purinoceptor desensitising dose of mATP (Figure 2:19). After the addition of mATP and nifedipine, the subsequent administration of prazosin (1mg/kg) and rauwolscine (1mg/kg) together, almost completely blocked the pressor responses at all frequencies.

Teprotide

The angiotensin converting enzyme (ACE) inhibitor, teprotide (1mg/kg) attenuated the purinergic component of the pressor response (that is, in the presence of adrenergic blockers): it significantly reduced the pressor response that remained after the addition of prazosin (1mg/kg) and rauwolscine (1mg/kg) at 20Hz stimulation frequency (Figure 2:22).

A comparison of responses to stimulation of different regions of the spinal outflow.

In all three regions studied, T_1 - T_4, T_6 - T_8, and T_{12} - L_1, the percentage contribution of the purinergic component (assessed as the percentage of the response that remained after adrenergic blockade) to the overall

size of the pressor response was larger than that of the alpha-adrenergic component of the response (Figure 2:24). In areas T_1 - T_4 & T_6 - T_8, this difference was significant.

The effect of mATP on the pressor response to noradrenaline.

The effects of the desensitising dose of mATP were studied on the pressor responses to 3 doses of NA. Single bolus doses of 0.1, 0.3, and 1 μg/kg NA gave control dose-dependent pressor responses of 20 ± 5, 36 ± 8, and 58 ± 6 mmHg respectively (n = 5, in each case). Within 10 minutes after the addition of mATP these same 3 doses of NA gave pressor responses of 22 ± 6, 35 ± 8, and 60 ± 5 mmHg and were not significantly different from the control responses. The sequence of the additions of the NA doses were alternated in each experiment since the blocking effect of mATP is time dependent (Figure 2:20).

The effect of mATP on vas deferens responses.

Longitudinal tension of the vasa deferentia were recorded in situ (Figure 6) by the method employed by Gillespie & McGrath (1974). Stimulation of the spinal sympathetic outflow from the pithing rod electrode (1cm electrode, T_{13}, 1sec, 5-20 Hz) gave contractile responses. The effects on these by the sequential

Figure 2:19

The effect of nifedipine on P_2-purinoceptor desensitised vasopressor responses to stimulation of the sympathetic outflow (T6-T8, for 1 sec at 1 - 20Hz) are measured as changes in diastolic blood pressure. Responses were measured after propranolol (1mg/kg) (⊡), then mATP (◆), nifedipine (0.3mg/kg i.a.) (■), and finally prazosin (1mg/kg) and rauwolscine (1mg/kg) together (◇). n=4

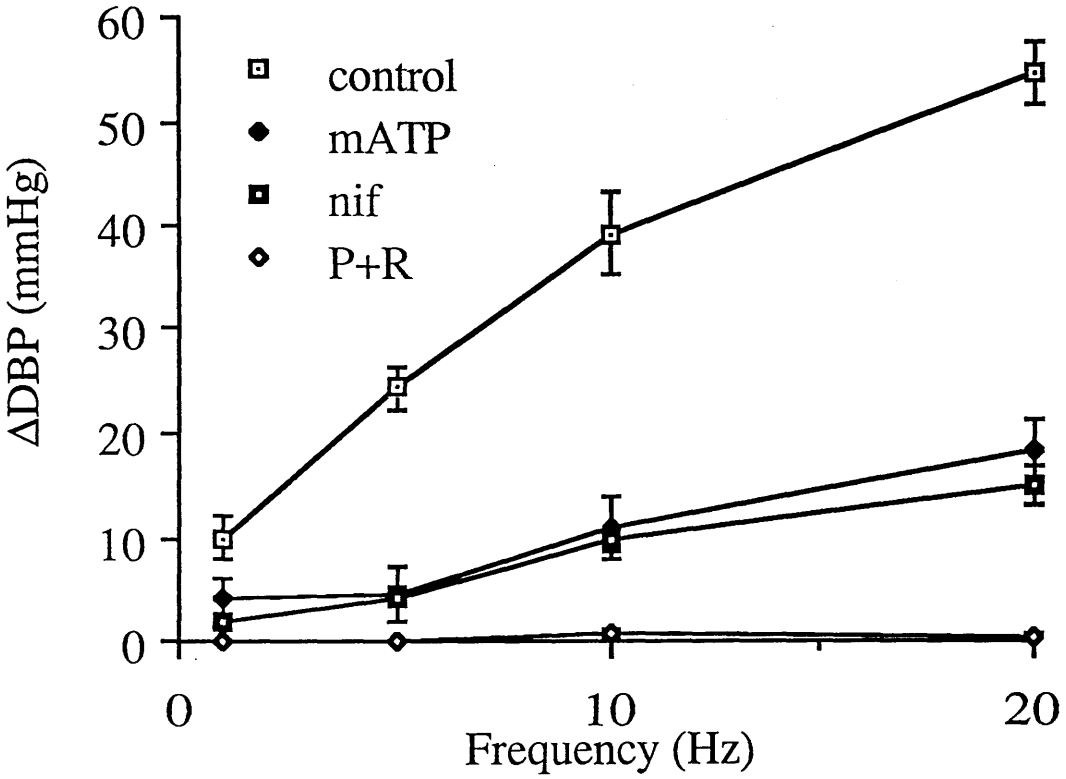
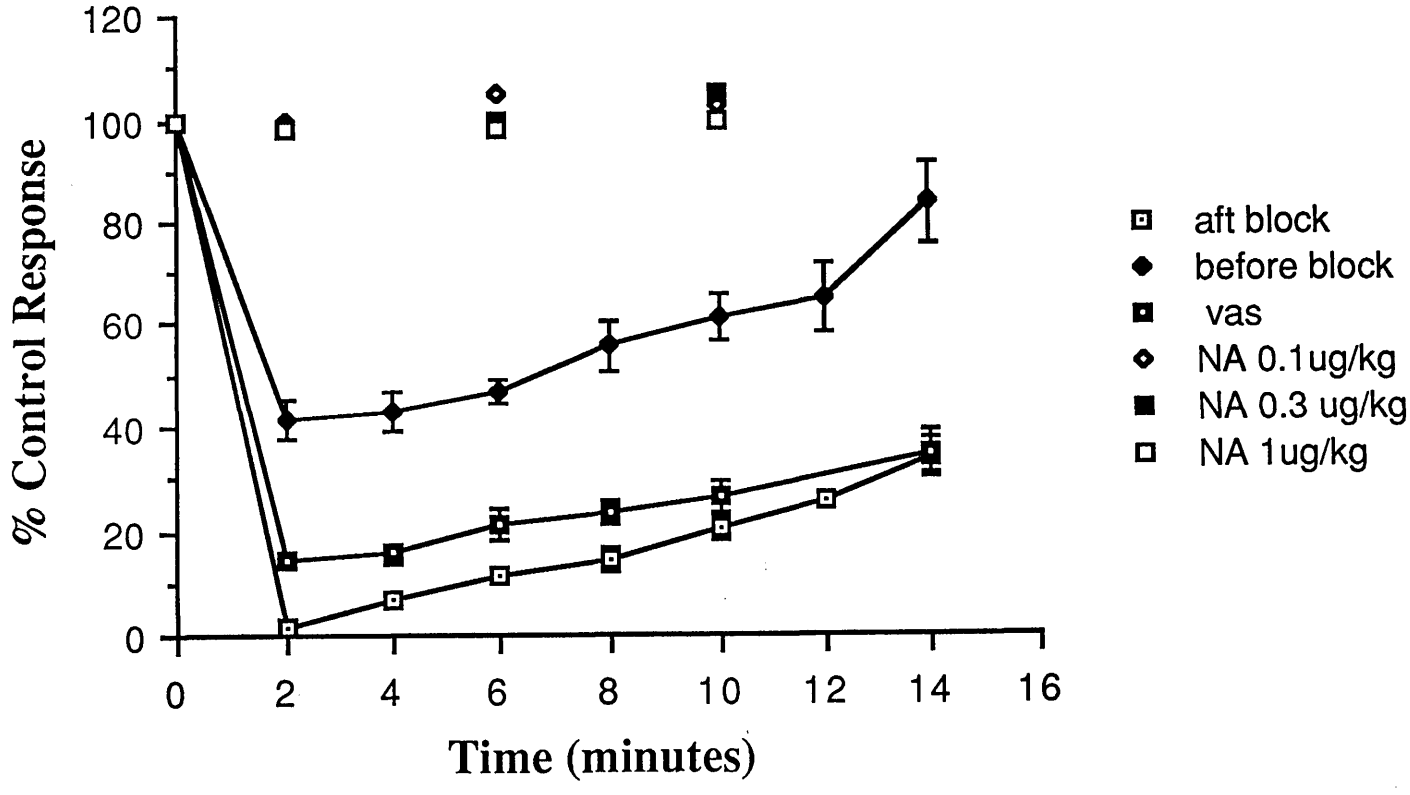

60
50
40
30
20
10
0
ΔDBP (mmHg)
control
mATP
nif
P+R
0
10
20
Frequency (Hz)

Figure 2:20

This graph illustrates the time dependent blocking effect of mATP in vivo on agonist induced and nerve mediated vasopressor responses (T6-T8, for 1 sec at 20Hz) and on nerve mediated motor responses of the vas deferens (T13, for 1 sec at 5Hz). These effects are plotted as % of control response of each preparation at two minute intervals after the administrations of mATP.

The vasopressor responses to NA (0.1µg/kg (◇); 0.3µg/kg (■); 1µg/kg (□)) were measured immediately after completion of the desensitisation schedule for P_2-purinoceptors by mATP. Dose response curves were completed in each preparation but the sequence of administration was rotated to take into account the short life of mATP in vivo. NA responses were measured in the presence of propranolol (1mg/kg).

Vasopressor responses to sympathetic nerve stimulation were measured both before (◆) and after (⊡) alpha-adrenoceptor antagonists had been added. Motor responses of the vas deferens were measured after alpha-adrenoceptor blockade (◘). These responses were measured in the presence of propranolol (1mg/kg) and gallamine (1mg/kg).n= 4- 6


120
100
80
60
40
20
0
% Control Response
0
2
4
6
8
10
12
14
16
Time (minutes)
aft block
before block
vas
NA 0.1ug/kg
NA 0.3 ug/kg
NA 1ug/kg

administration of propranolol, prazosin, rauwolscine and mATP were studied.

Neither propranolol (1mg/kg) nor prazosin (1mg/kg) affected the monophasic contractions of the vas deferens but rauwolscine (1mg/kg) did increase the responses (Figure 2:21).

The effects of mATP on the responses of the vas deferens were two-fold. The initial effects observed on addition of bolus injections of mATP (0.01- 0.1mg/kg) were increases in tone of the muscle accompanied by increases in contractions to the sympathetic stimulation. Within one minute this potentiating effect disappeared and was replaced by an attenuation of the responses (Figure 2:21).

This blocking effect of mATP was time dependent. These effects are illustrated in Figure 2:20 which shows the diminishing blocking effect of mATP on various responses with respect to time.

Figure 2:21

This trace shows the contractile responses obtained by stimulation of the vas deferens of the pithed rat in situ (1cm electrode, T13, 40v for 1sec at 5Hz at 1 minute intervals). Responses were measured after the sequential administration of propranolol (1mg/kg) (▲), prazosin (1mg/kg) (△), rauwolscine (1mg/kg) (□), mATP (0.01mg/kg) (○) and mATP (0.1mg/kg) (●).

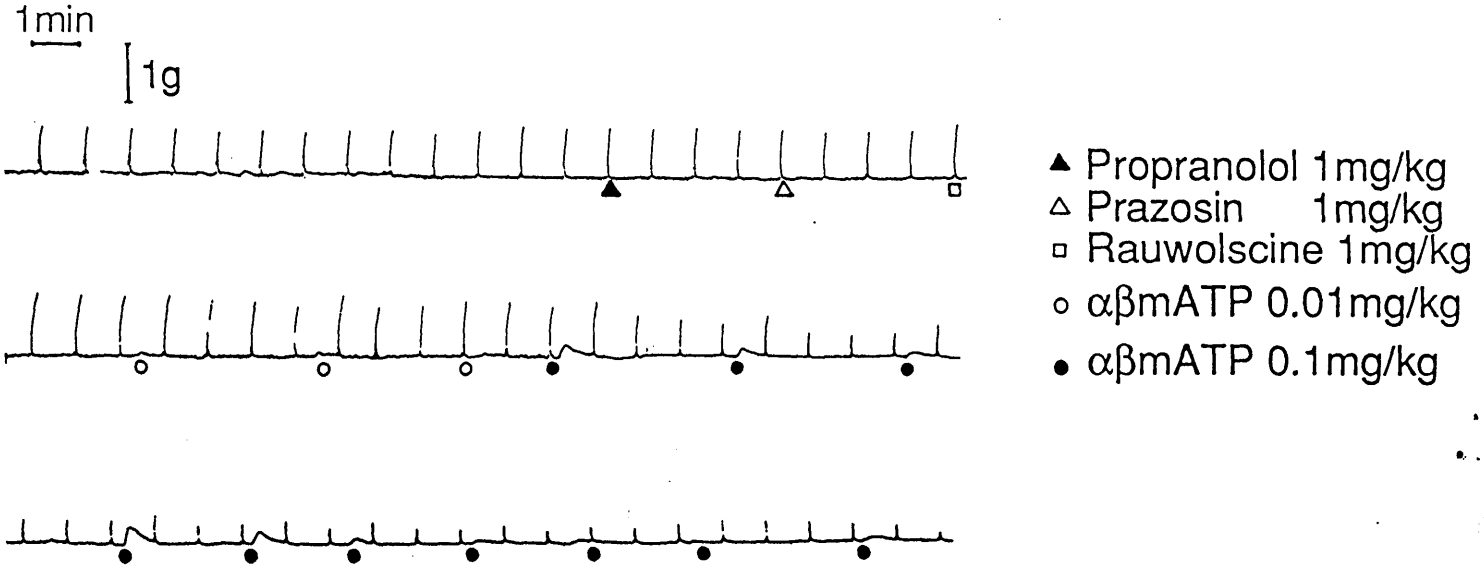

1min
1g
▲ Propranolol 1mg/kg
△ Prazosin 1mg/kg
▫ Rauwolscine 1mg/kg
○ αβmATP 0.01mg/kg
● αβmATP 0.1mg/kg

Figure 2:22

The direct component of the vasopressor response to sympathetic nerve stimulation (T6-T8) for 1sec at 20Hz were measured as changes in diastolic blood pressure ($\triangle$DBP) in control conditions (in the presence of propranolol (1mg/kg) and gallamine (10mg/kg)) and after the sequential administration of antagonists; prazosin (1mg/kg) & rauwolscine (1mg/kg) together, and then after the addition of teprotide (1mg/kg). $n=5$

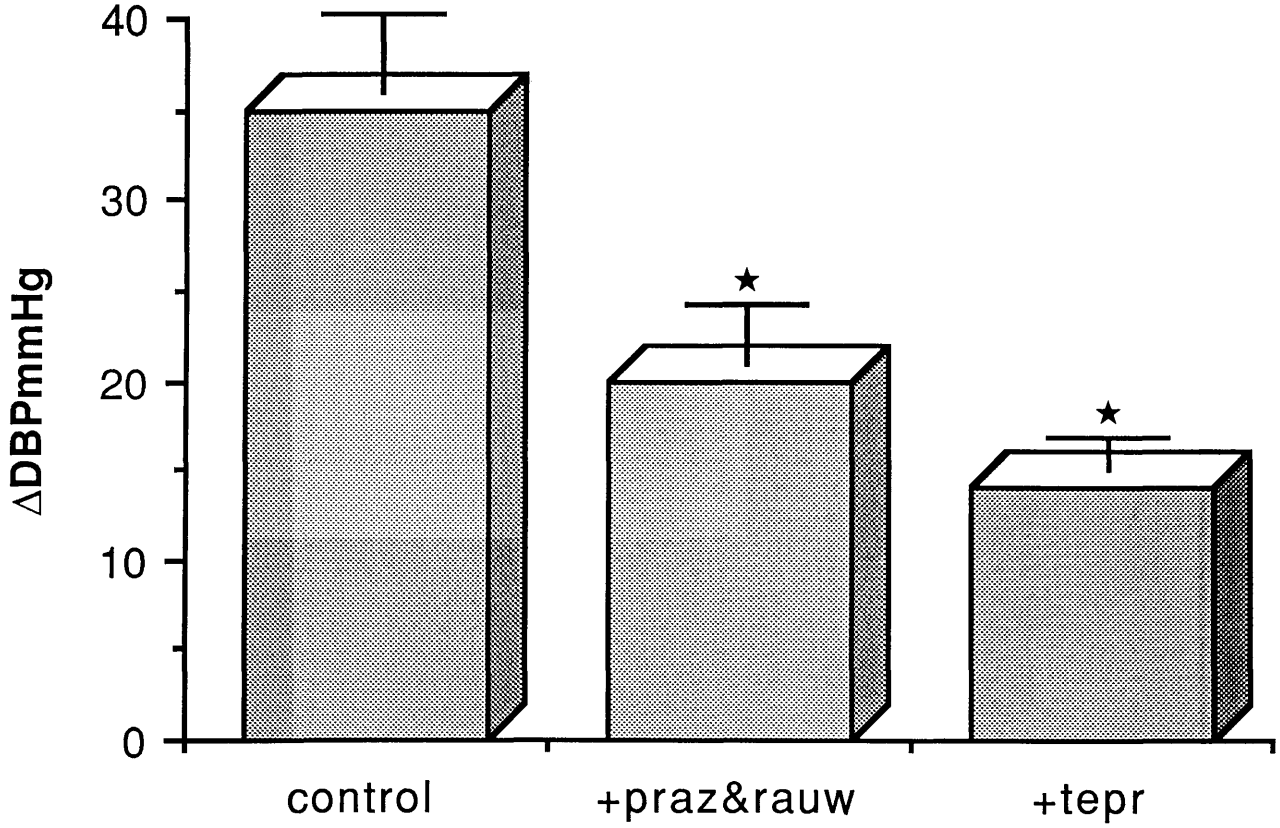
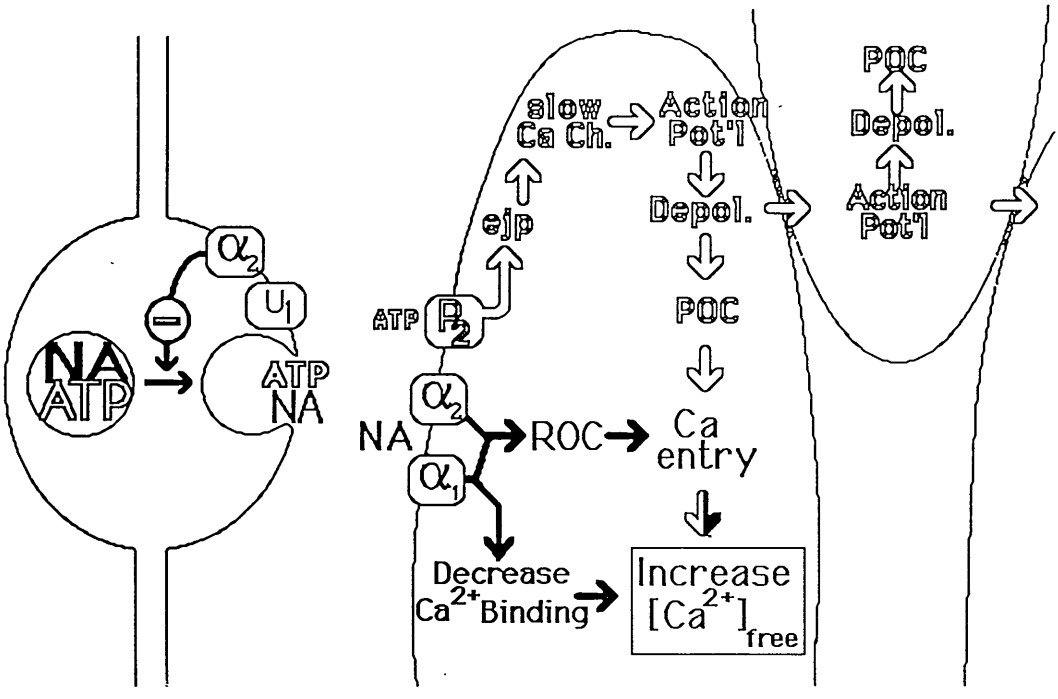

40
30
20
10
0
ΔDBPmmHg
control
+praz&rauw
+tepr

Figure 2:23

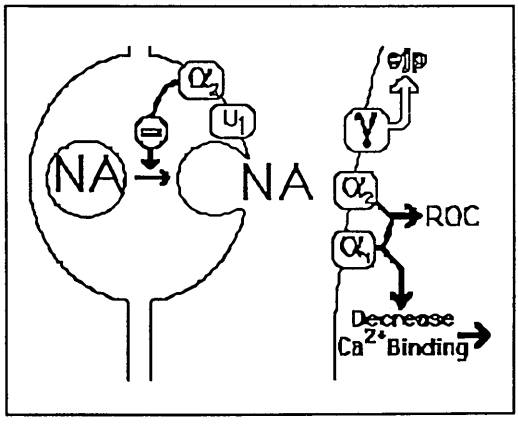
This representation of sympathetic neurotransmission shows how NA and ATP can act as co-transmitters. NA activates $alpha_1$- and $alpha_2$-adrenoceptors located on the effector smooth muscle cell to operate the receptor operated channels (R.O.C.s) as well as decreasing calcium binding. Both of these routes result in an increased free intracellular calcium concentraction since activation of ROCs allows calcium entry. This concept of having both types of alpha-adrenoceptor intra-junctionally has largely superceded the suggestion that only the $alpha_1$-adrenoceptor lies at the neuroeffector junction with the $alpha_2$-adrenoceptor located extra-junctionally. ATP acts through P_2-purinoceptors evoking excitatory junction potentials (e.j.p.s) and opening slow calcium channels to propagate action potentials, thus leading to contraction of the smooth muscle via potential operated channels (POCs) which allow calcium entry.

Another proposed mechanism exists but this time only one transmitter is released (NA) which acts through the $alpha_1$- and $alpha_2$-adrenoceptors or through a third type of adrenoceptor termed gamma-adrenoceptor. Gamma-adrenoceptor activation leads to smooth muscle contraction through the same pathway proposed for P_2-purinoceptor activation.

Sympathetic Neurotransmission



Gamma-adrenoceptor Hypoth.



Intra- & Extra-junctional Hypoth.

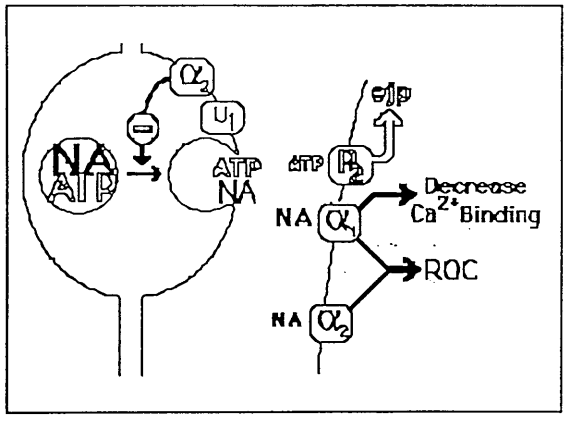
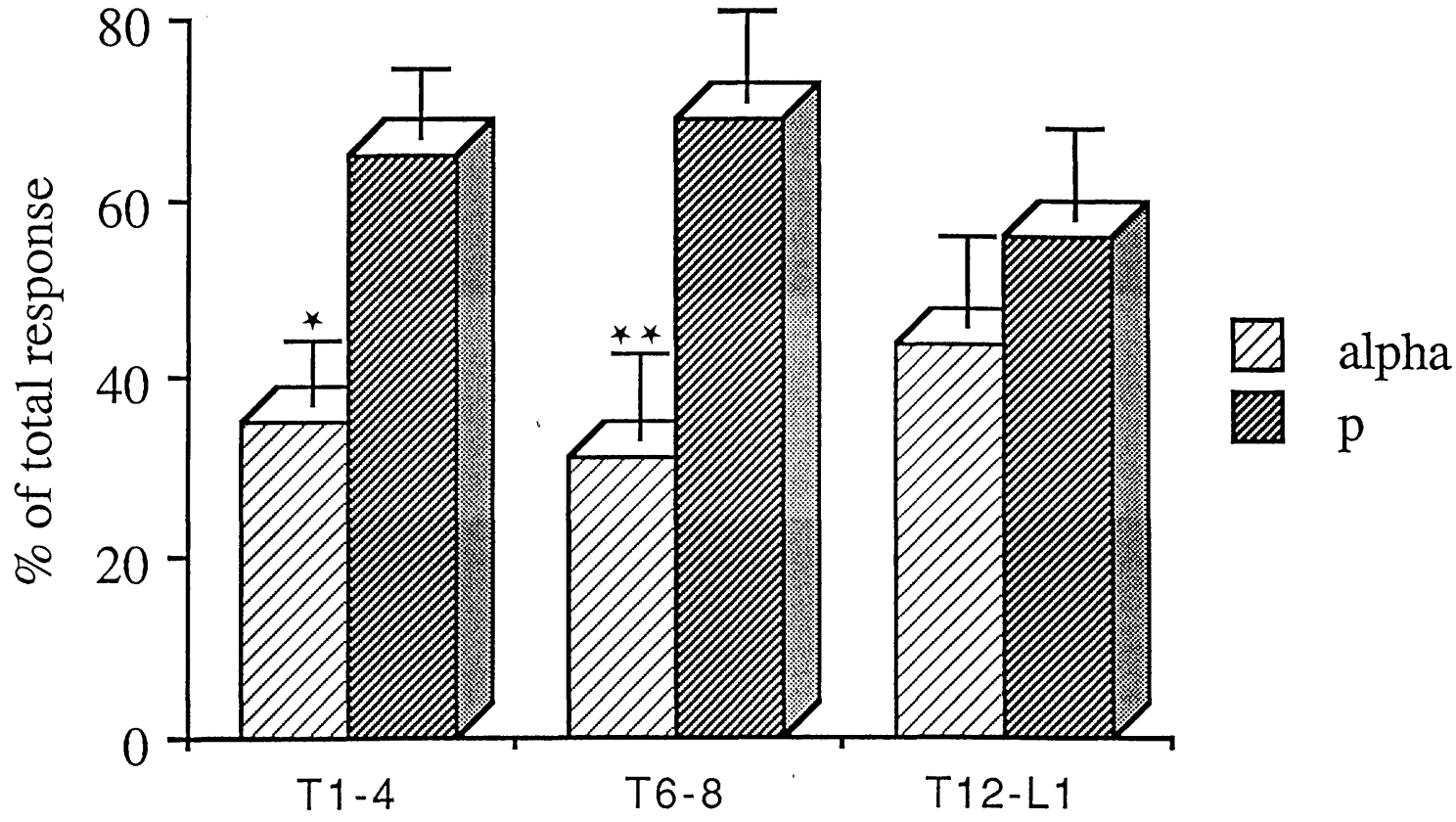


Figure 2:24

The percentage contribution of the adrenergic versus the purinergic component (the component remaining after adrenoceptor blockade) to the overall size of the pressor response to stimulation of the sympathetic outflow (1cm electrode, 20Hz, 1 sec, 0.05ms pulse width, 40v) at different regions of the spinal outflow (T_1 - T_4, T_6 - T_8, and T_{12} - L_1). $n>6$


80
60
40
20
0
% of total response
*
**
T1-4
T6-8
T12-L1
alpha
p

SECTION III

NERVE MEDIATED DEPRESSOR RESPONSES

Electrical stimulation of the spinal outflow in the pithed rat preparation produces vasopressor responses when the 1cm electrode tip of the pithing rod lies between the regions C6 to L5, or when the full sympathetic outflow is stimulated with the whole pithing rod as an electrode (Gillespie et al, 1970). When the sacral outflow only is stimulated, the changes in systemic arterial blood pressure are depressor (Dusting & Rand, 1972; McGrath, 1973) (Figure 1).

Depressor responses (Figure 3:1) were observed when the point of stimulation of the spinal outflow was between regions L5 and S2. In this region the vasopressor responses initiated by sympathetic nerve stimulation were either completely absent or were so small that the overall effect observed was a decrease in blood pressure. These depressor responses were not large when viewed against the systemic blood pressure, but they were reproducible and distinct. No depressor responses were found outside this region. There were no accompanying changes in heart rate with the decreases in blood pressure.

The stimulatory parameters used to obtain the depressor responses were 5 - 20 Hz frequency, 0.5ms pulse width, 40v, for 1 second. The responses were frequency dependent and maximal at 20Hz frequency (Figure 3:2) and reached a plateau approximately 10 seconds after the beginning of stimulation. Blood

pressure returned to the pre-stimulus control level between 30 seconds and 3 minutes after nerve stimulation had stopped, depending on frequency of stimulation.

Effect of adrenergic and cholinergic antagonists

In the absence of any antagonists the depressor response obtained at 20Hz was approximately 8.0mmHg. Atropine (1mg/kg), prazosin (1mg/kg), propranolol (1mg/kg), and rauwolscine (1mg/kg) given intravenously had no effect on the depressor responses either on their own or when administered sequentially (Figure 3:3). This confirms that these responses are non-adrenergic and non-cholinergic (McGrath, 1973; Gardner, 1977; Gardner & McGrath,1978).

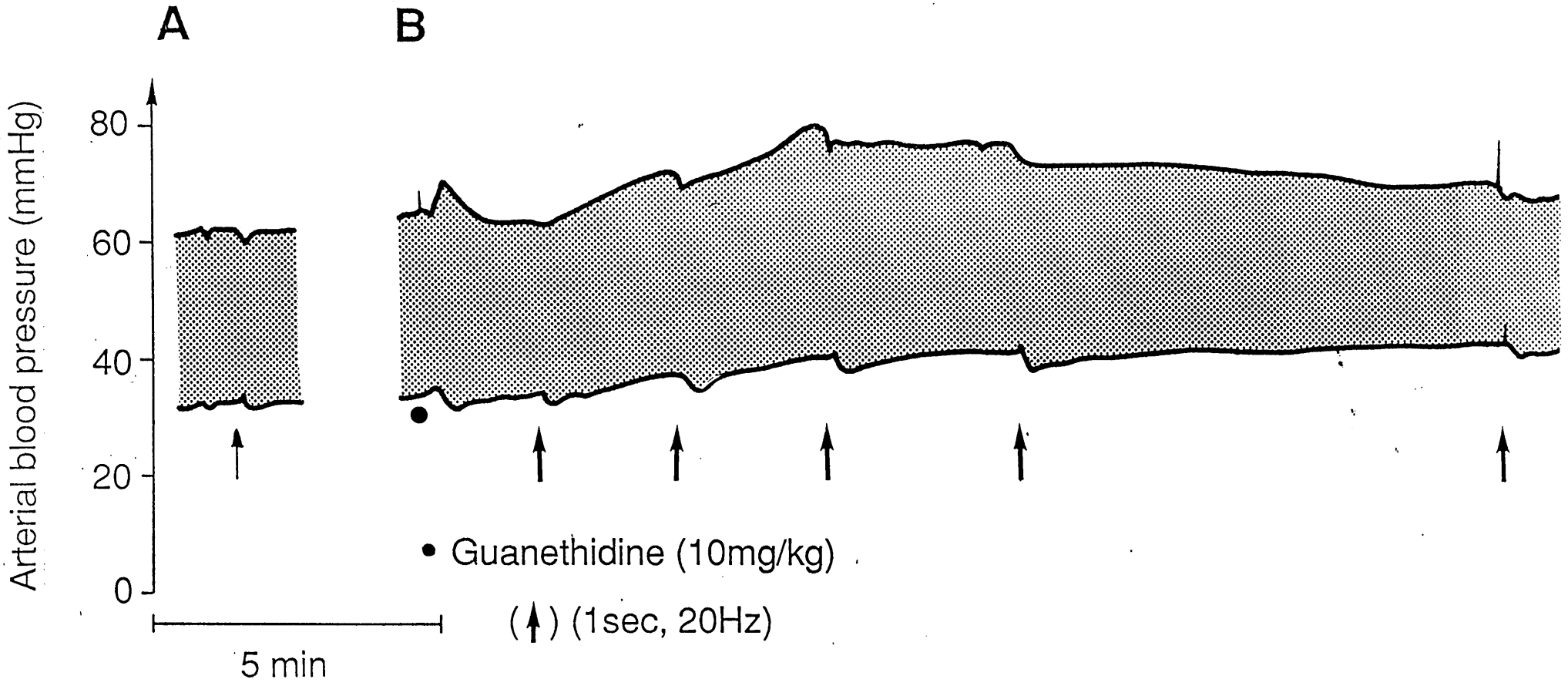
Effect of guanethidine

In the absence of other antagonists, the adrenergic neurone blocking agent guanethidine (10mg/kg) produced, on intravenous bolus injection, a large pressor response and accompanying increase in heart rate. These effects are due to its indirect sympathomimetic action.

Guanethidine (10mg/kg) did not attenuate the nerve mediated depressor responses and in most cases increased the drop in blood pressure caused by nerve stimulation (Figure 3:1). In conditions of adrenergic and cholinergic blockade, the subsequent administration of

Figure 3:1

Stimulation of the sacral outflow (L_5 - S_2, 20Hz, 1sec, 0.5ms) produces a depressor response. These responses increase in magnitude after the addition of guanethidine (•)(10mg/kg).


A
B
Arterial blood pressure (mmHg)
80
60
40
20
0
5 min
• Guanethidine (10mg/kg)
(↑) (1sec, 20Hz)

guanethidine (10mg/kg) did not produce a large pressor response since its indirect sympathomimetic action had been blocked by the adrenergic antagonists. Again the effect of guanethidine was to increase the size of the depressor response (Figure 3:4).

Effect of gallamine

Gallamine (10mg/kg i.v.) was added to prevent skeletal muscle twitching on stimulation of the spinal outflow. In all but 3 out of 18 animals studied, the intravenous administration of gallamine (10mg/kg) did not affect the nerve induced depressor responses. In the 3 animals that were affected by gallamine, no further depressor responses were noted in these preparations after gallamine addition.

Effect of purinergic antagonists

Neither the P_1-purinoceptor antagonist 8PT (10mg/kg) nor the P_2-purinoceptor desensitising agent mATP, when administered according to the desensitising schedule as discussed previously, had any significant effect on the nerve induced depressor responses, when used either on their own or in conditions of adrenergic and cholinergic blockade (Figure 3:5 & 3:3).

Figure 3:2

Stimulation of the sacral outflow (L_5 - S_2, 1sec, 0.5ms) in the absence of antagonists, produces depressor responses. These responses are frequency dependent,as illustrated by plotting the decrease in blood pressure produced by nerve stimulation (mmHg) against the frequency of stimulation. (n=6)

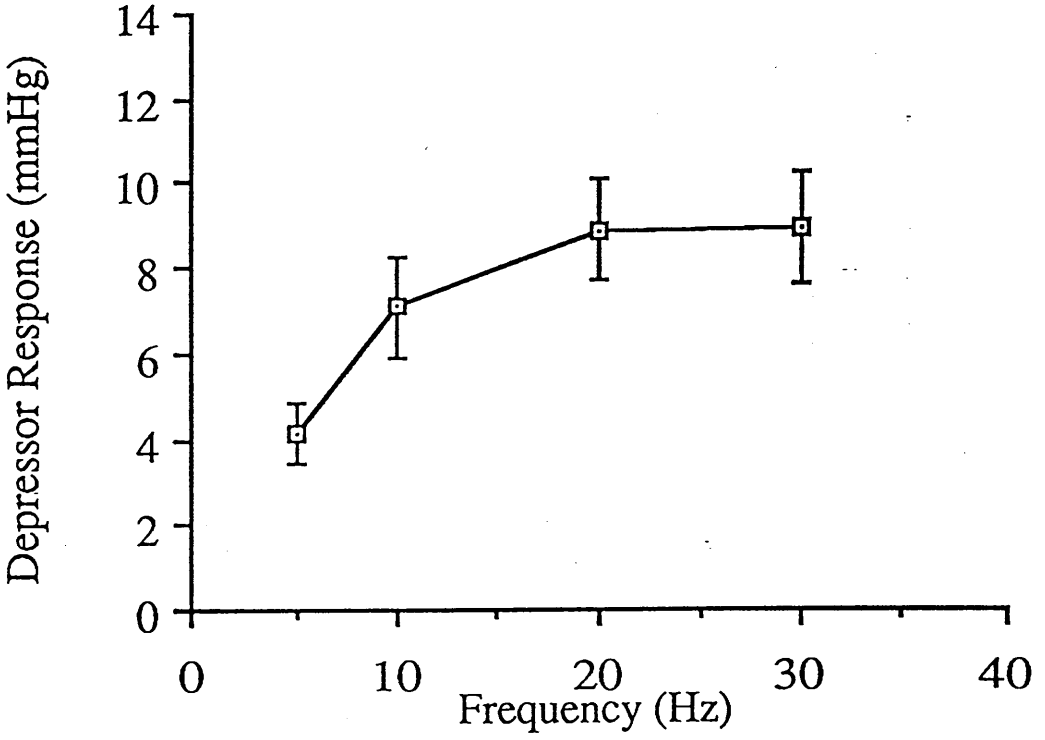

Depressor Response (mmHg)
14
12
10
8
6
4
2
0
0
10
20
30
40
Frequency (Hz)

Figure 3:3

Stimulation of the sacral outflow (L_5 - S_2, 20Hz, 1sec, 0.5ms) produces depressor responses which are unaffected by the sequential administration of prazosin (1mg/kg), propranolol (1mg/kg), rauwolscine (1mg/kg), atropine (1mg/kg), and finally α,β-methyene ATP (final concentration of 2.5mg/kg). (n=5)

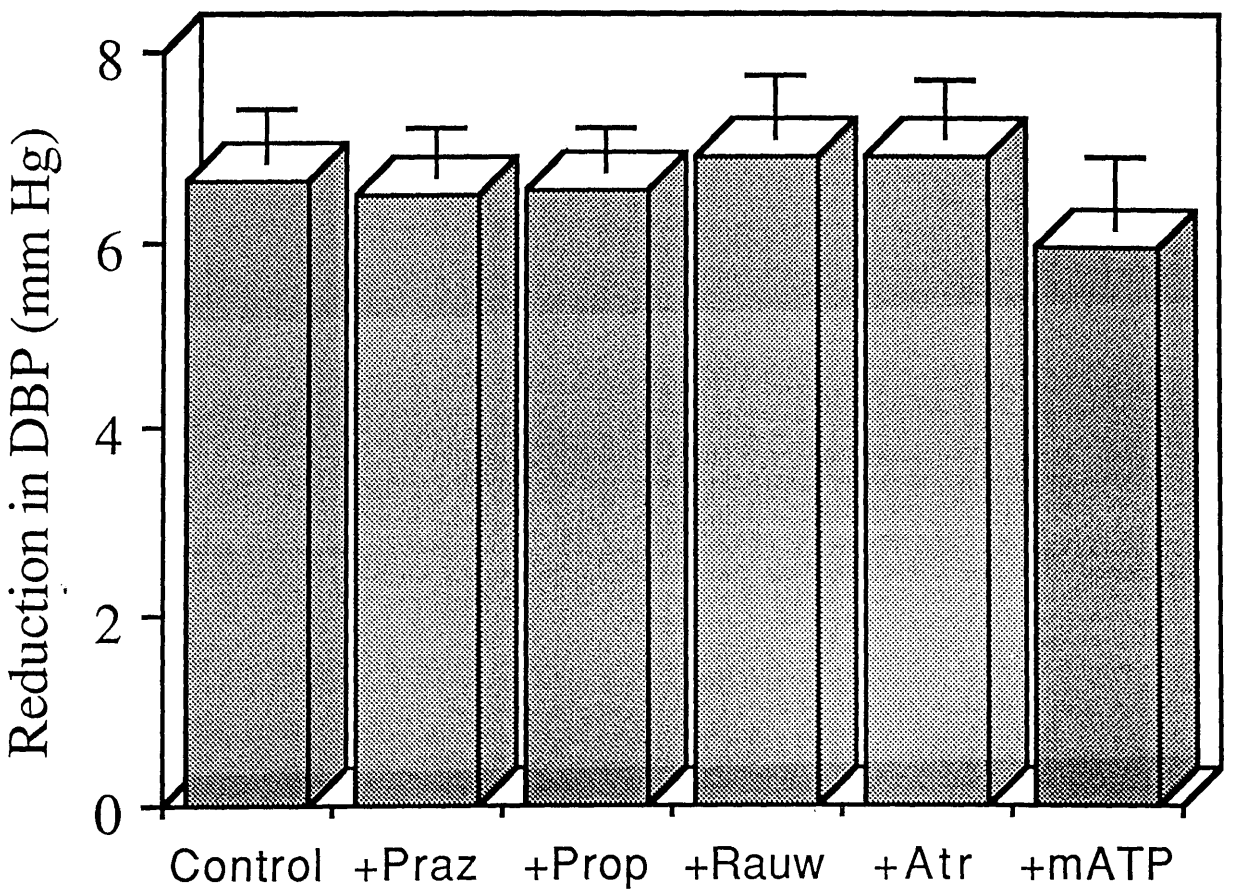

Reduction in DBP (mm Hg)
8
6
4
2
0
Control
+Praz
+Prop
+Rauw
+Atr
+mATP

Figure 3:4

This graph plots the depressor response (mmHg) against frequency of stimulation (L_5 - S_2, 1sec, 0.5ms) in control conditions (□)(n=10), after the addition of guanethidine (10mg/kg)(◆) (n=5), and the effect of guanethidine after adrenergic blockade (propranolol, prazosin and rauwolscine (each at 1mg/kg) (n=5)(■).

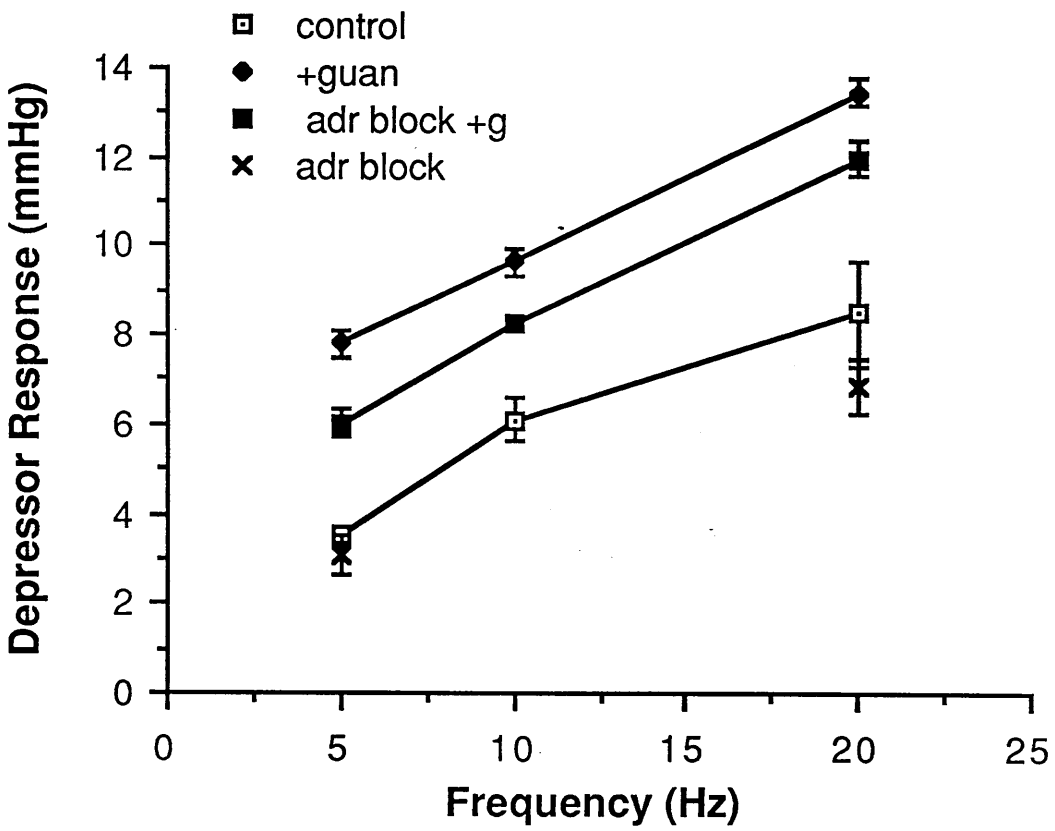
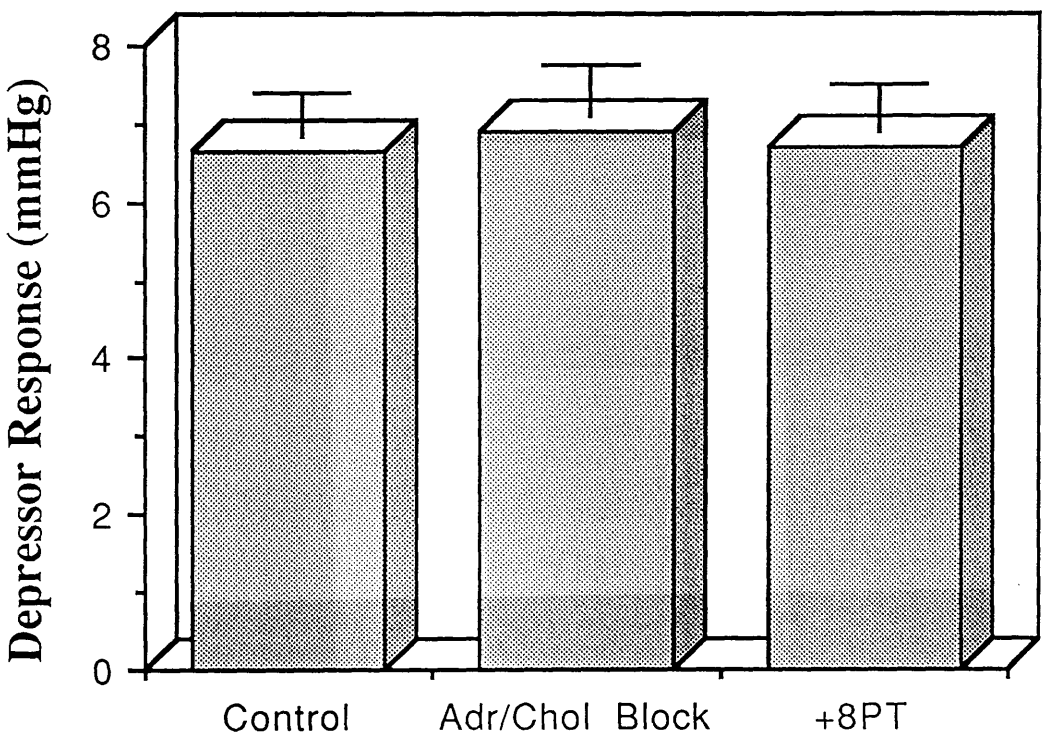

control
+guan
adr block +g
adr block
Depressor Response (mmHg)
14
12
10
8
6
4
2
0
0
5
10
15
20
25
Frequency (Hz)

Figure 3:5

Nerve mediated depressor responses (L_5 - S_2, 20Hz, 1sec, 0.5ms) plotted as the reduction in blood pressure (mmHg) is unaffected by the addition of 8PT(10mg/kg) in conditions of adrenergic and cholinergic blockade. n=4


Depressor Response (mmHg)
8
6
4
2
0
Control
Adr/Chol
Block
+8PT

Localisation of depressor response

The nerves mediating the vasodepressor response were found to have a spinal origin from L_5 to S_2 and within this region were found to be of the same magnitude wherever the electrode was placed. Attempted laminectomies in the lower lumbar and upper sacral regions were unsuccessful in abolishing the vasodepressor responses and therefore a more precise localisation of spinal outflow was not achieved.

In determining whether a specific vascular bed was innervated by the vasodepressor nerves, the effect of occluding the circulation of blood in different areas was investigated. This showed that whilst occluding blood flow to the hind limbs by ligating the femoral arteries had no effect on the depressor response, either completely removing the lower gut or occluding its blood supply by ligating the abdominal aorta just below the renal arteries but above the superior mesenteric artery, was successful in completely abolishing the depressor responses.

However occlusion of the large vascular bed of the gut itself produced a decrease in the resting blood pressure which might have masked any nerve-mediated depressor responses. The addition of guanethidine

increased the blood pressure to a level that was higher than before mesenteric occlusion. Vasodepressor responses did not return with this increase in blood pressure resting level.

SECTION IV

AN INVESTIGATION OF PURINERGIC INVOLVEMENT IN NERVE MEDIATED BRONCHOCONSTRICTION AND BRONCHODILATION IN THE ANAESTHETISED CAT.

The innervation of the lung and airways consists of three neuronal components: the cholinergic, the noradrenergic and the nonadrenergic noncholinergic inhibitory component. In the cat, as well as in man, this NANC system appears to provide the major inhibitory neural input to airways smooth muscle (Barnes, 1986). The study of the NANC component was therefore conducted in the anaesthetised cat.

When resting bronchial smooth muscle tone is low, vagal stimulation causes a cholinergic bronchoconstriction (Schwieler, 1966). However when the resting tone is high, stimulation of the vagus causes a non-adrenergic non-cholinergic bronchodilation (Diamond & O'Donnell, 1980; Irvin et al, 1980).

In the experiments where the bronchodilatory responses were studied, 5HT was continuously infused (5 - 20 μg/kg/min) into the cannulated left femoral vein to increase bronchial tone, by acting directly on the bronchial smooth muscle.

After tracheal intubation the cats were artificially ventilated on room air and respiratory measurements of airflow and transpulmonary pressure were made. Figure 4:1 illustrates the parameters measured in these experiments. Dynamic lung compliance and airways resistance could then be calculated from the resulting traces of these signals (Figure 4:2).

Figure 4:1

This is an illustration of the parameters measured in these experiments. The top trace is arterial blood pressure measured in mmHg. The second trace is a measure of transpulmonary pressure (TPP) in cm H_2O. Tidal volume (V_T) is also recorded. The airflow recording shows the positions of 0 - 80mls/sec flow corresponding to the expiratory part of the respiratory cycle. Time is displayed in seconds.

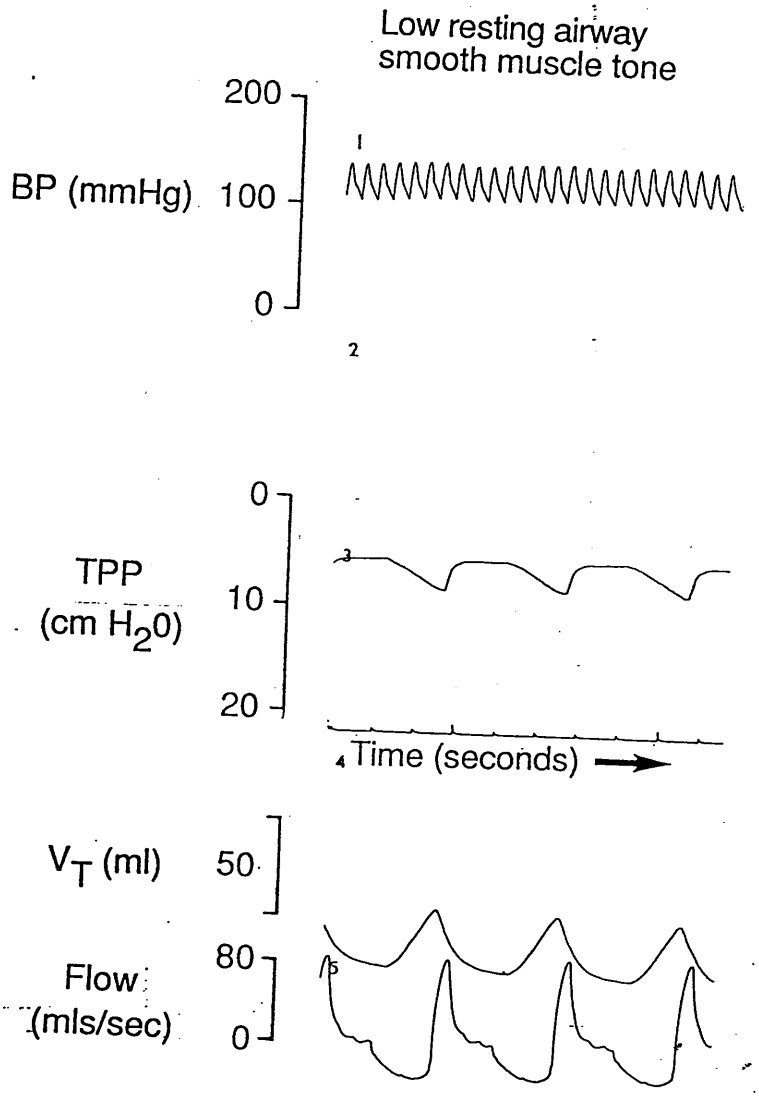

Low resting airway
smooth muscle tone
200
BP (mmHg)
100
0
1
2
0
TPP
(cm H_2O)
10
20
3
4 Time (seconds)
V_T (ml)
50
80
Flow
(mls/sec)
0
5
6

Figure 4:2

These traces are lettered according to different points in the respiratory cycle, enabling respiratory measurement of dynamic lung compliance (Panel I) and airway resistance (Panel II) to be calculated.

Compliance is measured at the beginning and end of inspiration when no air is being moved in or out (ie. no flow).

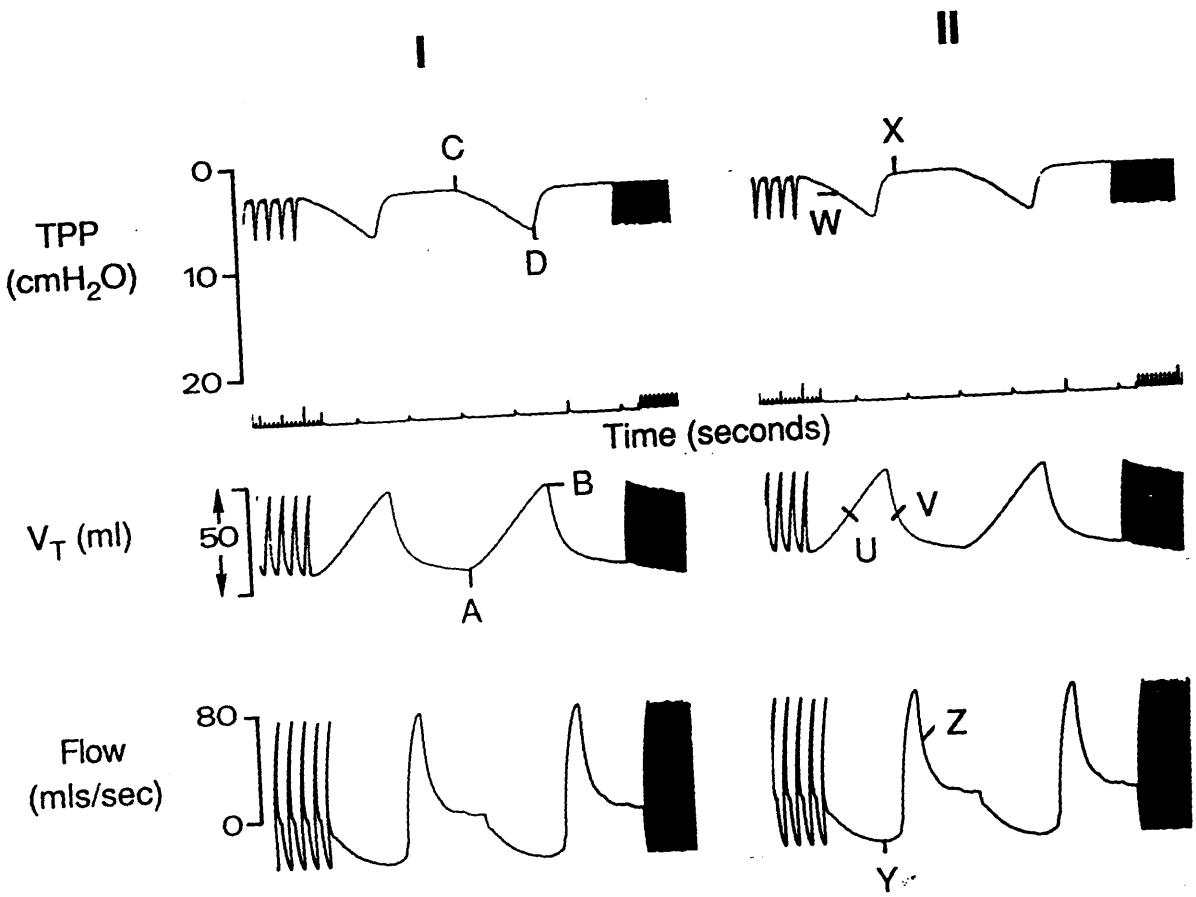
$$C_{dyn} = \frac{\text{Tidal Volume}}{\text{Change in Pressure}}$$

On the trace, A - B= the amount of air inspired
C - D= the corresponding change in pressure (TPP) during inspiration.

Resistance is calculated at points of equal lung volume (U & V), where elastic forces are approximately equal.

$$\text{Resistance} = \frac{\text{Change in Pressure (W - X)}}{\text{Change in Flow (Y - Z)}}$$

On the trace, W & Y lie at the corresponding positions in the pressure cycle and flow cycle respectively as the point U in the tidal volume. X & Z correspond to point V.


I
II
TPP
(cmH2O)
0
10
20
C
D
X
W
Time (seconds)
VT (ml)
50
B
A
U
V
Flow
(mls/sec)
80
0
Z
Y

Bronchoconstriction and bronchodilation cause changes in compliance and resistance. Compliance is a measure of the lung elasticity and reflects any changes in the lower airways, alveoli and pulmonary circulation. Changes in resistance are associated with changes in the airflow signal which in turn reflect changes in the upper airways. Mean values of TPP levels, resistance and compliance readings, for 12 preparations, in low resting airway smooth muscle tone and in high resting tone (produced by infusing 5HT) are given in Figure 4:3.

Bronchoconstriction can most easily be shown as increases in the TPP signal, and bronchodilation as decreases. This is because TPP (the pressure tending to inflate the lung) comprises both the resistant and compliant components. The cholinergic agonist ACh is a bronchoconstricting agent and the intravenous administration of this agonist leads to increases in TPP signals (Figure 4:4).

Profiles of agonists on the airways.

Intravenously administered ACh (10µg/kg - 100µg/kg), given either via jugular or femoral veins, (10µg/kg - produced large reproducible bronchoconstrictions (Figure 4:4). These responses were dose-dependent (Figure 4:19) and were completely blocked by 1mg/kg atropine.

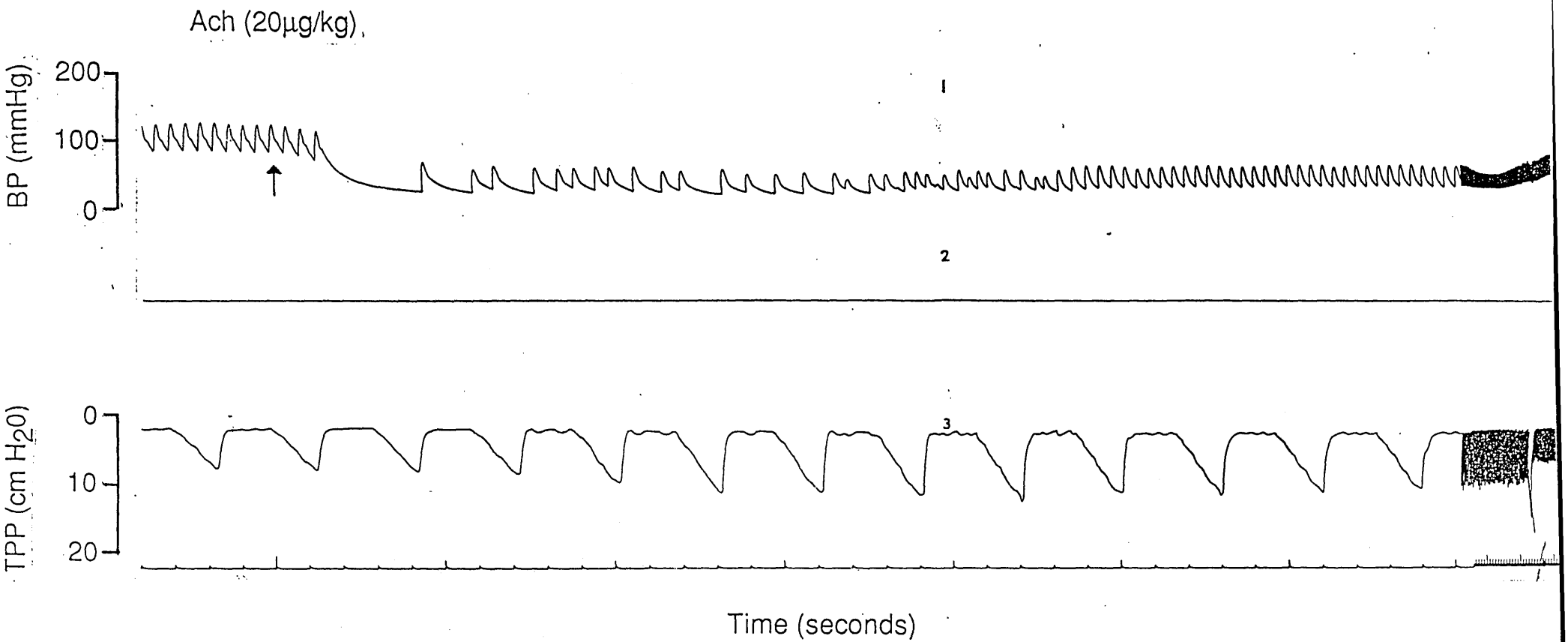
Figure 4:3

Mean values (N = 12) of TPP, resistance and compliance in the artificially respired animal in conditions of both low and high resting tone (as produced by 5HT infusions).

	TPP (cm H_2O)	Resistance (cm H_2O/l/sec)	Compliance (ml/cm H_2O)
Low Resting Tone	3.96 $\pm$ 1.3	26.5 $\pm$ 3.7	12.7 $\pm$ 2.4
High Resting Tone	20.9 $\pm$ 4.7	212 $\pm$ 33.2	5.4 $\pm$ 1.4

Figure 4:4

The intravenous administration of ACh (20ug/kg) (↑) produces a decrease in the blood pressure and heart rate as well as increasing the TPP signal.


Ach (20µg/kg)
BP (mmHg)
200
100
0
1
2
TPP (cm H_2O)
0
10
20
3
Time (seconds)

The intravenous administration of adenosine resulted in marked bronchoconstriction (Figures 4:20 & 4:21). This was slow in onset and long lasting and required clamping of the outlet of the respiration pump to force open the lungs before beginning to return to resting level. This bronchoconstriction was potentiated by dipyridamole (10mg/kg) (Figure 4:22) and was antagonised by either 8PT (10mg/kg) (Figure 4:22) or aminophylline (10mg/kg). The administration of the adenosine analogue 2-Chloroadenosine (1mg/kg) also produced bronchoconstriction, whereas inosine (a breakdown product of adenosine) at dose levels up to 10mg/kg had no effect on the resting levels of TPP, resistance or compliance.

Both 8PT (10mg/kg) and mATP (0.1mg/kg & 0.5mg/kg) caused initial evanescent bronchoconstriction immediately after their administration. The intravenous administration of mATP produced bronchoconstriction in both low and high tone (Figure 4:23 & 4:24). The bronchoconstrictor responses to mATP were subject to tachyphylaxis. Another effect noted on administering mATP was an increased mucus secretion from the lung.

8PT produced bronchoconstriction in both low and high tone, which was not blocked by aminophylline pretreatment. The responses produced by 8PT were not consistent and were sometimes accompanied by decreases in blood pressure (Figures 4:25 & 4:26). It was also

noted in some preparations in conditions of high resting tone that additional increases in tone caused by 8PT administration (10mg/kg) led to the onset of spontaneous breathing. The PEG/ NaOH vehicle for 8PT administration, was tested separately and had no effect on the lung.

The parameters of stimulation used for producing bronchoconstriction and bronchodilation.

Stimulation of the vagal nerve bundle at 15Hz/25v for 30sec at 0.5ms pulse width resulted in either bronchoconstriction (when the airway smooth muscle has low resting tone) (Figure 4:5) or bronchodilation (in high resting tone) (Figure 4:6).

For the investigation of the NANC component of the nerve induced bronchodilation, the cervical vagosympathetic nerve bundle (containing both sympathetic and parasympathetic nerves) was stimulated rather than just the parasympathetic branch (the vagus) and alpha and beta adrenoceptor antagonists were added to block any adrenergic effects. Phentolamine could not be used to block $alpha_1$ and $alpha_2$-adrenoceptors since it has anti-5HT activity and therefore reduced bronchial tone induced by the 5HT infusion. Thus prazosin was used to block $alpha_1$-adrenoceptors and either idazoxan or pretreatment with rauwolscine to block $alpha_2$-adrenoceptors.

Figure 4:5

Panel A shows a bronchoconstriction to stimulation of the vagus at 15Hz / 25v for 30 seconds (pulse width 0.5ms) in conditions of low resting airway smooth muscle tone and in the presence of propranolol (1mg/kg).

Panel B shows the response after the administration of the P_1-purinoceptor antagonist 8PT.

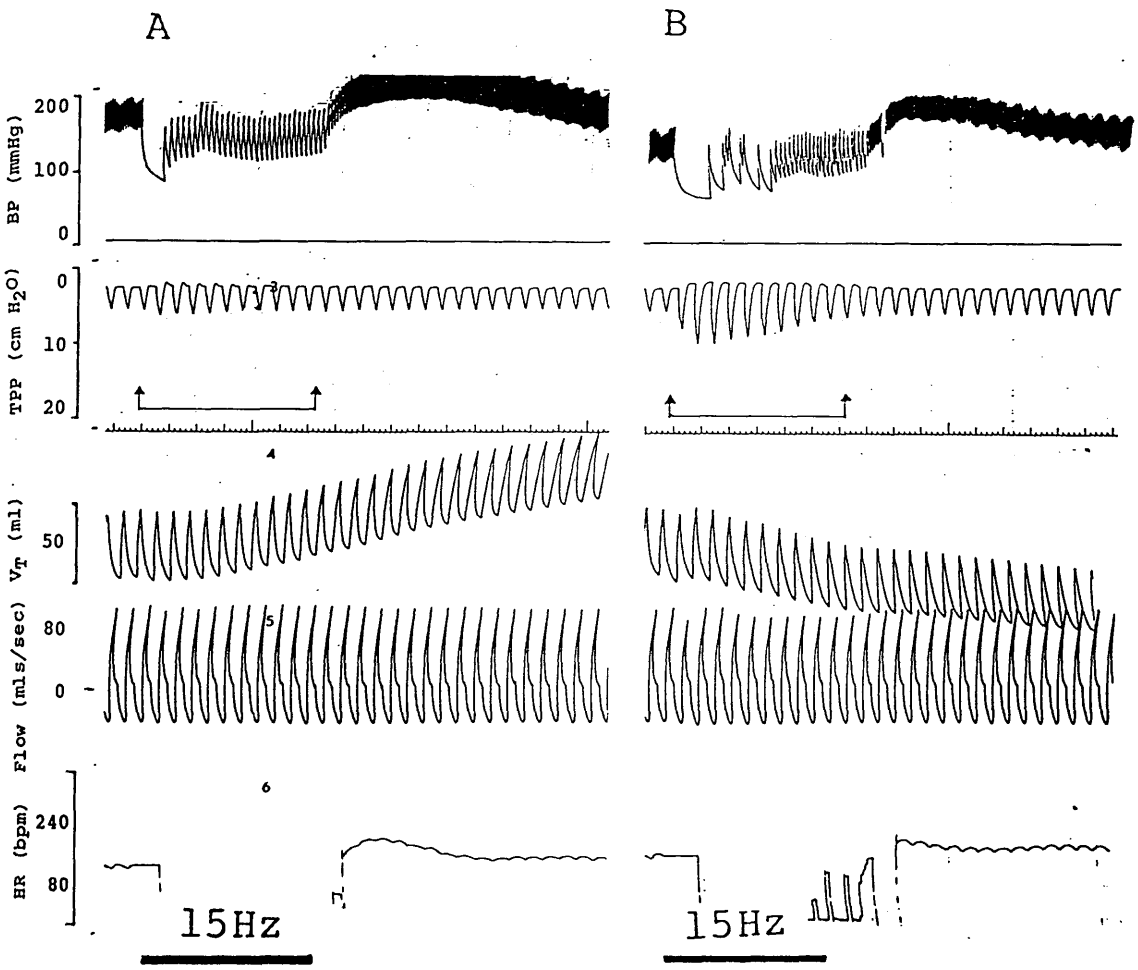
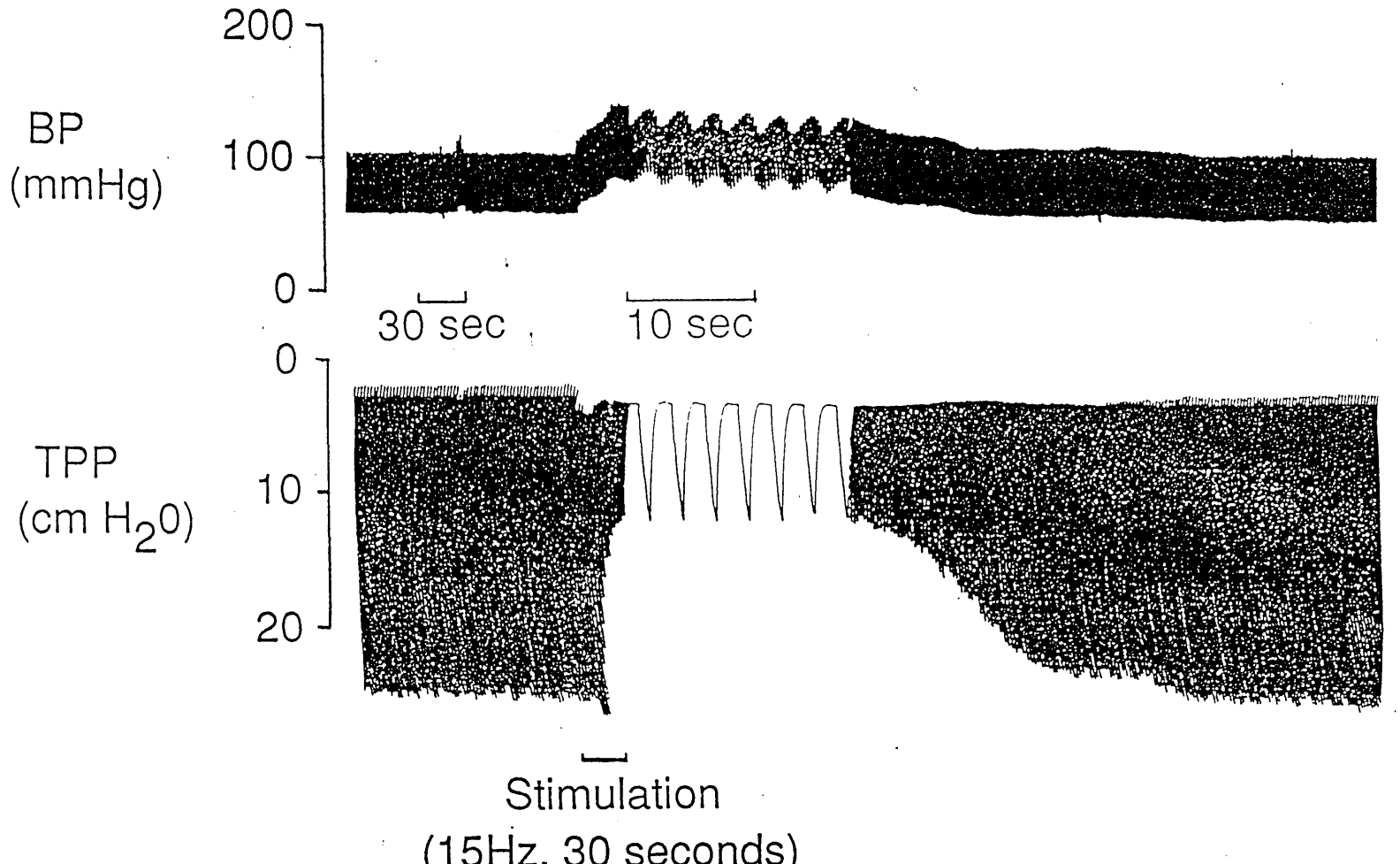

A
B
BP (mmHg)
200
100
0
TPP (cm H_2O)
0
10
20
V_T (ml)
50
Flow (mls/sec)
80
0
HR (bpm)
240
80
15Hz
15Hz

Figure 4:6

This bronchodilation was produced by stimulation of the vagal bundle at 15Hz for 30 seconds in a cat undergoing 5HT infusion to increase the bronchial tone. The cat was pretreated with atropine (1mg/kg). No other antagonists were present.


200
BP
(mmHg)
100
0
30 sec
10 sec
0
TPP
(cm H_2O)
10
20
Stimulation
(15Hz, 30 seconds)

Nerve induced bronchoconstriction in low resting airway smooth muscle tone: An investigation of the cholinergic and adrenergic involvement.

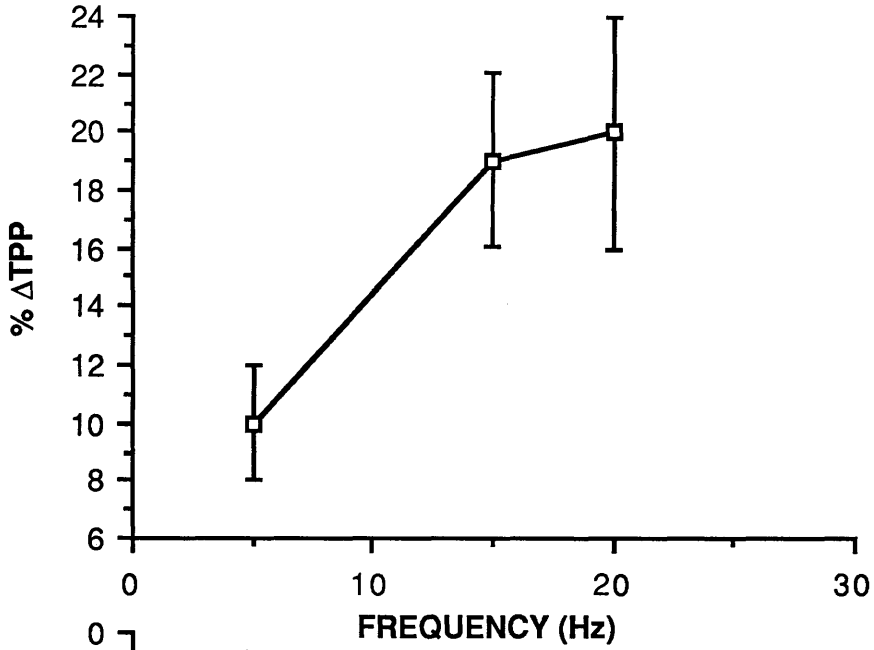
Stimulation of the vagus in conditions of low resting airway smooth muscle tone and in the absence of any antagonists produced bronchoconstriction (Figure 4:5). The degree of bronchoconstriction produced by nerve stimulation is frequency dependent (Figure 4:7) and is maximal around 15 Hz frequency. These bronchoconstrictory responses are comparable to those obtained by intravenous administration of ACh (Figure 4:4), with respect to time course and susceptibility to blockade by atropine (1mg/kg). The nerve induced bronchoconstriction was not blocked by adrenergic antagonists propranolol (1mg/kg), prazosin (1mg/kg) or rauwolscine (1mg/kg) but it was significantly enhanced after 8PT addition (10mg/kg) (Figure 4:5).

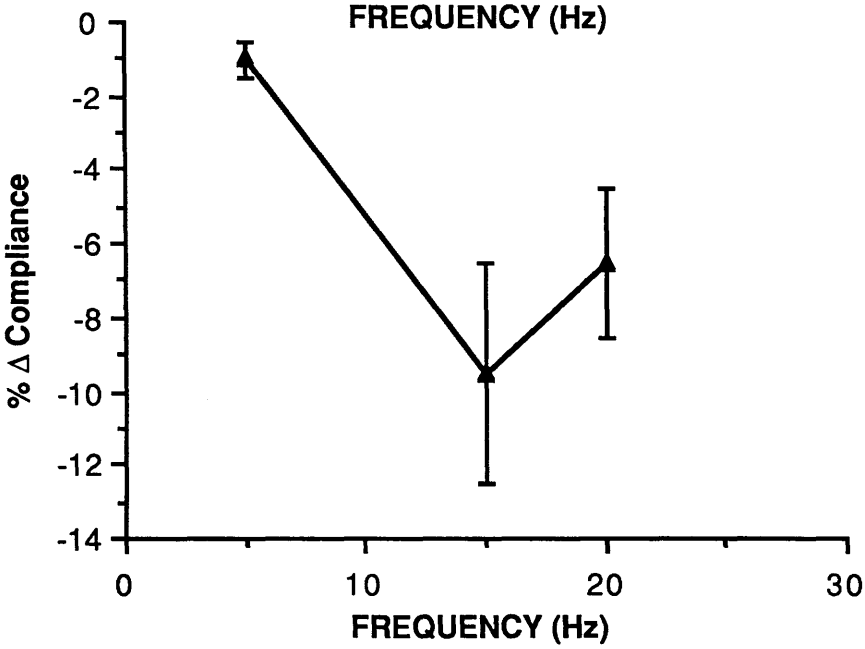
Nerve induced bronchodilation in high resting airway smooth muscle tone.

In conditions of high resting airway smooth muscle tone produced by infusing 5HT, stimulation of the sectioned left vagus nerve bundle, produced bronchodilatory responses which were frequency dependent. Maximal responses were obtained at frequencies between 15 and 20 Hz (Figure 4:8). These

Figure 4:7

All 3 graphs are frequency response curves to stimulation of the vagal bundle in conditions of low resting airway smooth muscle tone and are measured as percentage changes in TPP, compliance and resistance. Graphs i) and iii) are plotted as percentage increases in TPP and resistance, respectively and graph ii) as percentage decreases in compliance, from the prestimulus resting levels. (N=4)


24
22
20
18
16
14
12
10
8
6
% ΔTPP
0
10
20
30
FREQUENCY (Hz)


0
-2
-4
-6
-8
-10
-12
-14
% Δ Compliance
0
10
20
30
FREQUENCY (Hz)

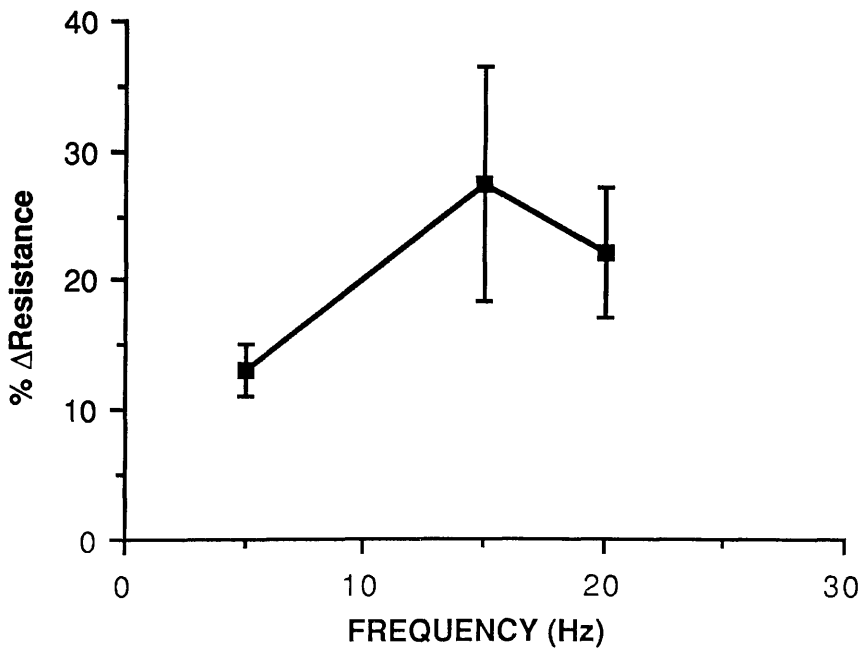
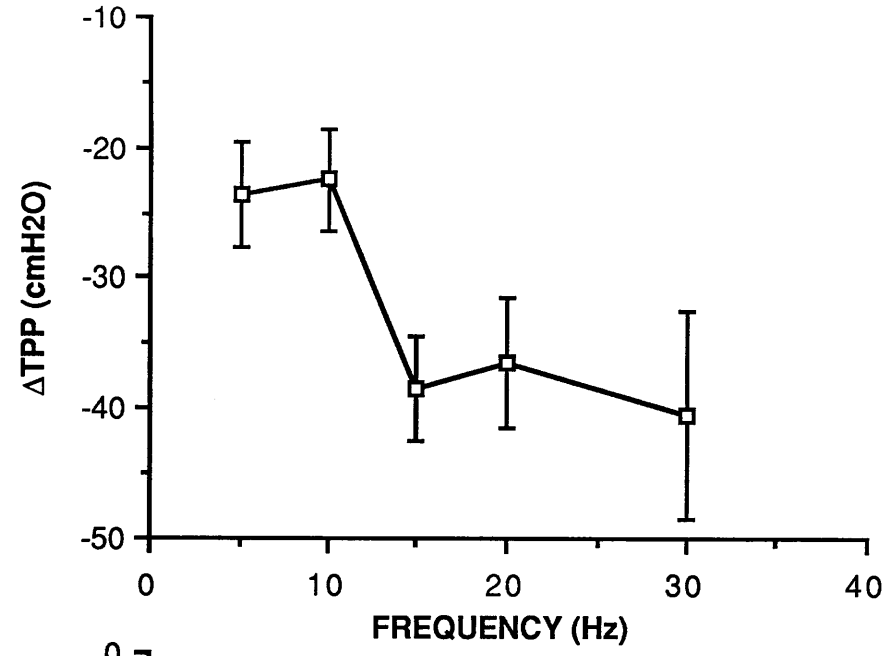
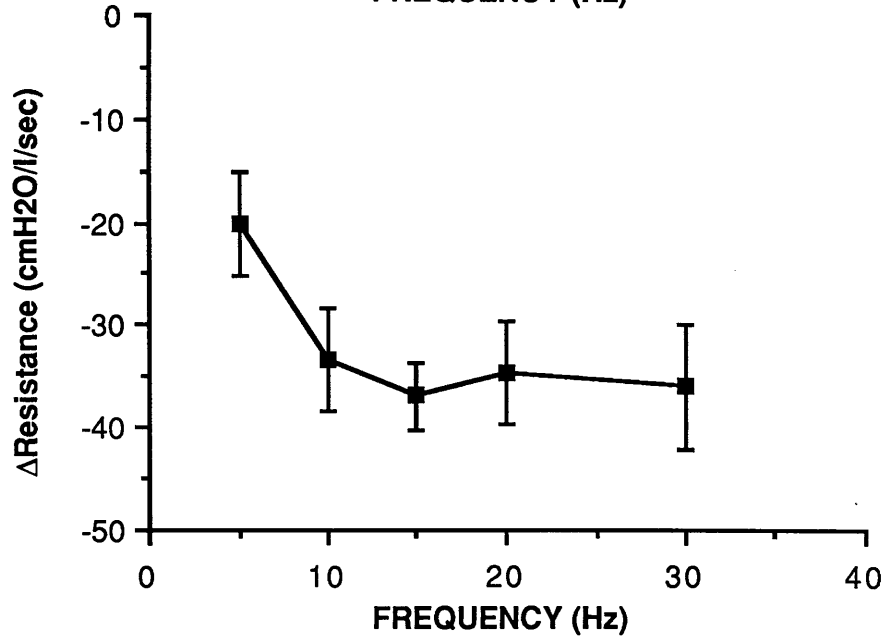
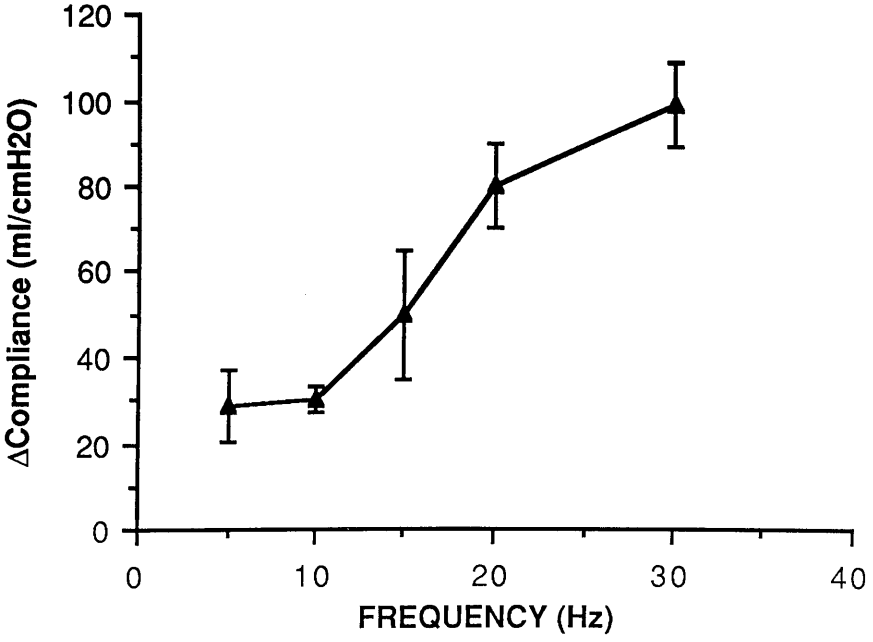

40
30
20
10
0
% ΔResistance
0
10
20
30
FREQUENCY (Hz)

Figure 4:8

All 3 graphs are frequency response curves to stimulation of the vagal bundle in conditions of high resting airway smooth muscle tone and are measured as percentage changes in TPP, resistance and compliance. Graphs i) and ii) are plotted as percentage decreases in TPP and resistance, respectively and graph iii) as percentage increases in compliance, from the prestimulus resting levels. (N=4)


-10
-20
-30
-40
-50
ΔTPP (cmH2O)
0
10
20
30
40
FREQUENCY (Hz)


0
-10
-20
-30
-40
-50
ΔResistance (cmH2O/l/sec)
0
10
20
30
40
FREQUENCY (Hz)


120
100
80
60
40
20
0
ΔCompliance (ml/cmH2O)
0
10
20
30
40
FREQUENCY (Hz)

bronchodilatory responses reached their maximum between 5 and 30 seconds after nerve stimulation had stopped and were not reduced by subsequent and sequential intravenous injections of atropine, propranolol, prazosin and idazoxan, each drug given at a dose levels of 1mg/kg (Figure 4:9).

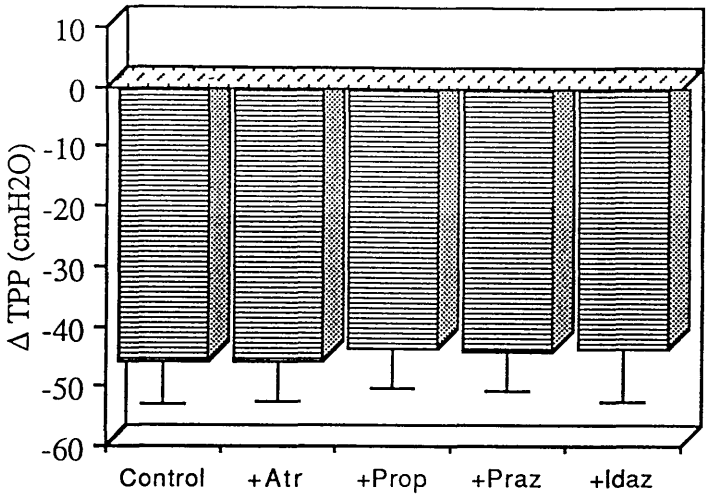
An investigation of purinergic involvement in the nerve mediated bronchodilator responses.

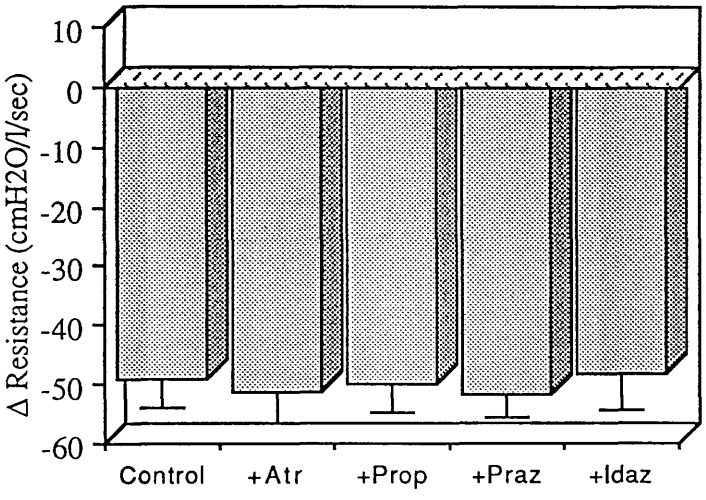
The P_1-purinoceptor antagonist 8PT (10mg/kg) caused a small reduction in the neurally evoked bronchodilatory response at 15Hz frequency in the presence of muscarinic and alpha and beta-adrenoceptor blockade (Figures 4:10;11;12;13). This was shown to be a dose-related inhibitory effect on bronchodilation in so far as 8PT at a dose level of 3mg/kg had no effect on the response but the higher dose of 10mg/kg did have. Following the administration of the adenosine uptake inhibitor dipyridamole (10mg/kg), it was found that 8PT markedly attenuated the bronchodilator response (Figure 4:14) at 15Hz when compared to 8PT's attenuation before dipyridamole addition. This combined dipyridamole/ 8PT attenuating effect was also observed at the submaximal frequency of 5Hz (Figures 4:15 & 4:16), but the bronchodilator response could not be completely blocked at either frequency.

Although the addition of dipyridamole (10mg/kg)

Figure 4:9

The sequential administration of adrenergic and cholinergic antagonists had no effect on the decreases in TPP and resistance, or increases in compliance from the control values for nerve induced bronchodilation responses (15Hz/25v/30sec/0.5ms pulse width) in conditions of high resting airway smooth muscle tone. (N=4)


10
0
-10
-20
-30
-40
-50
-60
Δ TPP (cmH2O)
Control
+Atr
+Prop
+Praz
+Idaz


10
0
-10
-20
-30
-40
-50
-60
Δ Resistance (cmH2O/l/sec)
Control
+Atr
+Prop
+Praz
+Idaz

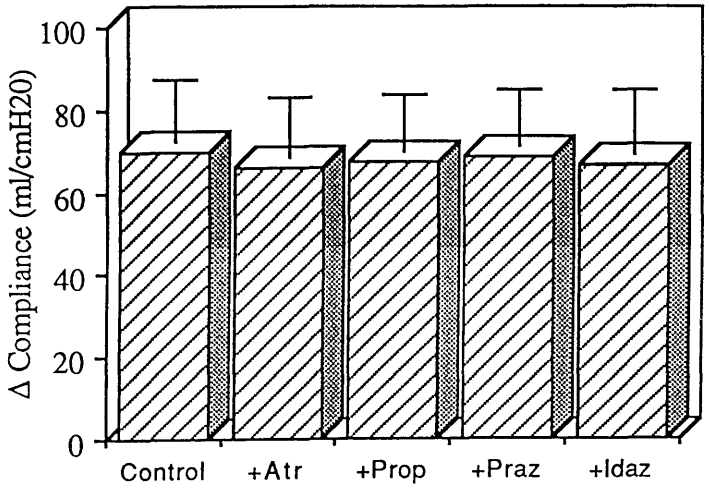

100
80
60
40
20
0
Δ Compliance (ml/cmH20)
Control
+Atr
+Prop
+Praz
+Idaz

Figure 4:10

Nerve induced bronchodilation responses (15Hz / 25v / 30sec / 0.5ms pulse width) in the presence of atropine (1mg/kg), propranolol (1mg/kg), prazosin (1mg/kg) and rauwolscine (1mg/kg), which were all administered before the start of 5HT infusion (Panel A); and after 8PT addition (10mg/kg).

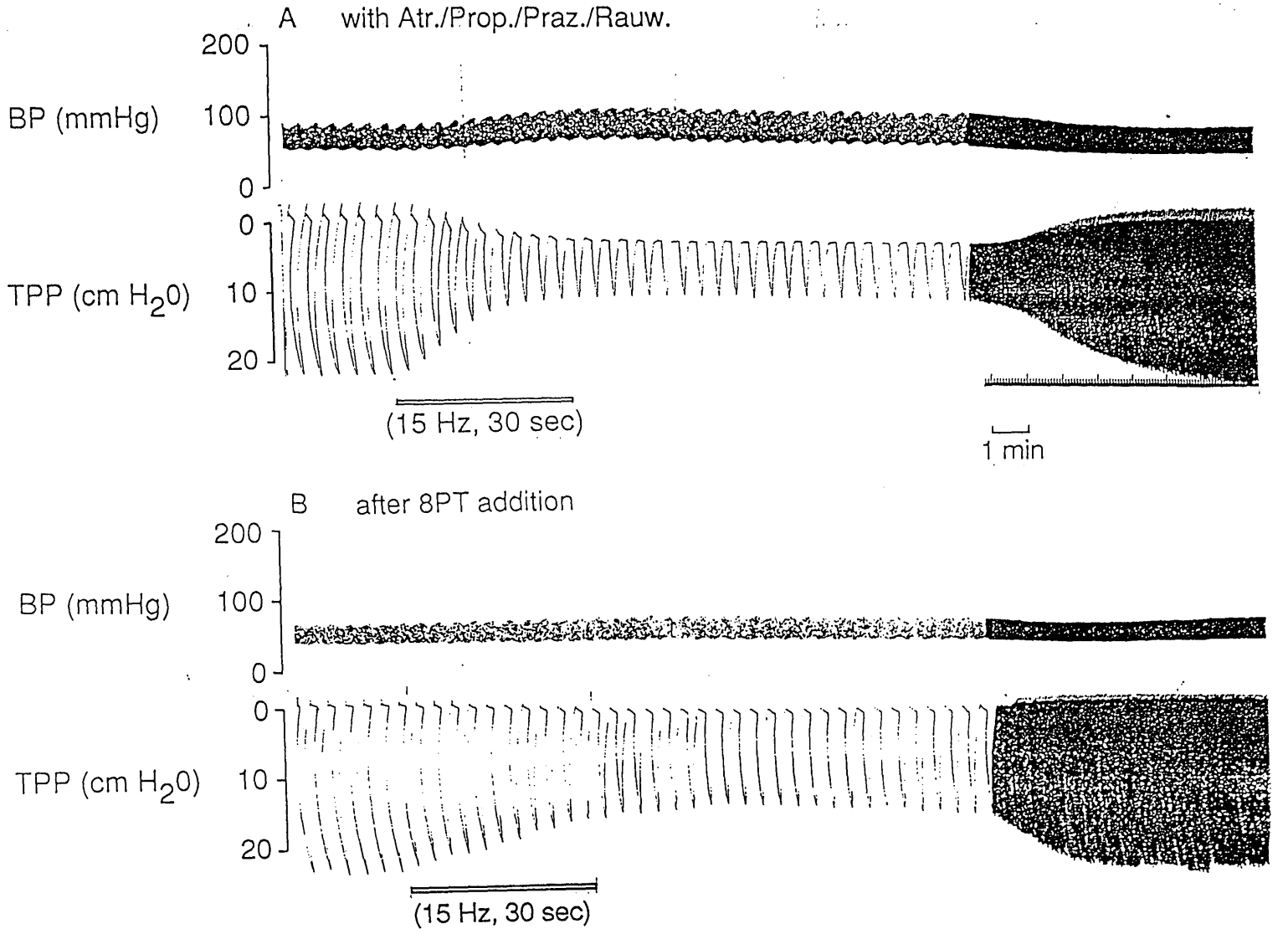

A with Atr./Prop./Praz./Rauw.
200
100
0
BP (mmHg)
0
10
20
TPP (cm $H_2$0)
(15 Hz, 30 sec)
1 min
B after 8PT addition
200
100
0
BP (mmHg)
0
10
20
TPP (cm $H_2$0)
(15 Hz, 30 sec)

Figure 4:11

In high resting airway smooth muscle tone, the change in TPP (cmH_2O) caused by vagal stimulation from the prestimulus resting level shows a significant difference when measured before and after the addition of 8PT (10mg/kg) (n=6).

CHANGE in TPP CAUSED by VAGAL STIMULATION from PRESTIMULUS RESTING LEVEL

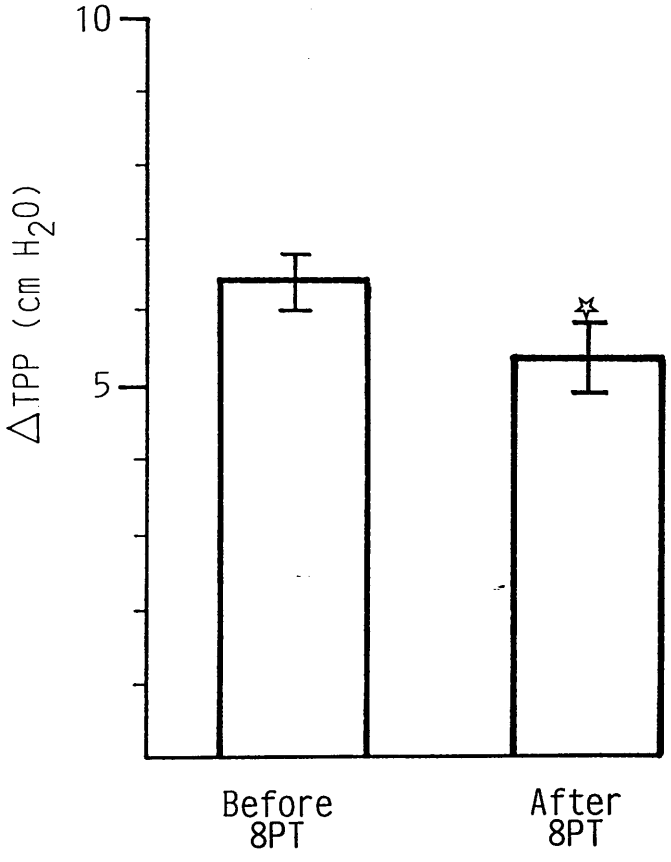


Figure 4:12

The percentage changes in TPP, resistance and compliance measurements caused by vagal stimulation (15 Hz) before and after 8PT (10mg/kg) in high resting airway smooth muscle tone. (n=6).

Differences in the change in TPP, resistance and compliance measurements caused by vagal stimulation before and after 8PT (10mg/kg). (n=6)

	Mean ΔTPP from prestimulus value	Mean Δresistance from prestimulus value	Mean Δcompliance from prestimulus value
Before 8PT	-41.5% ± 5.7	-57.0% ± 3.2	+66.0 ± 14.1
After 8PT	-30.8% ± 4.5	-39.5% ± 3.9	+42.8% ± 9.2
Significance level	*0.05	*0.035	0.268

Figure 4:13

The difference in TPP, resistance and compliance values caused by vagal stimulation (15Hz) before and after 8PT (10mg/kg) in high resting airway smooth muscle tone (n=6).

(cf. figure 4:15

* = significantly different from dipyridamole treated preparations)

The difference in TPP, resistance and compliance values caused by vagal stimulation before and after 8PT (10mg/kg). (n=6)

		TPP (cm H_2O)	Resistance (cm H_2O/l/sec)	Compliance (ml/cm H_2O)
Before 8 PT	Prestimulus value	16.4 ± 0.9	157.5 ± 21.9	2.94 ± 0.29
	Poststimulus value	9.8 ± 1.2	66.3 ± 6.5	5.04 ±0.71
	% age of Pre-stimulus value	58.5% ± 5.7	43.0% ± 3.2	166.0% ±14.1
After 8PT (10mg/kg)	Prestimulus value	19.5 ± 2.1	174.8 ± 29.0	2.60 ± 0.39
	Poststimulus value	14.0 ± 2.2	109.0 ± 22.6	3.80 ± 0.80
	% age of Pre-stimulus value	69.2% ± 4.5 *	60.5% ± 3.9 *	142.8% ±9.20 *

Figure 4:14

The change in TPP (cmH_2O) caused by vagal stimulation (15Hz) from the prestimulus resting level is measured in single animals in conditions of muscarinic and alpha- and beta-adrenergic blockade, and in the presence of dipyridamole (10mg/kg) before and after the addition of 8PT (10mg/kg).

CHANGE in TPP CAUSED by VAGAL STIMULATION from PRESTIMULUS RESTING LEVEL

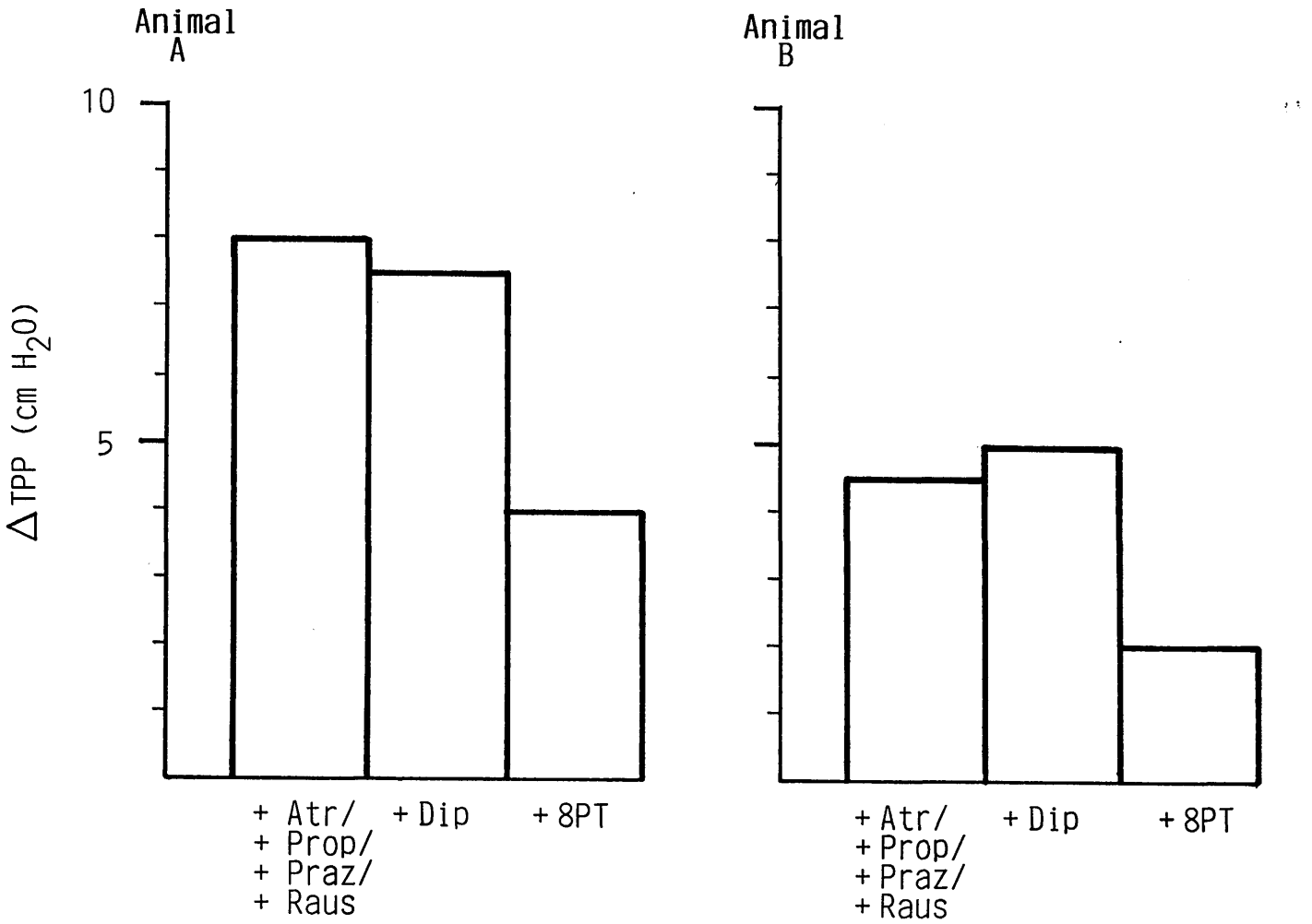


Figure 4:15

Differences in the change in TPP, resistance and compliance measurements caused by vagal stimulation (5 Hz) in conditions of muscarinic and alpha- and beta-adrenergic blockade and in the presence of dipyridamole (10mg/kg) before and after 8PT (10mg/kg) (n=4).

(cf. figure 4:13

* = significant difference)

		TPP (cm H_2O)	Resistance (cm H_2O/l/sec)	Compliance (ml/cm H_2O)
Before 8 PT	Prestimulus value	26.3 ± 0.9	210.5 ± 20.0	8.0 ± 0.57
	Poststimulus value	17.2 ± 1.1	125.0 ± 10.7	11.1 ± 0.7
	% age of Pre-stimulus value	65.1% ± 3.2	59.2% ± 8.1	37.7% + 20.0
After 8PT (10mg/kg)	Prestimulus value	28.1 ± 2.7	222.1 ± 17.0	7.3 ± 0.7
	Poststimulus value	22.8 ± 3.1	177.3 ± 15.0	10.7 ± 1.0
	% age of Pre-stimulus value	82.7% ± 2.9 ★	79.6% ± 8.0 ★	47.2% + 15.5 ★

Figure 4:16

The difference in TPP, resistance and compliance values caused by vagal stimulation (5Hz) in conditions of muscarinic and alpha- and beta-adrenergic blockade and in the presence of dipyridamole (10mg/kg) before and after 8PT (10mg/kg) (n=4).

	Mean ΔTPP from prestimulus value	Mean Δresistance from prestimulus value	Mean Δcompliance from prestimulus value
Before 8PT	-34.9% ±3.2	-40.8% ±8.1	+67.3% ±20.0
After 8PT	-17.3% ±2.9	-20.4% ±8.0	+52.8% ±15.0
Significance level	★	★	n.s.

enhanced the blocking effect of 8PT on the nerve induced bronchodilatory response, dipyridamole on its own did not affect the bronchodilatory responses using 15Hz (maximal frequency stimulation). At the sub-maximal frequency of 5Hz dipyridamole increased the duration of the response, but did not increase the peak size.

In the presence of adrenergic and cholinergic antagonists, another P_1-purinoceptor antagonist aminophylline (10mg/kg), slightly reduced the bronchodilatory response. The administration of aminophylline was carried out in one preparation to serve as an alternative to 8PT and its bronchoconstricting effects. The administration of aminophylline (10mg/kg) resulted in a transient decrease in the 5HT-induced tone (Figure 4:17).

The P_2-purinoceptor desensitising agent mATP, did not significantly antagonise the vagally induced bronchodilation either in the presence or in the absence of atropine , 8PT and the alpha and beta-adrenoceptor antagonists (Figure 4:18).

Figure 4:17

The intravenous administration of the P_1-purinoceptor antagonist, aminophylline (10 mg/kg) resulted in a transient bronchodilation, when airway smooth muscle has high resting tone.

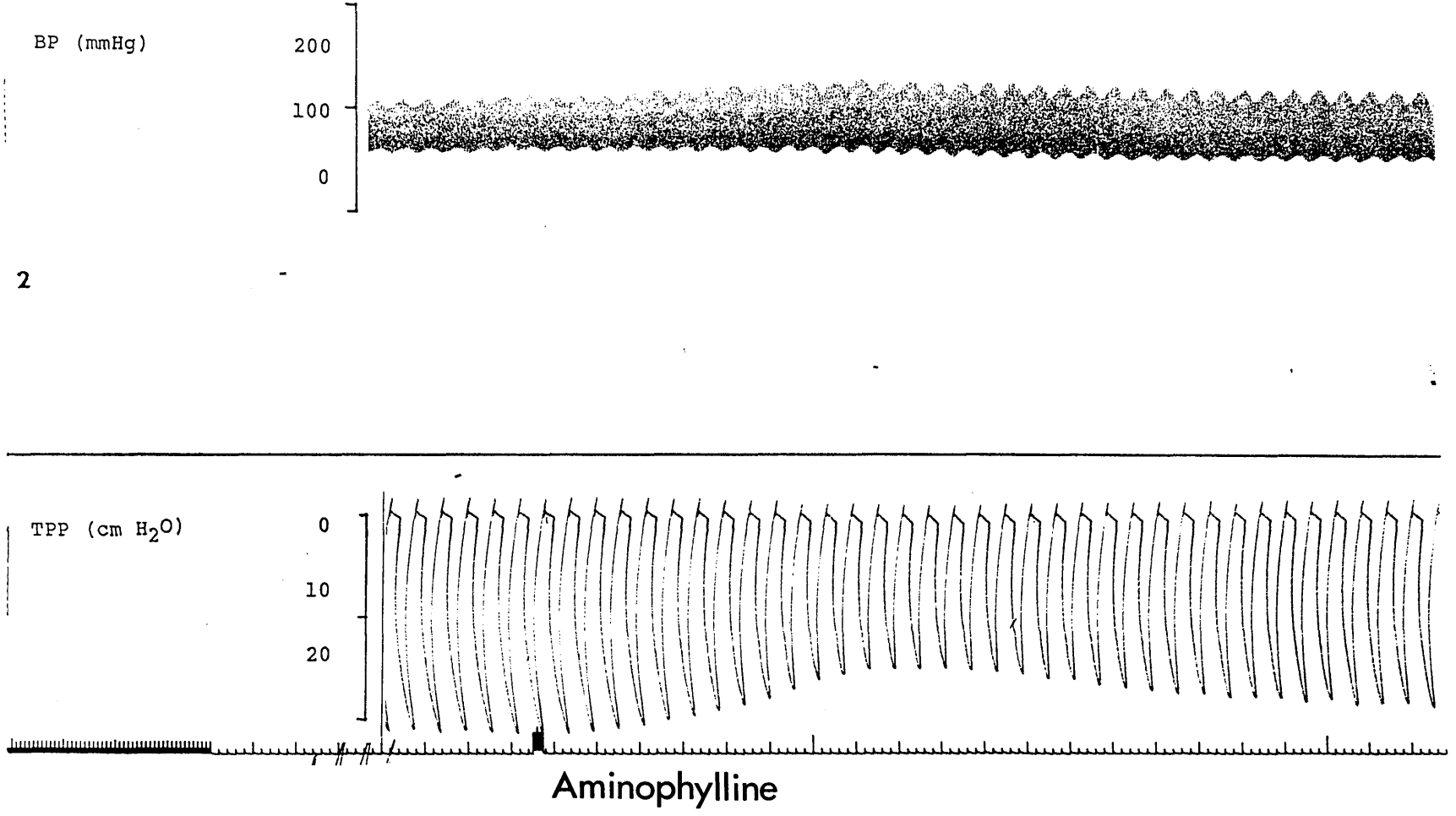

BP (mmHg)
200
100
0
2
TPP (cm H_2O)
0
10
20
Aminophylline

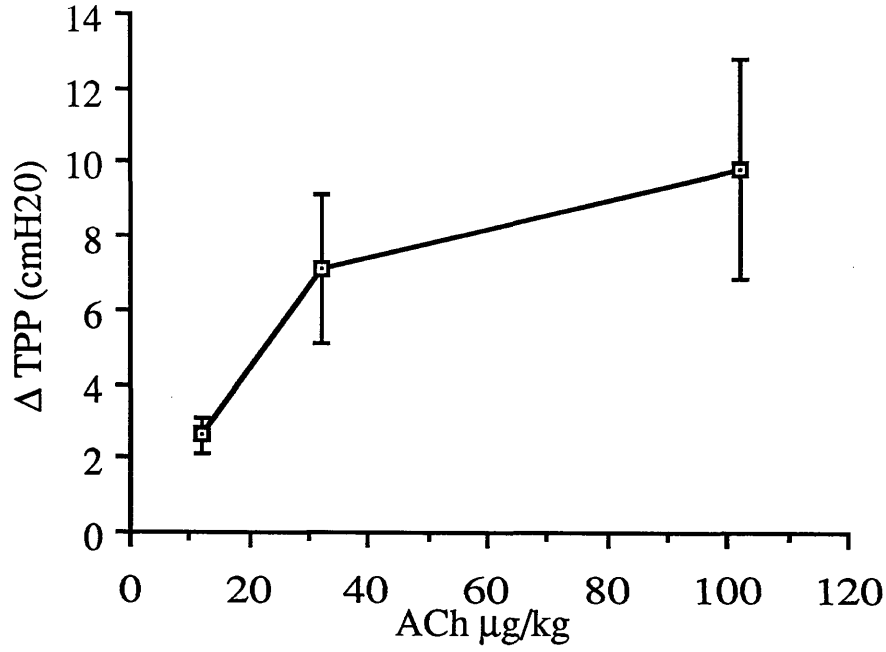
Figure 4:18

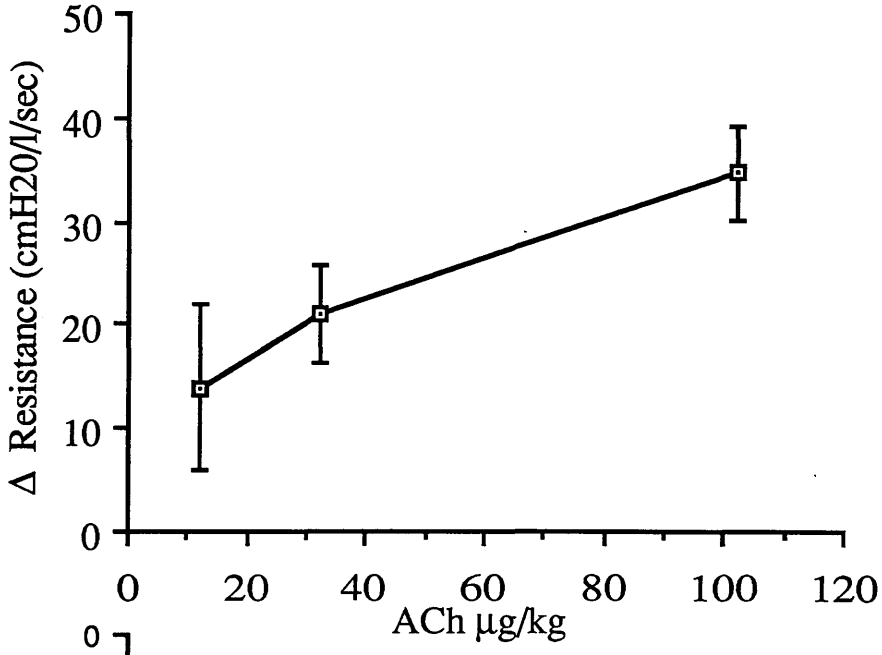
The difference in TPP, resistance and compliance values caused by vagal stimulation (15Hz) in conditions of muscarinic and alpha- and beta-adrenergic blockade measured before and after mATP (2.5mg/kg) (n=5).

	Mean ΔTPP from prestimulus value	Mean Δresistance from prestimulus value	Mean Δcompliance from prestimulus value
Before mATP	-49.4% ±4.8	-44.4% ±5.9	+79.0% ±12.1
After mATP	-49.4% ±4.7	-45.4% ±6.1	+72.0% ±17.1
Significance level	1.0	0.52	0.21

Figure 4:19

These graphs are dose/response curves to ACh in conditions of low resting airway smooth muscle tone (with no antagonists present) and are measured as percentage changes in TPP, compliance and resistance. Graphs i) and ii) are plotted as percentage increases in TPP and resistance, respectively and graph iii) as percentage decreases in compliance, from the control resting levels. (N=4)


14
12
10
8
6
4
2
0
Δ TPP (cmH20)
0
20
40
60
80
100
120
ACh µg/kg


50
40
30
20
10
0
Δ Resistance (cmH20/l/sec)
0
20
40
60
80
100
120
ACh µg/kg

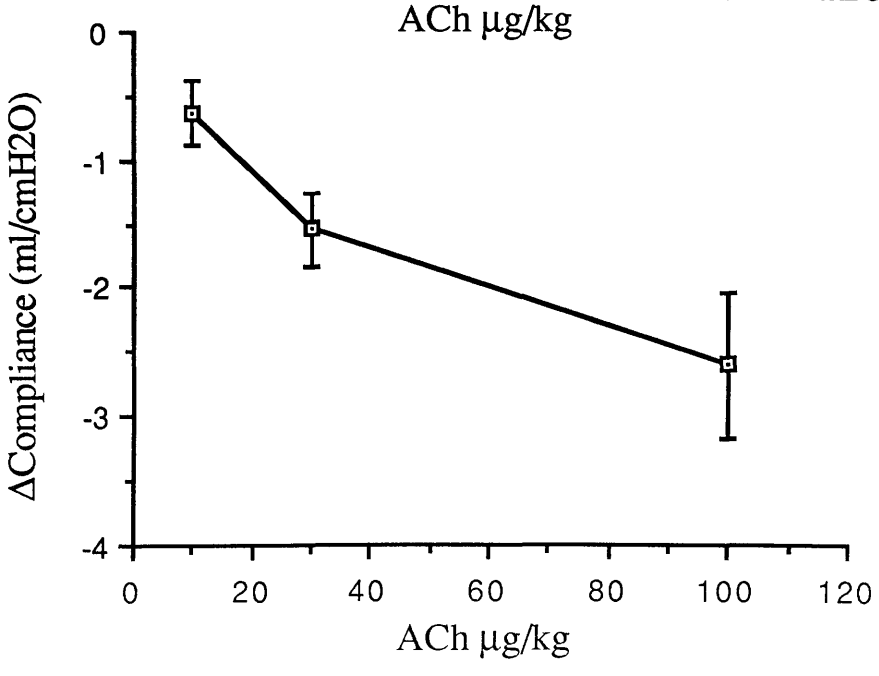

0
-1
-2
-3
-4
ΔCompliance (ml/cmH2O)
0
20
40
60
80
100
120
ACh µg/kg

Figure 4:20

The intravenous administration of Adenosine (10mg/kg) (↑) produces a decrease in the blood pressure level as well as increasing the TPP signal.

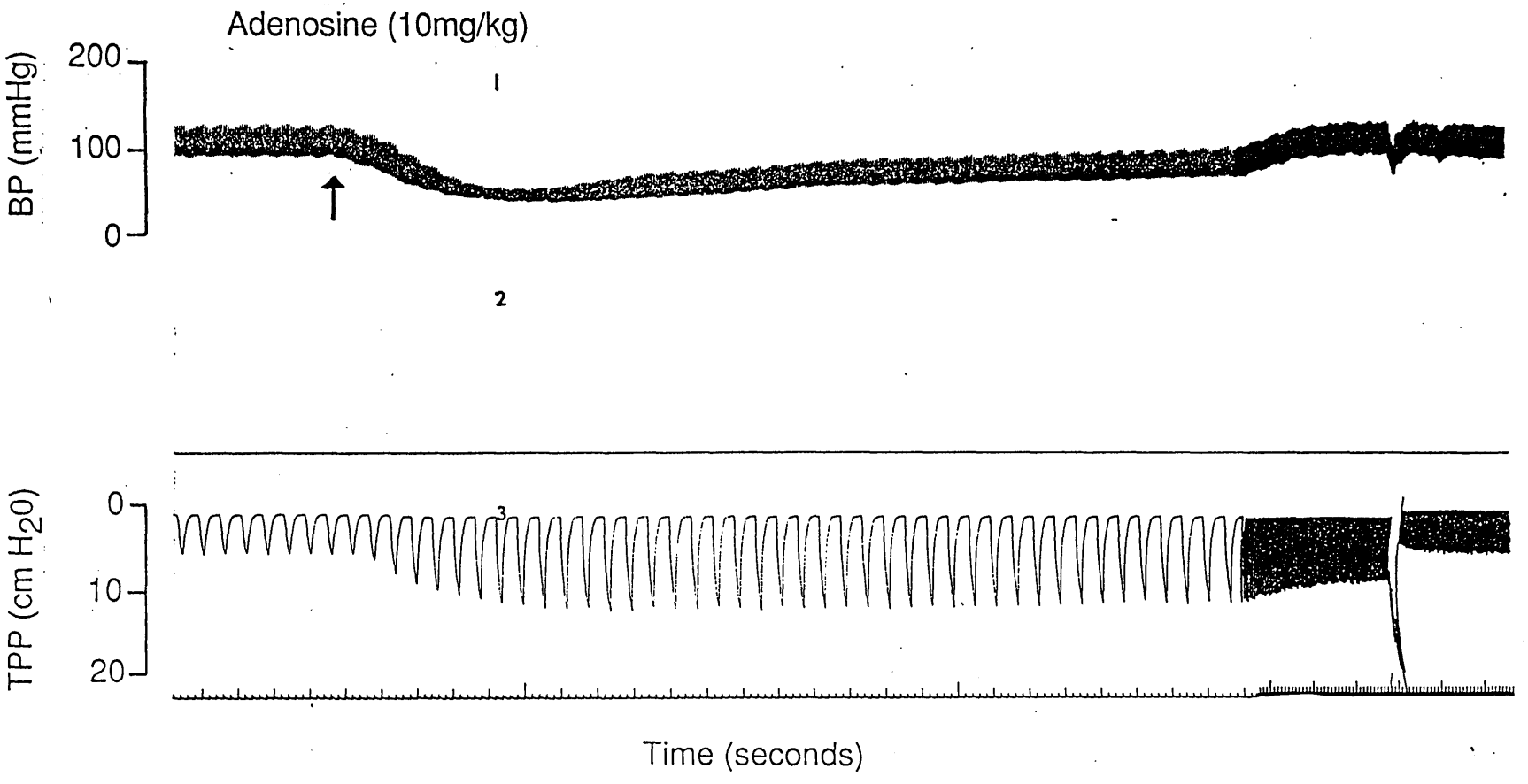

Adenosine (10mg/kg)
BP (mmHg)
200
100
0
1
2
TPP (cm H2O)
0
10
20
3
Time (seconds)

Figure 4:21

The intravenous administration of Adenosine (1mg/kg) produces a bronchoconstriction characterised by changes in the TPP (in cm H_2O), resistance (R) (in cm H_2O/l/sec), and compliance (C) (in ml/ cm H_2O) values.

(n=4)

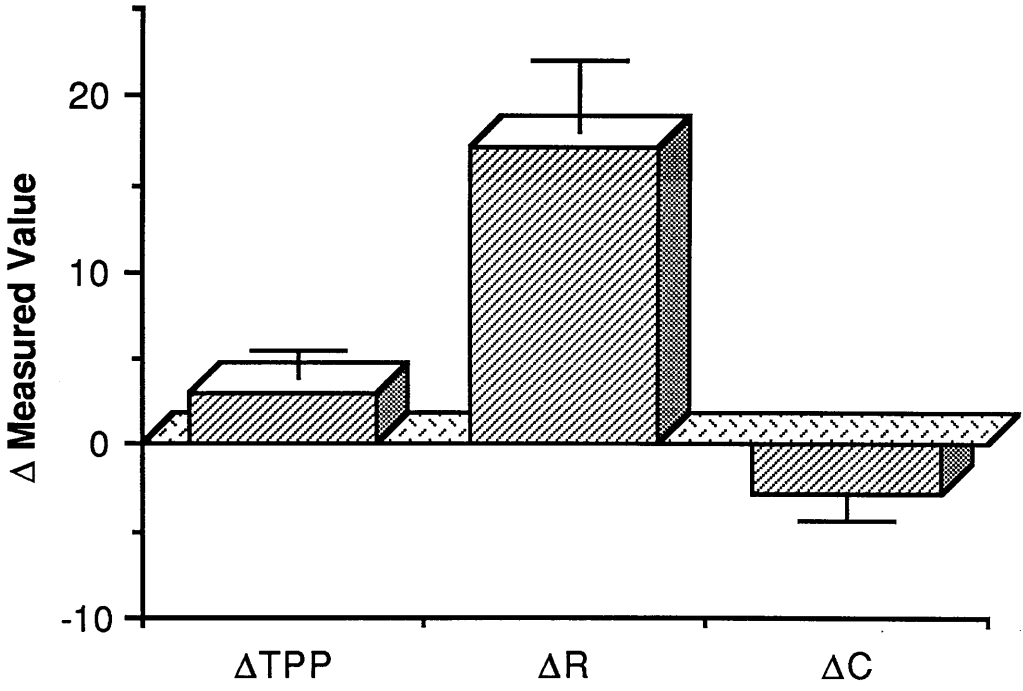
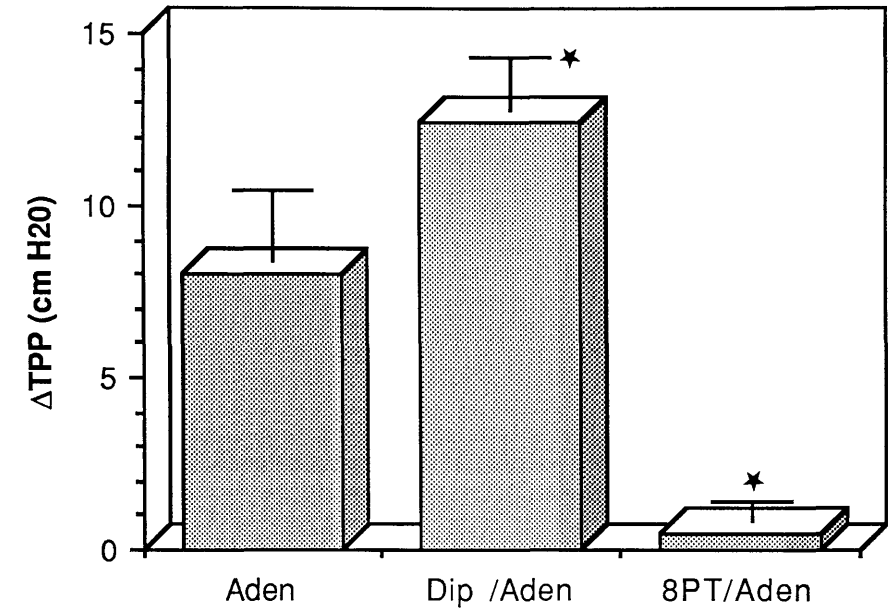
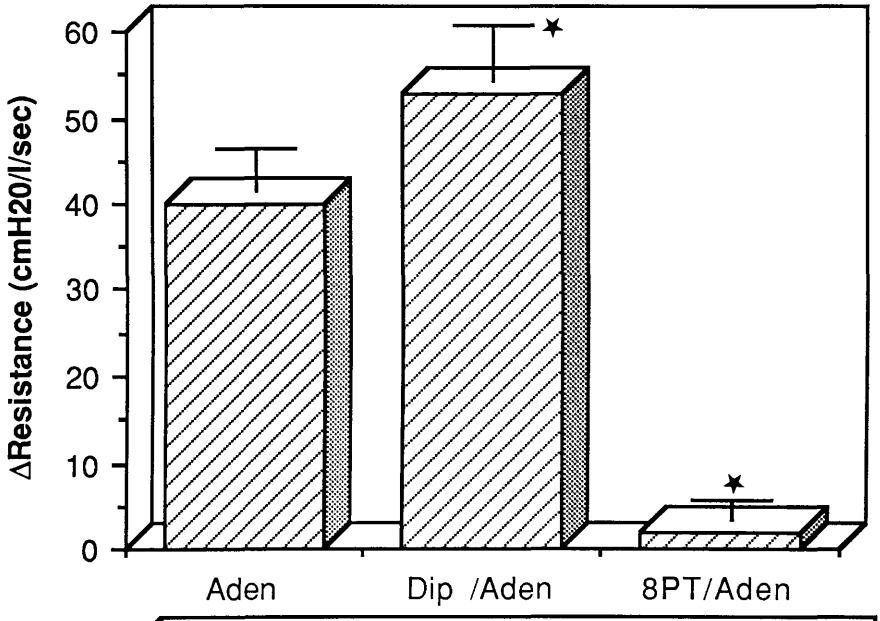

Δ Measured Value
20
10
0
-10
ΔTPP
ΔR
ΔC

Figure 4:22

The bronchoconstriction produced by intravenous administration of 10mg/kg adenosine and characterised by changes in TPP, resistance and compliance, is increased by the administration of dipyridamole (10mg/kg) and antagonised by 8PT (10mg/kg) (n = 4).


15
10
5
0
ΔTPP (cm H20)
Aden
Dip /Aden
8PT/Aden


60
50
40
30
20
10
0
ΔResistance (cmH20/l/sec)
Aden
Dip /Aden
8PT/Aden

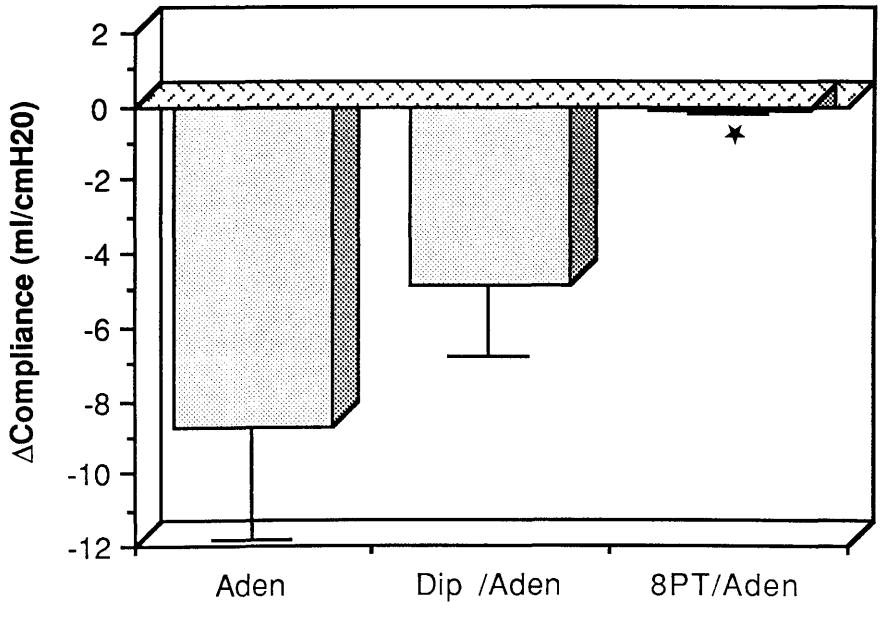

2
0
-2
-4
-6
-8
-10
-12
ΔCompliance (ml/cmH20)
Aden
Dip /Aden
8PT/Aden

Figure 4:23

The intravenous administration of mATP (0.1mg/kg) (↑) in conditions of low resting airway smooth muscle tone produces bronchoconstrictions shown by increases in the TPP signal. The blood pressure effects varied and were usually depressor, but pressor effects were noted in a few preparations.

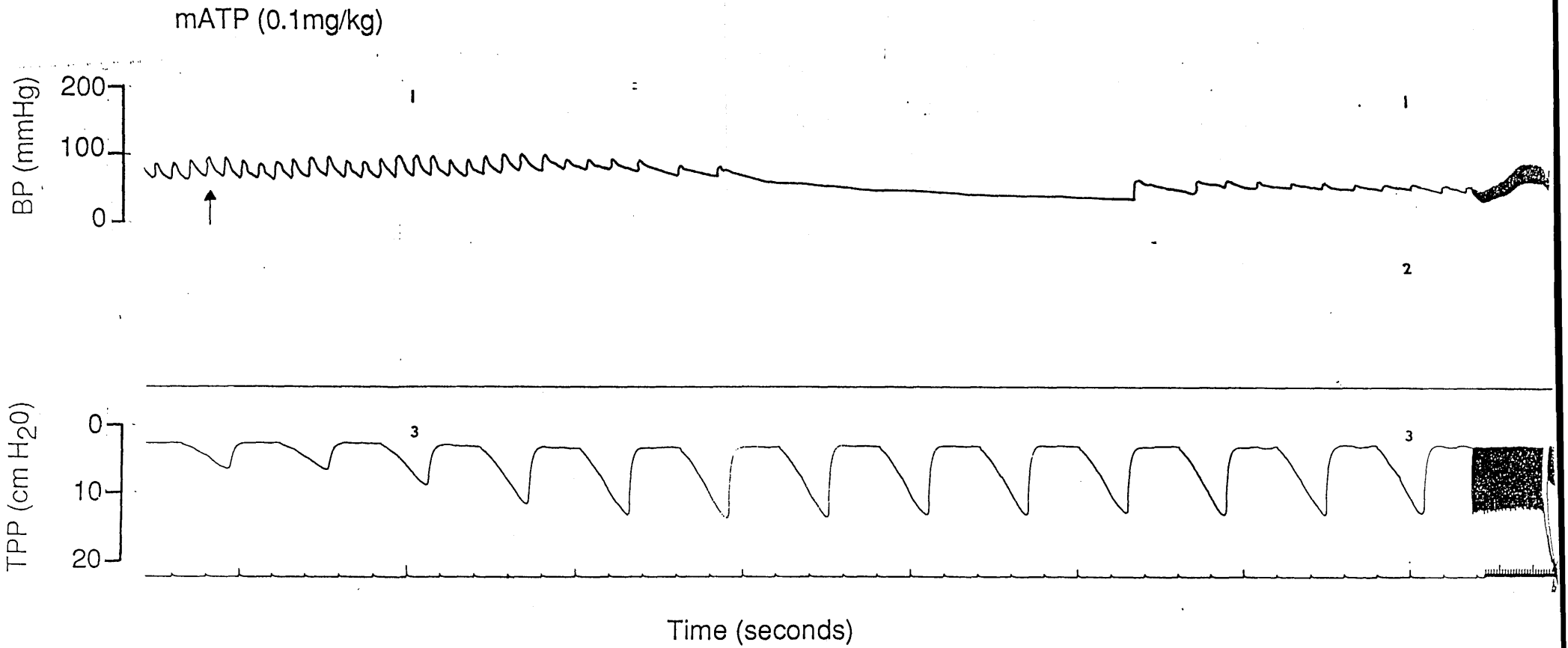

mATP (0.1mg/kg)
BP (mmHg)
200
100
0
1
1
2
TPP (cm H_2O)
0
10
20
3
3
Time (seconds)

Figure 4:24

The intravenous administration of mATP (0.1mg/kg) (↑) in high resting airway smooth muscle tone also produces bronchoconstrictions, shown here by the small increase in the TPP signal.

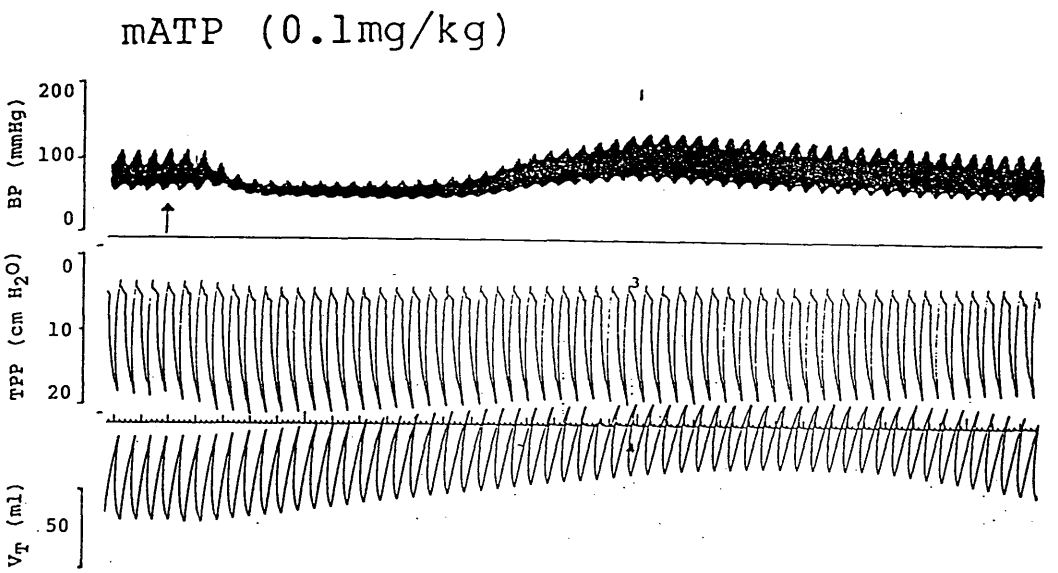

mATP (0.1mg/kg)
BP (mmHg)
200
100
0
TPP (cm H2O)
0
10
20
VT (ml)
50

Figure 4:25

The intravenous administration of the P_1-purinoceptor antagonist 8PT (10mg/kg) (↑) produced a large bronchoconstriction (shown by the increased TPP signal). In this preparation the bronchoconstriction was accompanied by a decrease in systemic arterial blood pressure.

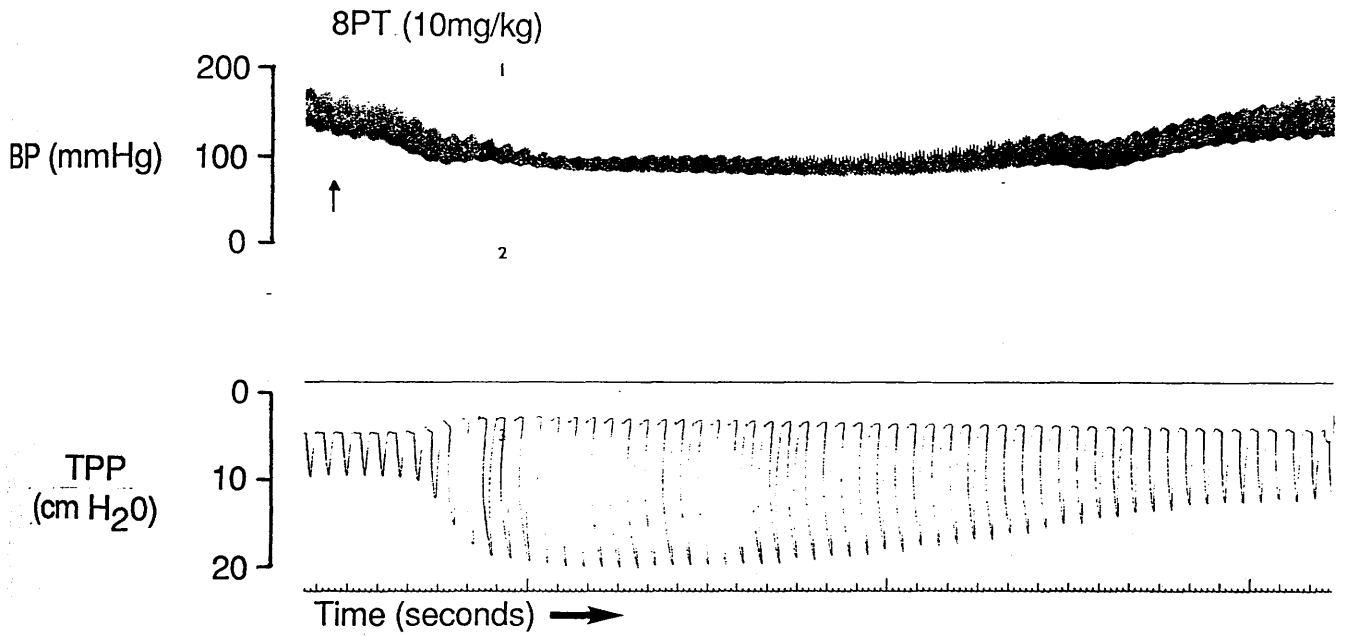
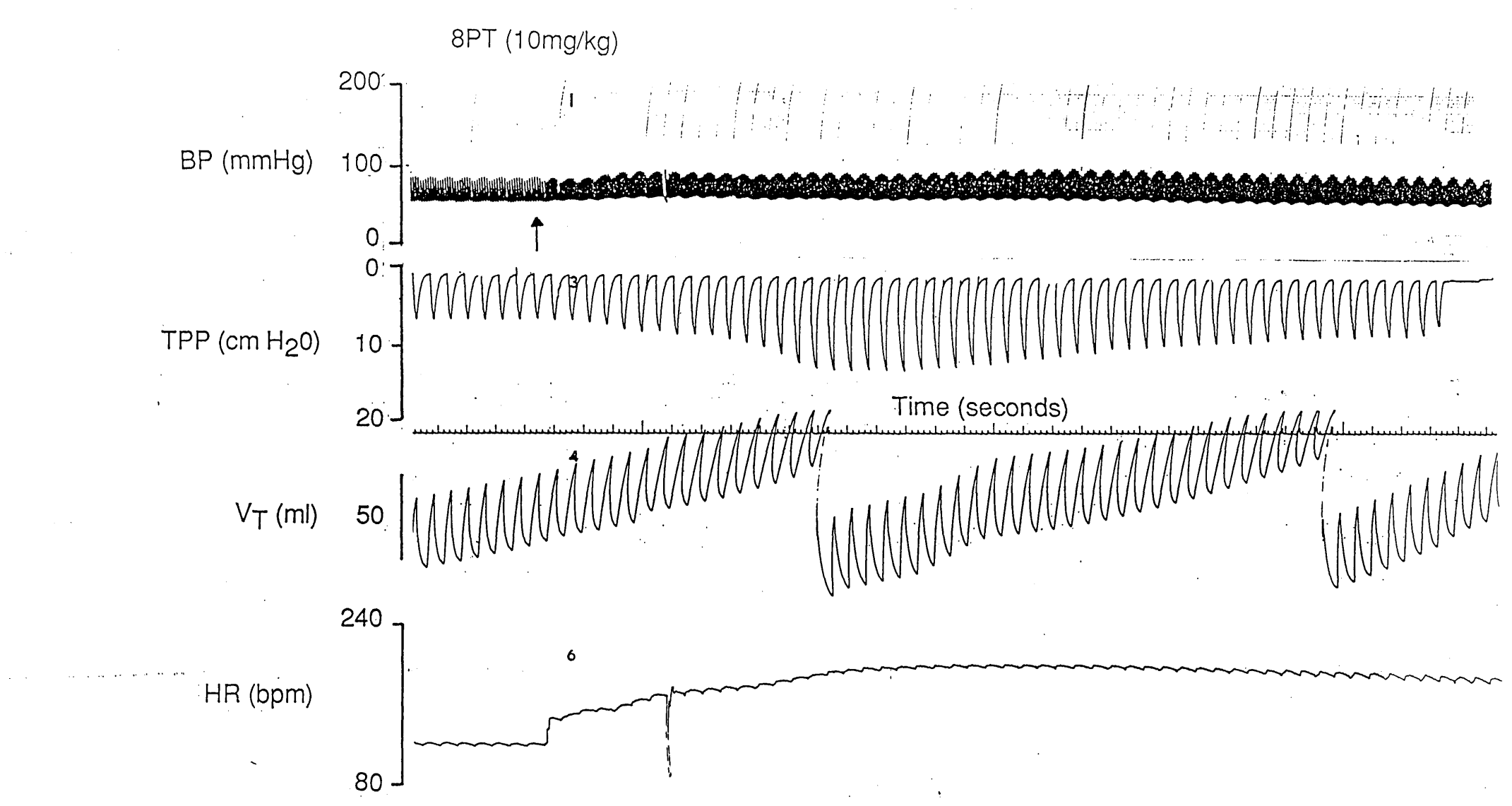

8PT (10mg/kg)
200
100
0
BP (mmHg)
1
2
0
10
20
TPP
(cm H2O)
Time (seconds)

Figure 4:26

The intravenous administration of the P_1-purinoceptor antagonist 8PT (10mg/kg) (↑) produced a large bronchoconstriction (shown by the increased TPP signal). In this preparation the bronchoconstriction was not accompanied by a decrease in systemic arterial blood pressure, however an increase in heart rate was observed.


8PT (10mg/kg)
200
100
0
BP (mmHg)
1
0
10
20
TPP (cm H_2O)
3
Time (seconds)
4
50
V_T (ml)
240
6
80
HR (bpm)

Section I: Discussion

The pressor effects of catecholamines or sympathetic nerve stimulation in pithed rats and rabbits, can be reduced by either $alpha_1$-adrenoceptor antagonists such as prazosin, or by $alpha_2$-adrenoceptor antagonists such as rauwolscine, imiloxan, Wy 26703 or yohimbine.

Difficulties can arise when classifying receptors by using relative potencies of agonists at producing pharmacological effects because the response produced depends on affinity, efficacy and on the ability of agonists to show tachyphylaxis, as well as on the stimulus-response coupling mechanism of the tissue involved. It was for this reason that antagonists rather than agonists were used in this classification.

However since both $alpha_1$ and $alpha_2$-adrenoceptors are involved in the response the quantitative effects of each antagonist depend not only on the dose of NA or frequency of nerve stimulation, but also on whether one of the receptor sub-types has already been blocked by another antagonist. The full effect of either an $alpha_1$ or $alpha_2$ selective antagonist is clear only after the influence of the other receptor has been removed. Thus in the pithed rabbit the addition of rauwolscine alone (Figure 1:1a&b), produced only a small shift in the NA dose/response curve, but in the presence of prazosin the shift to each dose of rauwolscine was much greater

indicating that although both alpha$_1$ and alpha$_2$ adrenoceptors participate in this pressor response, they do not do so by straightforward summation but rather in a non-additive way. The dominance of either one of the receptors can mask the role of the other receptor.

The rabbit, like the rat, has alpha$_1$- and alpha$_2$-adrenoceptors both of which can mediate pressor responses to noradrenaline and can be classified according to antagonist potencies. The selectivity of each antagonist, for the alpha-adrenoceptor sub-types in the rabbit, was established by its effects against phenylephrine. This is similarly shown in the rat. The effects of the more selective imiloxan (RS 21361) (Michel & Whiting, 1981) confirmed the alpha$_2$-adrenoceptor selectivity of rauwolscine and showed that the higher doses of rauwolscine had some alpha$_1$-adrenoceptor antagonism.

However against low doses of NA, rauwolscine is more potent in the rabbit than in the rat, whereas Wy 26703 is not (Table 1:2). This is consistent with the hypothesis that alpha$_2$-adrenoceptors in rabbit are different from those in rat with regard to relative antagonist potency and shows that this applies to resistance vessels in vivo. We have not analysed the alpha$_1$-adrenoceptors in the two species in the same way here. The potency of prazosin against phenylephrine (PE) in the rabbit (Figure 1:1c) was slightly less than

was found in the rat under similar conditions (in the presence of rauwolscine and propranolol, both at 1mg/kg) (Flavahan, 1983).

NA was more potent in the rat than in the rabbit at either alpha$_1$ or alpha$_2$-adrenoceptors but this was not reflected in the potency of the "selective" agonists phenylephrine and guanabenz (Table 1:1). This could be interpreted as a difference in metabolism or disposition of NA between the two species, or this may represent another difference between alpha-adrenoceptors in the two species so that both agonist and antagonist potencies differ. Together with the small difference in potency of prazosin mentioned above this might extend the difference to alpha$_1$-adrenoceptors as well as alpha$_2$-adrenoceptors. This has previously been suggested in isolated aorta by Ruffolo and Waddell (1982). However caution is necessary in interpreting absolute potency differences under non-equilibrium in vivo conditions (see Docherty & Hyland, 1985) particularly since some of the newer synthetic alpha$_2$-adrenoceptor antagonists have partial agonist activity which varies between species (Paciorek & Shepperson, 1983).

Responses to NA in rabbit have a higher proportion mediated by alpha$_2$-adrenoceptors at low doses compared with high doses at which alpha$_1$-adrenoceptors become dominant. However even at the lower part of the

dose/response curve a small shift due to prazosin was observed, indicating that even at this level the $alpha_1$ component plays some part, although $alpha_2$ is still dominant. A similar phenomenon occurs with pressor nerve induced responses which have a relatively greater $alpha_2$-adrenoceptor-mediated component at low frequencies of stimulation. This conclusion can be reached using either rauwolscine or imiloxan as $alpha_2$-adrenoceptor antagonist.

It is conceivable that other factors, such as differences in the way that the two species metabolise the agonists or antagonists could contribute to the apparent differences in antagonist potencies encountered in this study. However this seems unlikely since similar species differences in antagonist-ranking are apparent in *in vitro* preparations.

Because of the dual nature of the response parallel shifts of dose/response curves to $alpha_2$-antagonists could not be expected since a proportionally greater $alpha_1$ component emerged as the size of the response increased. Therefore $alpha_2$ antagonist potency was compared between the rat and rabbit using dose ratios, which covered different regions of the NA dose/response curve: a) the shallow initial part of the curve at diastolic pressor changes of 10 and 20 mmHg; and b) the steeply rising part at changes in diastolic blood pressure of 30 and 60 mmHg.

The potencies of the $alpha_2$ antagonists were studied in the presence of prazosin. The central observation was that the order of potencies of the $alpha_2$ antagonists differed between species and this difference was characterised by an increase in the potency of rauwolscine from rat to rabbit as well as a decrease for Wy 26703.

This suggests that the $alpha_2$-adrenoceptor involved in the pressor response to NA in vivo may be different in the rat and rabbit. Other recent investigations (Alabaster et al, 1984; Nahorski et al, 1985; and Waterfall et al, 1985) indicate that the $alpha_2$-adrenoceptor may have different pharmacological properties in different species. In vitro in the vas deferens, prejunctional $alpha_2$ receptors of rat and rabbit had different antagonist potency series, leading to the conclusion that $alpha_2$ receptors were a heterogenous population with respect to species rather than tissue type or location (Alabaster et al, 1984).

Recent work by Schumann & Lues (1983) proposes a role for AII in the vascular postsynaptic $alpha_2$-adrenoceptor-mediated response by showing that AII, acting postsynaptically, potentiates the contractile response of $alpha_2$-adrenoceptor agonists in the saphenous vein of the rabbit. However the administration of teprotide, captopril or enalapril to

the rabbit did not affect the responses to NA, UK 14304 or phenylephrine, but did reduce the response to AI by 80%. Therefore this proposal is not supported by the present study.

The results show that components of the response to vasopressor nerve stimulation can be partly blocked by alpha-adrenoceptor antagonists but that a resistant component remains. This indicates both alpha-adrenergic and non-alpha-adrenergic (possibly non-adrenergic) components of the vascular excitatory transmission process. This was further investigated in this present study (Section 2).

Against pressor responses to stimulation of the spinal outflow, low doses of prazosin (0.1 and 0.3 mg/kg), in the rabbit (Figure 1:6) and in the rat (Docherty & McGrath, 1980; Flavahan et al., 1985), produced the same degree of blockade as did the higher dose of 1 mg/kg. Another $alpha_1$-adrenoceptor antagonist, corynanthine, was also effective from 0.5 mg/Kg (Demichel et al., 1982). This shows that responses are susceptible to low doses of alpha-one blockers and provides no evidence that the higher dosage of prazosin (1 mg/kg) produces non-specific effects as has been suggested by Hirst et al (1985). Nevertheless there is a substantial component of the vasopressor response, particularly at higher frequencies, which in the rabbit as in the rat is

resistant to alpha-adrenoceptor blockers. In contrast the alpha$_2$-adrenoceptor-mediated component can be most easily demonstrated at low frequencies of stimulation. At high frequencies, the pre-junctional activity of the alpha$_2$-adrenoceptor antagonists complicated the interpretation of the nerve mediated pressor response data by interfering with the negative feedback mechanisms associated with the neurotransmitter. This would result in an increase of transmitter available to compete at the post-junctional receptor sites.

In conclusion, the results are consistent with the characteristics of the vascular postjunctional alpha$_2$-adrenoceptor being species dependent.

Section II:Discussion

Stimulation of the sympathetic spinal outflow between regions T_1 and L_4 produced pressor responses, whilst stimulation between regions L_5 and S_2 produced depressor responses. Responses to different agonists (alpha-adrenergic as well as purinergic) were compared to those obtained by nerve stimulation.

Vasopressor responses to NA and mATP compared well with those obtained on stimulation of the spinal outflow between regions T_1 and L_4. mATP, but not ATP, produced large pressor responses. This may be because ATP is rapidly broken down to adenosine (the intravenous administration of which results in vasodepressor responses) and therefore exerts its action via P_1-purinoceptors. Depressor responses to ATP and adenosine were compared to those obtained by stimulation of the spinal outflow between regions L_5 and S_2. The responses to adenosine and ATP were abolished by 8PT but the nerve mediated vasodepressor responses were unaffected by 8PT. The depressor response to ATP was therefore due to breakdown to adenosine and not via its interaction with P_{2y}-purinoceptors, which have been shown to produce vasodilation in the rabbit mesenteric artery (Burnstock & Warland, 1987).

In order to observe the properties of alpha-adrenoceptor mediated responses (excitatory), it was necessary to block the $beta_2$-adrenoceptors present on

the vascular smooth muscle, (whose activation results in vasodilation) by propranolol (1mg/kg). The contribution of skeletal muscle was kept to a minimum by employing low pulse widths (0.05ms) to minimise current spread by administering gallamine (10mg/kg).

The results suggest that the widespread sympathetic vasopressor response to stimulation of the sympathetic outflow in the rat comprises alpha-adrenergic and purinergic elements since it can be partly blocked by alpha-adrenoceptor antagonists or by a purinergic P_{2x} desensitising agent but can be completely blocked by a combination.

On its own, mATP significantly attenuated the vasopressor responses to nerve stimulation by about 60% of control values. After a combination of $alpha_1$ and $alpha_2$-adrenoceptor antagonists, the residual response was completely removed by mATP. This blocking effect of mATP was deemed to be specific against P_2-purinoceptors since a similar dose level of mATP had no effect on the pressor responses produced by the intravenous administration of noradrenaline. Taken together, these observations suggest that the response to this particular stimulus (20 pulses at 20Hz) consists of two additive elements, 40% alpha-adrenergic (mainly $alpha_1$) and 60% purinergic.

These conclusions contrast with those drawn made in

a separated study in the pithed rabbit (McGrath, unpublished observations), where the alpha-component was larger (up to 70% reduction in response after the addition of the alpha-adrenoceptor antagonists rauwolscine (1mg/kg) and prazosin (1mg/kg). The prazosin/rauwolscine resistant component in this case could only be slightly attenuated by additions of mATP. This might indicate that the rabbit has a very small purinergic component in comparison to the rat, which has a relatively small alpha-adrenergic component.

The evidence from the study using 1mg/kg prazosin, shows that the contribution of alpha-adrenoceptors in the pressor response to nerve stimulation in the pithed rat is in some cases a lot less than the arbitrary value of 50% quoted by Hirst & Lew (1987), since the percentage contribution of adrenergic and purinergic components to the overall response depends on stimulation parameters and position of stimulation. Nevertheless these values were larger than those reported for an in vivo study of the pressor responses in the anaesthetised rabbit (Hirst & Lew, 1987) using benextramine (10mg/kg) as the $alpha_1$-adrenoceptor antagonist instead of prazosin.

However the experimental set up used in this study was different from that used by Hirst & Lew. The present study used a pithed preparation (and hence has a lower resting blood pressure) and was stimulated pre-

ganglionically at the T_6 - T_8 outflow. The stimulation was therefore to a generalised area. In comparison, Hirst & Lew used an anaesthetised model with a higher resting blood pressure, and stimulated post-ganglionically to the hind limb only.

One further difference between pithed rat and anaesthetised rabbit preparations is that the pithed rats have increased Angiotensin II/ renin levels (Grant 1985). This species difference is more apparent when comparing the lower resting blood pressure levels of pithed rabbits with the higher levels of pithed rats. The addition of teprotide inhibited the nerve induced pressor response in the pithed rat by up to 30% after blockade of alpha-adrenoceptors (Figure 2:22) indicating that the non-alpha-mediated pressor response may be modulated by Angiotensin II. Thus differences in percentage contribution of alpha and non-alpha activity may be expected to vary between these preparations.

The results therefore demonstrate that stimulation of the sympathetic spinal outflow can produce vasopresor responses that are largely mediated at the P_{2x}-purinoceptor and to a lesser degree by $alpha_1$ and $alpha_2$-adrenoceptors. The relative contribution of these components is dependent on both position of stimulation and on parameters of stimulation applied, since the contribution of the purinergic component varied directly with the frequency of stimulation. Burnstock & Warland

(1987a) also found that in the rabbit saphenous artery in vitro, the purinergic component was the dominant component. However they concluded from this in vitro study that the relative contribution of the purinergic component, to the overall contractile response, was inversely related to the frequency of stimulation (low frequencies favouring the purinergic component of the response). It seems likely therefore that the frequency dependency of the relative contribution of the purinergic and adrenergic elements in sympathetic co-transmission varies from tissue to tissue, no general rule applying.

The administration of rauwolscine highlighted the potential difficulties encountered when using an antagonist which is not selective for postsynaptic $alpha_2$-adrenoceptors only. Rauwolscine's action on the presynaptic nerve terminal led to increases in the height of the responses by interfering with the feedback system. This therefore complicates interpretation of results since blockade of $alpha_2$-adrenoceptors blocks autofeedback and increases release of both transmitters, enhancing purinergic and adrenergic contibutions to transmission. Therefore in the presence of both prazosin and rauwolscine an exaggerated purinergic component may be observed. The action of rauwolscine also showed the problems faced when analysing results merely by measuring maximum heights of the responses rather than analysing the shape of the response (i.e. its time

course). Rauwolscine's most obvious action was to shorten its duration (Figure 2:7b) indicating perhaps a role for post-junctional alpha$_2$-adrenoceptor in maintaining the pressor response.

mATP attenuates the non-adrenergic component of nerve induced contractions of the vas deferens in vitro (Burnstock & Sneddon, 1984). We confirmed this in the pithed rat to verify the selectivity and the time course of blockade of mATP in vivo. Whereas a long-lived desensitisation can be maintained in vitro, in the pithed rat a parallel rapid loss of mATP's effect was clear in both vas deferens and resistance blood vessels, making this a critical consideration when employing mATP as a blocker of putative purinergic transmission.

Flavahan et al (1985) showed that nerve mediated pressor responses could be completely blocked by the ganglion blocker hexamethonium. Since the direct component of the nerve mediated response is almost abolished by guanethidine, this indicates that electrical stimulation via the pithing rod activates preganglionic autonomic nerves and that transmission can be stopped at the level of postganglionic adrenergic sympathetic nerve terminals, indicating that the response is mediated by a transmitter / or transmitters from the postganglionic sympathetic nerve terminals.

Previously it was shown that the direct pressor

response to stimulation of the sympathetic outflow could be only partially blocked by reserpine suggesting that NA is not the only transmitter. However transmission could be completely blocked by pretreatment with 6-OHDA suggesting that only nerves possessing uptake and accumulation sites for phenylethanolamines are involved (Flavahan et al, 1985). This was consistent with the hypothesis that the entire response arises from "adrenergic" nerves but part of it (the reserpine-resistant element) which does not require NA, is due to a co-transmitter. However the earlier failure to abolish the response with a combination of mATP and alpha-adrenoceptor antagonists left open the possibility of another co-transmitter or of an adrenergic response which was not mediated by alpha-adrenoceptors (however this was unlikely due to reserpine resistance). The present results show that this arose because of the failure to detect the transience of the blockade by mATP.

The calcium channel blocking agent, nifedipine, was also able to antagonise the sympathetic nerve mediated pressor response. This attenuating effect of nifedipine was best observed at higher frequencies of stimulation (20 Hz) and could be seen in both alpha-blocked and non-alpha-blocked preparations. In the presence of prazosin and rauwolscine, nifedipine reduced the remaining pressor response by 50% at 20Hz. Nifedipine also produced a marked reduction in the nerve mediated

pressor response in the absence of the alpha-adrenoceptor antagonists. However nifedipine had very little effect on the vasopressor response that remained after desensitisation with mATP. This perhaps indicates nifedipine and mATP are acting on the same component and since prazosin did not affect the blocking effects of nifedipine, then it is likely that prazosin and nifedipine are acting on different components of the vasopressor response.

At higher frequencies more of the non-adrenergic component of the pressor response in vascular smooth muscle is observed. These electrically dependent responses are mediated via e.j.p.s and slow Ca^{2+} channels and therefore were more susceptible to nifedipine. The adrenergic component of the response was largely unaffected by nifedipine as would be expected if the activation of alpha-adrenoceptors led to decreases in calcium binding therefore increasing the concentration of free calcium to produce contractile responses. Nifedipine's effects on alpha-adrenoceptor mediated responses might therefore only be `seen during prolonged agonist infusions, where prolonged contractile responses require more extracellular calcium (McGrath & O'Brien, 1987).

This action of nifedipine is analogous to its action in the vas deferens. The mechanical response of the vas deferens to single pulses or short trains of

stimuli comprises two distinct phases: (Ambache & Zar, 1971; McGrath, 1978) an initial twitch, which may be attributed to purinergic involvement; and a slow well maintained secondary contraction attributed to NA release. Nifedipine abolishes the initial twitch component but leaves the secondary component intact (French & Scott, 1981). In the vas deferens nifedipine acts to abolish the smooth muscle action potential and the initial twitch response, without reducing the excitatory junction potentials (e.j.p.s) which were measured by intracellular electrodes (Blakely et al, 1981). This suggests that there was a difference in the receptor-response coupling of the two components of the response.

The sites of action for nifedipine in preventing the initiation of the smooth muscle action potential and hence in blocking the initial twitch, can be seen from the digrammatic representation of the theory behind sympathetic neuro-effector coupling in smooth muscle in Figure 2:23. Ca^{2+} is the ion carrying most of the inward current during the rising phase of the action potential (Bennet, 1967). The initial twitch corresponds to the contraction associated with the smooth muscle action potential. Since in the vas deferens, the e.j.p.s which summate to initiate the smooth muscle action potential and associated contraction, are resistant to alpha-adrenoceptor antagonists, it seems that the alpha-adrenoceptor mediated contractile response of the vas

deferens does not require an action potential and thus does not work through the potential operated channel pathway (Figure 2:23).

By analogy this explains the results obtained in the pithed rat preparation with nifedipine, especially since similar electrophysiological results to those in the vas deferens, have now been found in arteriolar smooth muscle (Hirst & Neild, 1980) where e.j.p.s are resistant to $alpha_1$-adrenoceptor blockade. In the isolated arteriolar smooth muscle, iontophoretic application of NA gave contractile responses which occurred without any detectable change in membrane potential, again comparing well with the adrenergic mediated response to nerve stimulation in the vas deferens which occurred independently of the action potential.

Hirst & Neild (1980) explained these results in vascular smooth muscle by postulating the existence of a gamma-adrenoceptor which could not be antagonised by alpha-adrenoceptor antagonists but which could be activated by neuronally released NA and could mediate contraction via e.j.p.s and hence action potential propagation. However they had no direct evidence that the nerve induced e.j.p. was caused by NA.

A more likely explanation of the results is that ATP and NA are released together as co-transmitters from

the sympathetic nerve terminal (Figure 2:23). This explains the observations made in the vas deferens and isolated vascular smooth muscle as well as explaining the results of the present study of sympathetic mediated vasopressor responses of rat resistance vessels in vivo. Neuronally released NA activates $alpha_1$- and $alpha_2$-adrenoceptors on the effector smooth muscle to operate the 'receptor operated channels' (R.O.C.s) and possibly also cause a decrease in Ca^{2+} binding, which results in an increase in free intracellular Ca^{2+}. In contrast noradrenaline's co-transmitter ATP acts through P_2 receptors to elicit e.j.p.s which initiates propagated action potentials, which open V.O.C.s, allowing Ca^{2+} entry and hence leading to contraction of smooth muscle.

Evidence from Sneddon & Burnstock (1984) is compatible with this theory since they showed that the P_2-purinoceptor desensitising agent mATP, blocked the e.j.p.s in the guinea pig vas deferens.

All of the data is now consistent with the hypothesis that NA and ATP can act as co-transmitters at vascular neuroeffector junctions and produce additive responses through only alpha-adrenoceptors and P_2-purinoceptors, respectively.

SECTION III

DISCUSSION

In attempting to localise the vascular bed or beds involved in the depressor responses to stimulation of the spinal outflow between regions L_5 and S_2, the vascular beds of the lower gut (colon) and the hind limb being the most obvious with respect to location and relative contribution to systemic blood pressure, were studied, but clear conclusions could still not be drawn.

The involvement of the gut in these responses seemed the more likely since the vasodepressor response occurs in similar circumstances to the inhibitory nerve-mediated response of the anococcygeus muscle (McGrath, 1973). However since occlusion of the mesenteric vasculature led to a drop in the resting blood pressure level (presumably by decreasing the venous return and cardiac output) then this would complicate interpretation of data obtained. Such a drop in blood pressure itself is might have contributed to the disappearance of nerve induced depressor responses, if accompanied by a decrease in vascular smooth muscle tone, without considering the effect of removing the mesenteric circulation from the system. However since the subsequent increase in blood pressure, caused by adding guanethidine (10mg/kg) to these preparations, still did not bring back the depressor responses, then it may be reasonable to assume that the mesenteric vasculature is involved in the nerve induced

vasodepressor response.

Dusting & Rand (1972) found that when the blood pressure of the pithed rat was raised by NA infusion, the response to spinal stimulation had a more distinct depressor component. Subsequent studies (McGrath, 1973; Gardner, 1977) employed either guanethidine or the indirectly acting sympathomimetic tyramine, to increase resting blood pressure levels before study of the vasodepressor responses commenced.

In the present study it was possible to obtain depressor responses without the addition of such agents. However the intravenous administration of guanethidine (10mg/kg) led to increases in the magnitude of the depressor responses obtained on stimulation of the spinal outflow. These increases were not due only to the increased resting blood pressure level caused by guanethidine, since this effect was also observed in preparations pretreated with atropine, propranolol, prazosin and rauwolscine. In these preparations with adrenergic and cholinergic blockade, guanethidine did not produce the large pressor responses observed in the untreated animals. Therefore guanethidine's effect was not simply due to an increased blood pressure level. One possible explanation could be that the additions of adrenergic antagonists did not all reach the site of action or were successfully competed against by the neurotransmitters, so that complete blocked of alpha-

and beta-adrenoceptors was not attained. This seems unlikely due to the high dosage of antagonists employed. Alternatively guanethidine may also be blocking the excitatory actions of a co-transmitter released alongside NA from the adrenergic nerve terminals. This would therefore unmask the full depressor effect of the inhibitory transmitter.

Since guanethidine did not block the depressor responses, it is likely that the inhibitory transmitter is not a co-transmitter in adrenergic nerve terminals but rather is released at another nerve type, either as the main neurotransmitter (eg. 'pure' purinergic nerves) or as a co-transmitter (eg. released alongside Ach).

Although 8PT (10mg/kg) and mATP had no effect on the vasodepressor response, it is still not possible to completely rule out purinergic involvement in these responses. Since mATP is a P_{2x} desensitising agent, thus blocking the excitatory action of ATP, then any inhibitory responses of ATP acting through P_{2y}-purinoceptors will remain unaffected. Burnstock & Warland (1987) showed that ATP relaxes rabbit mesenteric artery via the P_{2y}-purinoceptor and that this inhibitory action of ATP could be blocked by Reactive Blue 2.

In the majority of preparations, gallamine (10mg/kg) had no effect on the depressor responses. This

is in agreement with a previous study by McGrath (1973). In the few preparations where the depressor responses were abolished it could be that gallamine was acting non-specifically in causing ganglionic blockade (Blaber et al, 1985). Dusting & Rand (1972) suggested that the depressor response is a result of skeletal muscle causing an 'arterial clamping effect', thus increasing peripheral resistance and causing an increase in blood pressure, therefore leading to the secondary depressor response following stimulation attributed to a functional hyperaemia in the stimulated muscles, however this seems unlikely. Other evidence against the depressor response being a result of functional hyperaemia is that the vasodepression occurs between 1 and 2 seconds after the start of stimulation, which would be too rapid onset for this type of mechanism to occur. Neither is it likely that overflow of ACh from ganglionic synapses and skeletal neuromuscular junctions could exert a vasodilator action since atropine had no effect on these responses.

McGrath (1973) showed that these responses were susceptible to blockade by the ganglion blocking agent hexamethonium, suggesting that the depressor fibres are organised in the pattern of the autonomic nervous system with a ganglion relay. It is also possible that the vasodepression could be due to the effect of endothelium derived relaxant factor (EDRF) which would be released from the endothelium of the blood vessels by a non-

adrenergic neurotransmitter (since adrenergic transmission would be blocked by guanethidine). This effect of EDRF may be present throughout the spinal outflow but is apparent only in this region where there are no large pressor effects to hide any dilator action. Stimulation of the sacral outflow in the presence of an agent which will block the action of EDRF in vivo might throw more light on this possibility.

Peptidergic involvement may also be a possibility. Substance P is not only a potent releasing agent of EDRF but it can also mediate, via a local axon mechanism in the gut, vasodepressor responses in the intact animal (Lembeck & Skofitisch, 1982). However the peptidergic involvement in vasodepressor responses has yet to be fully elucidated and awaits further development of specific antagonists of Substance P.

Dorsal root vasodilator fibres cause a vasodilation in skeletal muscle and superficial mucus membrane (Holton, 1959). Their physiological role is to produce arteriolar dilation as a response to local injury. The sensory C fibres supplying the skeletal muscle branch peripherally and sensory stimulation of these fibres causes impulses to pass both centrally and by the peripheral branch to arterioles in the vicinity. These dilate and their response causes the 'flare' of the 'triple response' (Lewis, 1927). Although the peripheral transmitter is unknown, Substance P involvement in at

least the afferent pathway is likely. In a study of the sacral parasympathetic outflow to the colon (S_2 - S_3) Hulten and co-workers (1969) found that stimulation produced colonic contraction, a large vasodilation in the vascular bed of the colon and an associated intense flushing of the colonic mucosa. The vasodilation response was unaffected by atropine and was abolished by ganglionic blockade, indicating an autonomic non-cholinergic mechanism of transmission.

Other substances studied in relation to the nerve induced vasodepressor responses include bradykinin and histamine (Gardner, 1977) as well as prostaglandins (Murray, 1978), none of which seem to play a role in the nerve mediated depressor responses.

Therefore it seems that these depressor responses obtained by stimulation of the spinal outflow at regions L_5-S_2, are NANC, are mediated through a ganglionic pathway but the peripheral transmitter has yet to be identified.

SECTION IV: DISCUSSION

PROFILES OF AGONISTS ON THE AIRWAYS

The first agent tested in these preparations was ACh. This served as a test for the viability of the preparation before proceeding with the experimental protocol. ACh (10 - 100µg/kg) administered intravenously produced dose dependent bronchoconstrictions which were antagonised by the muscarinic antagonist atropine as were the bronchoconstrictor responses to nerve stimulation in low resting airway smooth muscle tone.

The intravenous administration of adenosine resulted in marked bronchoconstriction which lasted over 5 minutes. This bronchoconstriction was potentiated by dipyridamole (10mg/kg) and was blocked by either 8PT (10mg/kg) or aminophylline (10mg/kg). No bronchodilator responses were observed to intravenously administered adenosine even in preparations undergoing 5HT infusions. Administration of the adenosine analogue, 2-chloroadenosine and the P_{2x}-purinoceptor desensitising agent also produced bronchoconstrictions.

The major effect of adenosine and its nucleotides on airway musculature, has in the past been reported to be relaxatory (Bennet & Drury, 1931; Coleman, 1976) both in vivo and in vitro. However in this study, the effect of adenosine (and mATP) was excitatory. A recent report by Pauwels and Van der Straeten (1986) shows that the in

vivo intravenous administration of adenosine caused bronchoconstriction in the rat. This bronchoconstricting effect was also observed in human airways (Holgate et al, 1986) when adenosine was administered as an aerosol to patients with asthma.

Since the P_1-purinoceptor antagonist 8PT (10mg/kg) attenuated the nerve-mediated bronchodilations and increased the nerve-mediated bronchoconstrictions it may be said that the neuronal responses attributable to adenosine are inhibitory and are probably mediated via P_1 purinoceptors, therefore there is the possibility that the excitatory responses produced by intravenous administrations of adenosine may not be mediated via the same population of receptors and could be mediated via a non-receptor mechanism. For example Welton & Simko (1980) suggested that adenosine-mediated effects in lung tissue in sensitised animals were a result of antigen-induced histamine release, unlike those to ATP, which causes histamine release from mast cells. This would explain observations made in patients with asthma but does not explain the results of the present study with non-sensitised animals. Therefore adenosine may be interacting with the lung epithelium to release bronchoconstricting agents, like prostaglandins, as suggested by Kamikawa & Shimo (1976). This could explain the bronchoconstrictor action observed when administering adenosine intravenously. Pauwels & van Der Straeten (1986) also suggested that adenosine action in

the lung is indirect and that adenosine induced bronchoconstriction is antagonised by high doses of atropine.

Therefore in the lung adenosine could well have more than one effect , for example: relaxation of airway smooth muscle (probably via postsynaptic P_{1a}-purinoceptor); contraction (by releasing prostaglandins from the lung epithelium, or alternatively by activating a P_{1i}-purinoceptor to produce contraction); and a presynaptic effect on the parasympathetic nerve terminal. This is all consistent with the pharmacological from this present study.

These effects may occur together but the dominating effect would depend on the tone of the smooth muscle and on the localisation adenosine and its accessibility to P_1-purinoceptors, and thus would depend on whether the r`ather labile adenosine was given i.v. or was released during nerve stimulation. The following examples illustrate how this can occur and explain our main experimental observations.

1) In high tone, stimulation of the vagal nerve bundle may release adenosine (probably as a co-transmitter) which acts on P_{1a}-purinoceptors to cause relaxation. Any excitatory effects of neurally released adenosine would be masked in this instance.

2) In low tone, nerve released adenosine acting on P_{1a}-purinoceptors might produce no detectable effect since the smooth muscle tone is too low to see any relaxation, but intravenously administered adenosine could cause release of histamine or 5HT from mast cells and hence produce contractions.

3) Consider low tone where the nerve induced bronchoconstriction is increased after the addition of 8PT. The latter could be acting to block the negative feedback effect of adenosine (via P_1-purinoceptors) on ACh release on the parasympathetic nerve terminal. Therefore more ACh will be released. Alternatively this could be explained by postsynaptic mechanisms again assuming that ACh and adenosine are co-transmitters, but this time the released ACh acts on muscarinic receptors in the airway smooth muscle to constrict it whilst the adenosine released along with it acts on P_1-purinoceptors to produce relaxation of smooth muscle. Therefore by blocking these post-synaptic P_1-purinoceptors an increase in the excitatory response occurs.

Previous studies have shown that both ATP and adenosine have a direct action on smooth muscle and that this can be excitatory (Collier et al, 1966), inhibitory (Coleman, 1976) or biphasic (Kamikawa & Shimo, 1976). The responses often are dependent on intrinsic smooth muscle tone.

Early studies showed that adenosine and adenine nucleotides caused bronchial dilatation in guinea pigs in vivo (Bennet & Drury, 1931). In vitro studies have also shown this (Bianchi et al, 1963). For example adenosine and adenine nucleotides caused bronchial dilatation in perfused guinea pig lungs and in isolated guinea pig trachea (Coleman, 1976).

Farmer & Farrah (1976) found that adenosine and adenine nucleotides all produced a contraction followed by a relaxation in the high spontaneous tone guinea pig tracheal tube preparation. They explained this biphasic response on the basis that the direct effect of these compounds was excitatory but they were then broken down to inosine and AMP which would then mediate the relaxation. Kamikawa & Shimo (1976) showed that the biphasic action of ATP on guinea pig tracheal strips was antagonised by a prostaglandin antagonist and mimicked by PGE_2, therefore they concluded that the response to ATP was indirect and mediated by PGE_2 released via stimulation of its biosynthesis.

An unexpected observation was made when adding the P_1-purinoceptor antagonist 8PT, in that it produced a bronchoconstriction. The bronchoconstriction to 8PT could have a number of causes.

Firstly, 8PT could be a purinergic partial agonist.

However this seems to be the least likely of the possibilities since aminophylline in a dosage which blocked the adenosine response did not block this 8PT response.

Secondly, 8PT could be acting in an antagonistic capacity. Parasympathetic co-transmission of excitatory ACh and inhibitory adenosine together could keep the resting tone of the airway smooth muscle lower than if ACh was the sole transmitter. Assume that the lung is under the constant neural inhibitory influence of adenosine. 8PT in antagonising the inhibitory action of adenosine would thus increase the tone of the lung. Consequently it would appear as if 8PT itself was causing a contraction by acting as an agonist whereas it would be removing an inhibition. Similar observations were made by Baer and coworkers in 1983. They suggested that increased contractile activity in the DNECA (an adenosine analogue) relaxed isolated longitudinal muscle of rabbit small intestine on addition of 8PT, was due to 8PT antagonising DNECA and so reversing the relaxation.

Alternatively, the effect may be independent of purinoceptors. 8PT may increase calcium sensitivity and or internal calcium moblization and so cause contraction of the muscle much in the same way as does the structurally similar caffeine (Weber & Herz, 1968).

An 8PT vehicle effect (PEG/NaOH) was also ruled out as being responsible for bronchoconstriction. The vehicle on its own had no effect on the respiratory system. As the drug is so difficult to dissolve another possibility is that after intravenous administration, the 8PT crystallises out and so may produce an effect by a physical mechanism. This could also explain the large decrease in blood pressure that often (but not always) accompanied these bronchoconstrictor responses.

In some preparations, in conditions of high resting tone, additional increases in tone caused by 8PT administration led to the onset of spontaneous breathing. This may have been a result of abnormal blood gas levels (caused by a prolonged increase in tone), or by stimulation of J-receptors or irritant receptors (Barnes, 1986). Stimulation of these irritant receptors might be responsible for the bronchoconstricting effect of 8PT in low tone.

The P_2-purinoceptor desensitising agent mATP, may be causing bronchoconstriction by the same mechanisms as reported for ATP (Diamant & Kruger, 1967; Sugiyama & Yamasaki, 1969; Sugiyama, 1971). In these reports ATP was identified as a histamine liberator via action on mast cells. However an additional mechanism has been reported for ATP: that is, the ability of ATP to release prostaglandins from the lung endothelium (Kamikawa & Shimo,1976). This report suggests that the ATP-induced

response in the lung was induced and mediated by prostaglandins since the response could be antagonised by indomethacin and mimicked by PGE_2. However this mechanism is associated with bronchodilation rather than bronchoconstriction.

It was not possible to construct dose/response curves for mATP since the bronchoconstrictor responses to mATP were subject to tachyphylaxis. Another problem encountered in this part of the study was the ability of mATP to cause the secretion of large amounts of mucus from the lung. A recent report by Rice & Singleton (1986) proposed P_2-purinoceptor involvement in the regulation of surfactant secretion from rat isolated alveolar cells. It is possible that P_2-purinoceptors are involved in the production of mucus also.

Evidence indicates that compounds such as adenosine and ATP may act as modulators in some tissues (Fredholm & Hedqvist, 1980; Ribeiro, 1979; Stone, 1981). These modulating roles include those on neurotransmission, receptor sensitivity, smooth muscle reactivity and local blood flow, all of which are of importance in this present study. It has been suggested that bronchial asthma may be a result of an abnormality of these purinergic modulations.

Bronchial asthma is induced mainly by antigen-antibody reaction and, when allergens bind to specific

immunoglobulin (IgE), chemical mediators such as histamine are released from mast cells and basophils. These mediators cause the clinical symptoms observed during an asthmatic attack as well as contributing to hyperreactivity of airway smooth muscle. Another factor contributing to this hyperreactivity of airway smooth muscle could be the hyperfunction of parasympathetic nerves (Widdicombe, 1979). This hyperfunction of parasympathetic nerves could be comparable to the increased bronchoconstriction induced by stimulation of the vagus after the addition of 8PT.

Apart from this possible purinergic involvement in the hyperfunction of parasympathetic nerves there are other links between purines and asthma. For example, ATP itself is released during the antigen-antibody reaction and it can cause many of the symptoms of an asthmatic attack. Aerosol administered ATP has been reported to cause bronchoconstriction in asthmatic humans (Holgate et al, 1986). ATP can liberate histamine from mast cells directly through a calcium dependent process, hence releasing histamine which contributes to asthmatic symptoms (Sugiyama, 1971). Adenosine, unlike ATP does not release histamine itself but it does induce the antigen-induced histamine release from tissue (Welton & Simko, 1980) and mast cells (Fredholm & Sydbom, 1980). Adenosine also is released during the antigen challenge.

NERVE MEDIATED RESPONSES.

Exploration of parameters suitable for vagal bundle stimulation showed that a frequency of 15Hz at 25v for 30sec (0.5ms pulse width) gave a good bronchoconstriction when the airway smooth muscle had low resting tone as well as giving good bronchodilation in conditions of high resting tone of the airway smooth muscle. Previous investigations of the NANC bronchodilation response have used similar stimulatory parameters. Diamond & O'Donnell (1980) obtained bronchodilations at frequencies between 1 and 100 Hz at 30v for 5 sec (pulse width 1ms), whereas Irvin et al (1980) stimulated at a frequency of 30Hz (0.5ms pulse width) throughout but varied the voltage (10-40v). When studying the effects of the adenosine uptake inhibitor, dipyridamole, on the nerve induced bronchodilations, it became apparent that stimulation at frequencies of 15Hz were not suitable for this particular part of the study. The bronchodilations produced under these conditions were usually maximal, reaching a level comparable to the resting tone of the airway smooth muscle before the start of the 5HT infusion. Therefore in order to leave the possibility of an increase in the bronchodilation by any agent (such as dipyridamole), a sub-maximal frequency of 5Hz was employed.

Rather than just stimulating the parasympathetic

branch (the vagus), the isolated and sectioned cervical vagosympathetic nerve bundle was stimulated and alpha and beta adrenoceptor antagonists were added to block out the adrenergic effects. This overcame any difficulties which could have arisen due to damage of the parasympathetic branch, which is thought to be responsible for both the NANC transmitter as well as the excitatory transmitter ACh, or even VIP (Polak & Bloom, 1986) or the consequences of including stray sympathetic fibres which would have made experimental interpretations difficult.

The bronchoconstriction produced by stimulation of the vagal bundle in low resting airway smooth muscle tone was certainly cholinergic, and thus likely to be mediated by the parasympathetic branch, since propranolol, prazosin and idazoxan did not affect the responses and atropine at a dose level of 1mg/kg completely blocked them. Also the bronchoconstrictor responses produced by vagal stimulation compared well with those obtained by intravenous administration of ACh.

The effect of the P_1-purinoceptor antagonist 8PT, on the nerve induced bronchoconstriction, was to enhance the level of bronchoconstriction produced. One possible explanation of this could be that 8PT is acting by inhibiting an adenosine negative feedback system which would normally inhibit the release of the

neurotransmitter (ACh) or co-transmitters (ACh with ATP or adenosine), thus more of the excitatory transmitter is released. Alternatively 8PT may be acting to reduce the effective adenosine component of transmission (either pre-junctionally by blocking the release of adenosine, or post-junctionally by blocking the inhibitory action of adenosine on the smooth muscle via P_1-purinoceptors). The bronchoconstriction produced by the intravenous administration of ACh was not affected by 8PT, therefore it is unlikely that 8PT was acting to potentiate the action of released ACh post-junctionally. Gustaffson and co-workers (1986) reported an adenosine/ cholinergic interaction in transmission in the rabbit bronchi but they concluded that this interaction resulted in an adenosine enhancement of cholinergic transmission and not inhibition.

NERVE MEDIATED RESPONSES IN HIGH RESTING AIRWAY SMOOTH MUSCLE TONE

The bronchodilatory responses reached their maximum 5 - 30 seconds after nerve stimulation had stopped. This may indicate that some process must occur before activation of the receptor on the smooth muscle cell which leads to the response. For example there may be either breakdown of the released transmitter to the actual receptor ligand (e.g. ATP to adenosine, if ATP rather than adenosine was the released co-transmitter) or the release of another substance (e.g. a

prostaglandin from the lung epithelium (Kamikawa & Shimo, 1976)), which would then mediate the bronchodilation rather than adenosine itself. Alternatively the receptor inhibition-coupling may be a slow process. This is not unusual for an inhibitory process since it requires the removal of the active tone and may for example involve the spontaneous loss of some substance or the active pumping or binding of another (e.g. Ca^{2+} ions).

These responses were not reduced by sequential intravenous injections of atropine,propranolol, prazosin and idazoxan. This confirmed other peoples findings that these response were non-adrenergic non-cholinergic (Diamond & O'Donnell, 1980; Irvin et al, 1980).

Diamond & O'Donnell (1980) reported that the bronchodilation produced by vagal stimulation appeared to increase after atropine had been added. This was probably caused by taking elimination of the contractile part of the biphasic response which they observed in this preparation, therefore the full effect of bronchodilation was unmasked.

Propranolol made no significant difference to the observed bronchodilation, indicating that this bronchodilation is unlikely to be due to release of NA from the sympathetic branch. Stimulation of beta-adrenoceptors by release of NA from sympathetic nerves

has been reported to cause bronchodilation in the cat in vivo (Diamond & O'Donnell, 1980; Diamond & Gillespie, 1982). However the NANC bronchodilation is the larger (both in magnitude and in time course) and dominates over the smaller sympathetic modulated bronchodilation (Irvin et al, 1980).

A combination of prazosin and idazoxan (or rauwolscine) used for alpha$_1$- and alpha$_2$-adrenoceptor blockade, had no effect on the bronchodilation responses, therefore eliminating the likelihood of the involvement of alpha-adrenoceptors in this response.

The P_1 antagonist 8PT caused a small reduction in the neurally evoked bronchodilatory response in the presence of muscarinic and alpha and beta-adrenoceptor blockade. However following the administration of the adenosine uptake inhibitor dipyridamole (10mg/kg), it was found that attenuation of the bronchodilator response caused by 8PT was more marked a frequency of stimulation of 15Hz. The attenuating effect of dipyridamole /8PT pretreatment is best shown at a submaximal frequency of 5Hz.

8PT (10mg/kg) could have reduced the nerve induced bronchodilation by antagonising an inhibitory effect of adenosine (which is released by nerve stimulation) on the airway smooth muscle. For example if adenosine is acting postjunctionally as an inhibitory transmitter

relaxing the precontracted airway smooth muscle 8PT will antagonise this. Alternatively, 8PT may act presynaptically reducing the output of a bronchoconstricting transmitter such as Substance P (Barnes, 1986) rather than ACh.

The addition of the adenosine uptake inhibitor dipyridamole (10mg/kg) enhanced the blocking effect of 8PT on the nerve induced bronchodilatory response. Dipyridamole on its own did not affect the bronchodilatory responses at 15Hz stimulation, but responses were probably maximal so that increases in bronchodilation could not be expected. At submaximal bronchodilation produced by 5Hz stimulation, the potentiating effects of dipyridamole on the bronchodilation could be observed. In explaining this, perhaps 8PT is a substrate for the cellular uptake process for adenosine and dipyridamole, by blocking this, makes more 8PT available at the post-synaptic P_1-purinoceptor sites on the airway smooth muscle. Alternatively, dipyridamole might potentiate pre and postsynaptic effects of adenosine resulting in reduced transmitter output therefore 1) less response is mediated by other transmitters, and 2) more of the response is ATP/adenosine mediated, so overall, 8PT will block more effectively, since the response is maintained by a lower concentration of ATP/adenosine acting at more sites (due to loss of uptake) therefore the antagonist 8PT can compete more effectively.

Another P_1-purinoceptor antagonist, aminophylline (10mg/kg), also slightly reduced the bronchodilatory response, again indicating a P_1 component to the NANC response. The administration of aminophylline produced a short lasting decrease in 5HT-induced tone. Aminophylline is used in asthma therapy for relaxation of bronchial smooth muscle. The mechanism of action of aminophylline and other theophylline type drugs is regarded as mainly through cyclic nucleotide phosphodiesterase inhibition and only partly through adenosine antagonist action (Fredholm et al, 1979). This would explain why a relaxation was not observed with 8PT, since 8PT is thought to be virtually without phosphodiesterase action (Smellie et al, 1979).

The P_2-purinoceptor desensitising agent mATP did not significantly antagonise the vagally induced bronchodilation either in the presence or in the absence of atropine, 8PT and the alpha and beta-adrenoceptor antagonists. Since the P_{2x}-desensitising agent, mATP, was ineffective, the response under study is another example of an inhibitory NANC response which does not involve P_{2x}-purinoceptors. This might therefore indicate that these responses have P_1 rather than P_2-purinoceptor mediated components. However since mATP is specific for the excitatory P_{2x}-purinoceptors, it will have no effect on P_{2y}-purinoceptors through which ATP acts to produce relaxatory/inhibitory effects. It

therefore is not possible to rule out P_2-purinoceptor involvement in these responses until a suitable P_{2y}-purinoceptor antagonist can be found and tested against these responses.

In conclusion, it is not yet possible to state the exact physiological and pathophysiological importance of purines in the lung, but since the purinergic agonists adenosine and mATP show that purinergic receptors are present and functional, from the findings of the present study, it can be said that purines do play a role in lung function, since the NANC inhibitory nervous system in the airways of the cat is at least partly purinergic mediated.

REFERENCES

Abel, J. J. & Crawford, A. C. (1879). On the blood pressure raising constituent of the suprarenal capsul. Bull. Johns. Hopk. Hosp., 8, 151-157.

Abel, J. J. & Taveau, R. (1905). On the decomposition products of epinephrine hydrate. J. biol. chem., 1, 1-32.

Ahlquist, R. P. (1948). A study of the adrenotropic receptors. Am. J. Physiol., 153, 586-600.

Alabaster, V.A., Keir, R.F.& Peters, C.J. (1985). Comparison of activity of alpha-adrenoceptor agonists and antagonists in dog and rabbit saphenous vein. Naunyn-Schmeidebergs Arch. Pharmac.330: 33-36

Alabaster, V. A., Peters, C. J. & Keir, R. F. (1986). Comparison of potency of alpha$_2$ antagonists in vitro: evidence for heterogeneity of alpha$_2$ adrenoceptors. Br. J. Pharmac.88, 607-615.

Aldrich, T. B. (1901). A preliminary report on the active principal of the suprarenal gland. Am. J. Physiol.5, 457-461.

Ambache, N.(1951). Unmasking after cholinergic paralysis by botulinum toxin, of a reversed action of nicotine on the mammalian intestine, revealing the probable presence of local inhibitory ganglion cells in the enteric plexus. Br. J. Pharmac.6, 51-67.

Ambache, N. & Zar, M. A. (1970). Non cholinergic transmission by post-ganglionic motor neurones in the mammalian bladder. J. Physiol. (London), 210, 761-783.

Ambache, N. & Zar, M. A. (1971). Evidence against adrenergic motor transmission in guinea-pig vas deferens. J. Physiol. (Lond.) 216, 359-389.

Amdur, M. O. & Mead, J. (1958). Mechanics of respiration in unanaesthetized guinea pigs. Am. J. Physiol. 192, 364-368.

Baer, H. P. & Drummond, G. I. (1968). Catabolism of adenine nucleotides by the isolated perfused rat heart. Proc. Soc. exp. Biol. Med.,127,33-36.

Baer, H. P., Muller, M. J., & Vriend., R.(1983). Adenosine receptors in smooth muscle. p77-83. In: Physiology and Pharmacology of Adenosine Derivatives. Raven Press , New York.

Banks, B. E. C., Brown, C., Burgess, G. M., Burnstock, G., Claret, M., Cocks, T. M. & Jenkinson, D. H. (1979). Apamin blocks certain neurotransmitter-induced increases in potassium permeability. Nature 282,415-417.

Barger, G. & Dale. H. H. (1910). Chemical structure and sympathomimetic action of amines. J. Physiol., 41, 19-59.

Barnes, P.J. (1984). The third nervous system in the lung: physiology and clinical perspectives. Thorax39, 561-567.

Barnes, P. J. (1986). Non-adrenergic non-cholinergic neural control of human airways. Arch. int. pharmacodyn. 280, Suppl.,208-228.

Barnes, P. J. (1986). Asthma as an axon reflex. Lancet.1, 242-244.

Bennet, D.W. & Drury, A.N. (1931). Further observations relating to the physiological activity of adenine compounds. J. Physiol. (London).72, 288-320.

Berne, R.M. (1963). Cardiac nucleotides in hypoxia: possible role in regulation of coronary blood flow. Am. J. Physiol.204, 317-322.

Berne, R.M. (1980). The role of adenosine in the regulation of coronary blood flow. Circulation Res.,47, 807-813.

Berne, R.M., Rubio, R. & Dobson, J.G. (1975). Adenosine and adenine nucleotides as possible mediators of cardiac and skeletal muscle blood flow regulation. Circ. Res. (Suppl. 1),28, 115-119.

Bianchi, A., De Natale, G. & Giaquinto, S. (1963). The effects of adenosine and its phosphorylated derivatives upon the respiratory apparatus. Arch. Int. Pharmacodyn.145, 498-517.

Blaber, L. C., Fryer, A. D. & Maclagan, J. (1985). Neuronal muscarinic receptors attenuate vagally-induced contraction of feline bronchial smooth muscle. Br. J. Pharmac. 86, 723-728.

Blakely, A. G. H., Brown, D. A., Cunnane, T. C., French, A. M., McGrath, J. C. & Scott, N. C. (1981). Effects of nifedipine on electrical and mechanical responses of rat and guinea pig vas deferens. Nature.294 759-761.

Boyd, I.A. & Forrester, T. (1968). The release of adenosine triphosphate from frog skeletal muscle in vitro. J. Physiol. 199, 115-135.

Braun, S. & Levitski, A. (1977). Mode of coupling adenylate cyclase to the adenosine receptor. Isr. J. Med. Sci.15,82.

Brown, C. M. & Burnstock, G. (1981). Evidence in support of the P_1/P_2 purinoceptor hypothesis in the guinea-pig taeni coli. Br. J. Pharmac. 73, 617-624.

Brown, D. A., Docherty, J. R., French, A. M., MacDonald, A.,McGrath, J. C. & Scott, N. C. (1983). Separation of adrenergic and non-adrenergic contractions to field stimulation in the rat vas deferens. Br. J. Pharmac. 79, 379-393.

Brown, G.L. & Gillespie, J.S. (1957). The output of sympathetic transmitter from the spleen of the cat. J. Physiol. 138, 81-102.

Bunger, R., Haddy, F.J. & Gerlach, E. (1975). Coronary responses to dilating substances and competitive inhibition by theophylline in the isolated perfused guinea-pig heart. Pflugers Arch.,358, 213-224.

Burn, J.H. & Rand, M.J. (1965). Acetylcholine in adrenergic transmission. Ann. Rev. Pharmacol. 5, 163-182.

Burnstock, G. (1978). A basis for distinguishing two types of purinergic receptor. In: Cell membrane receptors for drugs and hormones: a multidisciplinary approach. Ed. L. Bolton & R.W. Straub ,ppl07-118. Raven Press, New York.

Burnstock, G. (1982a). Adenine nucleotides and nucleosides in cerebral hypoxia. In: Cerebral hypoxia in the pathogenesis of migraine (Progress in Neurobiology) Ed. F.C. Rose & W.K. Amery, pp77-81, Pitman Press, Bath.

Burnstock, G. (1982b). The co-transmitter hypothesis, with special reference to the storage and release of ATP with noradrenaline and acetylcholine. In: Co-transmission. Ed. A.C. Cuello, ppl51-163, MacMillan Press, London.

Burnstock, G., Campbell, G., Bennett, M. & Holman, M.E. (1963). Inhibition of the smooth muscle of the taeni coli. Nature, London.,200, 581-582.

Burnstock, G., Campbell, G., Bennett, M. & Holman, M.E. (1964). Innervation of the guinea-pig taenia coli: are there intrinsic inhibitory nerves which are distinct from sympathetic nerves? J. Neuropharmacol. 3, 163-166.

Burnstock, G., Campbell, G. & Rand, M.J. (1966). The inhibitory innervation of the taenia of the guinea pig caecum. J. Physiol. 182, 504-526.

Burnstock, G., Campbell, G., Satchell, D.G. & Smythe, A. (1970). Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenegic inhibitory nerves in the gut. Br. J. Pharmac. 40, 668-688.

Burnstock, G., Cocks, T., Crowe, R. & Kasakov, L. (1978). Purinergic innervation of the guinea-pig urinary bladder. Br. J. Pharmac.63, 125-138.

Burnstock, G., Cocks, T., Kasakov, L. & Wong, H. (1978). Direct evidence for ATP release from non-adrenergic non-cholinergic (purinergic) nerves in the guinea-pig taenia coli. Eur. J. Pharmac.49, 145-149.

Burnstock, G., Dumsday, B. & Smythe, A. (1972). Atropine resistant excitation of the urinary bladder: the possibility of transmission via nerves releasing releasing a purine nucleotide. Br. J. Pharmacol.44, 451-461.

Burnstock, G. & Holman, M.E. (1964). An electrophysiological investigation of the actions of some autonomic blocking drugs on transmission in the guinea-pig vas deferens. Br. J. Pharmacol. 23, 600-612.

Burnstock, G. & Iwayama, T. (1971). Fine structural and experimental studies on autonomic nerve endings in smooth muscle. In: Histochemistry of nervous transmission (Progress in Brain Research). Ed. O. Eranko, Vol. 34.pp389-404. Elsevier, Amsterdam.

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

Burnstock, G & Sneddon, P. (1984) Inhibition of excitatory junction potentials in guinea-pig vasdeferens by α,β-methylene ATP: further evidence foe ATP and noradrenaline as cotransmitters. Eur. J. Pharmac>100,85-90.

Burnstock, G & Sneddon, P. (1985). Evidence for ATP and noradrenaline as cotransmitters in sympathetic nerves. Clinical Science.68 (Suppl.10), 89s-92s.

Burnstock, G. & Warland, J.L.I. (1987a). A Pharmacological study of the rabbit saphenous artery in vitro: a vessel with a large purinergic contractile response to sympahtetic nerve stimulation. Br. J. Pharmac. 90, 111-120.

Burnstock, G. & Warland, J.L.I. (1987b). P_2-purinoceptors of two subtypes in the rabbit mesenteric artery: reactive blue 2 selectively inhibits responses mediated via the P_{2y}- but not the P_{2x}-purinoceptor. Br. J. Pharmac. 90, 383-391.

Byrne, N. G. & Large, W. A. (1986). The effect of α,β-methylene ATP on the depolarization evoked by noradrenaline (α-adrenoceptor response) and ATP in the immature rat basilar artery. Br. J. Pharmac. 88, 6-8.

Cannon, W. B. & Rosenbleuth, A. (1933). Studies on

conditions of activity in endocrine organs. XXXIX. Sympathin E and Sympathin I. Am. J. Physiol.,104, 557-574.

Cannon, W. B. & Uridil, J. E. (1921). Some effects on the denervated heart of stimulating the nerves of the liver. Am. J. Physiol. 58,353-364.

Chaudry, I.H. & Gould, M.K. (1970). Evidence for the uptake of ATP by rat soleus muscle in vitro. Biochem. Biophys. Acta.196, 320-326.

Clanachan, A.S. (1979). Modulation of release by adenosine and adenine nucleotides. In: The release of catecholamines from adrenergic neurones. Ed. D.M. Paton, pp263-273. Pergammon Press, Oxford.

Clanachan, A.S., Johns, A. & Paton, D.M. (1977). Presynaptic inhibitory actions of adenine nucleotides and adenosine on neurotransmission in the rat vas deferens. Neuroscience. 2, 597-602.

Clanachan, A. S. & McGrath, J. C. (1976). The effects of ketamine on the peripheral autonomic nervous system of the rat. Br. J. Pharmac. 58, 247-252.

Coburn.,R. F. & Tomita, T. (1973). Evidence for nonadrenergic inhibitory nerves in the guinea pig trachealis muscle. Am. J. Physiol. 224 (5), 1072-1080.

Coleman, R. A. (1976). Effects of some purine derivatives on the guinea-pig trachea and their interaction with drugs that block adenosine uptake. Br. J. Pharmac. 57, 51-57.

Coleman, R. A. & Levy, G. P. (1974). A non-adrenergic inhibitory nervous pathway in guinea-pig trachea. Br. J. Pharmac. 52, 167-174.

Collier, H. O. J., James, G.W.L. & Sneider, C. (1966). Antagonism by aspirin and fenamates of bronchoconstriction and noiciception induced by adenosine 5'-triphosphate. Nature, 212, 411-412.

Dakin, H. D. (1905). The synthesis of a substance allied to adrenaline. Proc. Roy. Soc.B. 76,49-497.

Dale, H. H. (1906). On some physiological actions of ergot. J. Physiol.XXXIV, (3), 163-206.

Dean, D.M. & Downie, J.W. (1978). Contribution of adrenergic and purinergic neurotransmission to contraction in rabbit detrusor. J. Pharmacol. exp. Ther. 207, 431-445.

Delbro et al (1985) Potent vasoconstrictor actions of α,β-methylene ATP, a stable analogue of ATP, on the rat vasculature, in vivo. Acta Physiol. Scand. 123,501-503.

Demichel,P., Gomond,P. & Roquebert, J.(1982). -adrenoceptor blocking properties of raubasin in pithed rats. Br.J. Pharmac.77,449-454.

Diamant, B. & Kruger, P.G. (1967). Histamine release from isolated rat peritoneal mast cells induced by adenosine 5'-triphosphate. Acta. Physiol. Scand. 71, 291-302.

Diamond, L. & Gillespie, M. (1982). The lung nonadrenergic inhibitory nervous system. Trends Pharmacol. Sci. 3,237-239.

Diamond, L. & O'Donnell, M. (1980). A nonadrenergic vagal inhibitory pathway to feline airways. Science. 208, 185-188.

Docherty, J.R. & Hyland, L. (1985). No evidence for differences between pre- and post-junctional alpha$_2$-adrenoceptors. Br. J. Pharmac. 86:335-339.

Docherty, J. R., MacDonald, A., & McGrath, J. C. (1979). Further subclassification of alpha adrenoceptors in the cardiovascular system, vas deferens and anococcygeus of the rat. Br. J. Pharmac.67, 421P-422P.

Docherty, J. R. & McGrath, J. C. (1979). An analysis of some factors influencing -adrenoceptor feed-back at the sympathetic junction in the rat heart. Br. J. Pharmac. 68, 55-63.

Docherty, J. R. & McGrath, J. C. (1980). A comparison of pre- and post-junctional potencies of several alpha-adrenoceptor agonists in the cardiovascular system and anococcygeus of the rat. Evidence for two types of post junctional alpha-adrenoceptor. Naunyn Schmiedebergs Arch. Pharmacol.312, 107-116.

Douglas, W.W. (1968). Stimulus-secretion coupling: the concept and clues from chromaffin and other cells. Br. J. Pharmac. 34, 451-476.

Douglas, W.W. & Poisner, A.M. (1966). On the relation between ATP splitting and secretion in the adrenal chromaffin cell: extrusion of ATP (unhydrolysed) during release of catecholamines. J. Physiol. (London).183, 249-256.

Dowdle, E.B. & Maske, R. (1980). The effects of calcium concentration on the inhibition of cholinergic neurotransmission in the myenteric plexus of the guinea pig ileum by adenine nucleotides. Br. J. Pharmac. 71, 245-253.

Drew, G. M. (1980). Postsynaptic alpha$_2$-adrenoceptors mediating pressor responses to 2-N, N-dimethylamino-5, 6-dihydroxy-1,2,3,4-tetrahydronapthalene (M7). Eur. J. Pharmac., 85-87.

Drew, G. M. & Whiting, S. B. (1979) Evidence for two distinct types of postsynaptic -adrenceptor in vascular smooth muscle in vivo.Br. J. Pharmac.67,207-215.

Drury, A.N. & Szent-Gyorgi, A. (1929). The physiological activity of adenine compounds with special reference to their action upon mammalian heart. J. Physiol. 68, 213-237.

Dusting & Rand (1972). Depressor responses to spinal stimulation in the pithed rat. Br. J. Pharmac.45,218-228.

Eccles, J.C. (1976). From electrical to chemical transmission in the central nervous system. Proc. Of Sir Henry Dale Centennial Symposium. J. Physiol. Lond.

Elliot, T. R. (1905). The action of adrenalin. J. Physiol., 32., 401-467.

Euler, von U. S. (1946) A specific sympathomimetic ergone in adrenergic nerve fibres (sympathin) and its relations to adrenaline and noradrenaline. Acta. Physiol. Scand., 12,73-97.

Ewald, D.A. (1976a). Modulation of postjunctional cholinergic sensitivity of rat diaphragm muscle by cyclic adenosine monophosphate. J. Memb. Biol. 29, 67-79.

Ewald, D.A. (1976b). Potentiation of postjunctional cholinergic sensitivity of rat diaphragm muscle by high energy phosphate adenine nucleotides. J. Memb. Biol. 29, 47-65.

Farmer, J.B. & Farrah, D.G. (1976). Pharmacological studies with adenine, adenosine and some phosphorylated derivatives on guinea pig tracheal muscle. J. Pharm. Pharmacol.28, 748-752.

Fedan, J.S., Hogaboom, G.K., O'Donnell, J.P., Colby, J. & Westfall, D.P. (1981). Contribution by purines to the neuro genic response of the vas deferens of the guinea pig. Eur. J. Pharmac.69,41-53.

Finkleman, B. (1930). On the nature of inhibition of the intestine. J. Physiol. 70, 145-157.

Flavahan, N.A. (1983) Alpha-adrenoceptor subtypes in the cardiovascular system. Ph.D. Thesis. University of Glasgow.

Flavahan, N. A., Grant, T. L., Greig, J. & McGrath, J. C.(1985). Analysis of the alpha-adrenoceptor-mediated, and other components, in the sympathetic vasopressor responses in the pithed rat. Br. J. Pharmac. 86,265-274.

Flavahan, N. A. & McGrath, J. C.(1980). Blockade by yohimbine of prazosin resistant pressor effects of

adrenaline in the pithed rat. Br. J. Pharmac. 69,355-357.

Fredholm, B.B. (1976). Release of adenosine-like material from isolated perfused dog adipose tissue following sympathetic nerve stimulation and its inhibition by adrenergic alpha-receptor blockade. Acta. Physiol. Scand. 96, 422-430.

Fredholm, B.B. & Hedqvist, P. (1978). Release of ^{3}H purines from ^{3}H adenine-labelled rabbit kidney following sympathetic nerve stimulation and its inhibition by alpha-adrenoceptor blockade. Br. J. Pharmac. 64, 239-246.

Fredholm, B.B. & Hedqvist, P. (1980). Modulation of neurotransmission by purine nucleotides and nucleosides. Biochem. Pharmac.29,1635-1643.

Fredholm, B.B. & Hjemdahl, P. (1979). Uptake and release of adenosine in isolated rat fat cells. Acta. Physiol. Scand.105, 257-267.

Fredholm, B.B. & Sydbom, A. (1980). Are there anti-asthmatic actions of theophylline due to antagonism at the adenosine receptor? Agents. Actions.10, 145-147.

Fredholm, B. B., Brodin, K. & Strandberg, K. (1979). On the mechanism of relaxation of tracheal muscle by theophylline and other cyclic nucleotide phosphodiesterase inhibitors. Acta. Pharmacol. et Toxicol. 45, 336-344.

French, A.M. & Scott, N.C. (1981). A comparison of the effects of nifedipine and verapamil on rat vas deferens. Br. J. Pharmac. 73, 321-323.

Frew, R. & Lundi, P. (1982). Effect of arylazido aminopropionyl ATP ($ANAPP_3$). A putative ATP antagonist on ATP responses of isolated guinea pig smooth muscle. Life. Sci. 30, 259-267.

Gardner, C. B.(1977). A study of the vasodepressor response to stimulation of the sacral outflow in the pithed rat. Honours Thesis. University of Glasgow.

Gardner, C.B. & McGrath, J.C. (1978). Depressor responses to stimulation of the sacral outflow in the pithed rat. J. Physiol.277, 76P.

Giles, R.W. & Wilken, D.E.L. (1977). Reactive hyperaemia in dog heart- interrelations between adenosine, ATP and aminophylline and effect of indomethacin. Cardiovasc. Res.11, 113-121.

Gillespie, J.S. (1980). Presynaptic receptors in the autonomic nervous system. In: Adrenergic activators and inhibitors, Part I. Ed. L. Szkeres, pp353-425., Springer Verlag, Berlin.

Gillespie, J.S., Maclaren, A. & Pollock, D. (1970). A method of stimulating different segments of the autonomic outflow from the spinal column to various organs in the pithed cat and rat. Br. J. Pharmac. 40,257-267.

Gillespie, J. S. & Muir, T. C. (1967). A method of stimulating the complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat. Br. J. Pharmac. 30, 78-87.

Gillespie, J.S. & McGrath (1974) The effect of pithing and of nerve stimulation on the depletion of noradrenaline by reserpine in the rat anococcygeus muscle and vas deferens. Br.J.Pharmac.52,585-590.

Grant, T. L. (1985). Influence of angiotensin II on adrenergic pressor responses in the rat. Ph.D. Thesis, University of Glasgow.

Grant, T. L., Flavahan, N. A. Greig, J., McGrath, J. C., McKean C. E., & Reid, J. L. (1985). Attempts to uncover subtypes of alpha-adrenoceptors and associated mechanisms by using sequential administration of blocking drugs. Clin. Sci., 68 (Suppl. 10), 25S-30S.

Grant, T. L. & McGrath, J. C. (1984). The effects of teprotide on pressor responses to alpha-agonists and sympathetic nerve stimulation. Br. J. Pharmac. 83: 368P.

Green, H.N. & Stoner, H.B. (1950). Biological actions of the adenine nucleotides. Lewis . London.

Griffith, S. G., Meghji, P., Moody, C. J. , Burnstock, G. (1981). 8-phenyltheophylline: a potent P_1-purinoceptor antagonist. Eur. J. Pharmac. 75, 61-64.

Grundstrom,N.,& Andersson, R.G.G. (1985). In vivo demonstration of the excitatory non-cholinergic neurotransmission in guinea pig airways. Naunyn-Schmiedeberg's Arch. Pharmacol. 328, 236-240.

Gustafsson, L. E., Wiklund, N. P., & Cederqvist, B. (1986) Apparent enhancement of cholinergic transmission in rabbit bronchi via adenosine A_2 receptors. Eur. J. Pharmacol. 120, 179-185.

Haddy, F.J. & Scott, J.B. (1968). Metabolically linked vasoactive chemicals in local regulation of blood flow. Physiol. Rev. 48, 688-707.

Hedquist, P. & Fredhlom, B.B. (1976). Effects of adenosine on adrenergic transmission; prejunctional inhibiton and postjunctional enhancement. Naunyn Scmiedebergs Arch. Pharmacol. 293, 217-223.

Hirst, G.D.S. & Lew, M.J. (1987). Lack of involvement of alpha-adreneoceptors in sympathetci neural

vasoconstriction in the hindquarters of the rabbit. Br. J. Pharmac. 90, 51-60.

Hirst, G. D. S. & Neild, T. O. (1980) Evidence for two populations of excitatory receptors for noradrenaline on arteriolar smooth muscle. Nature283,767-768.

Hogaboom, G.K., O'Donnell, J.P. & Fedan, J.S. (1980). Purinergic receptors: Photoaffinity analogue of adenosine triphosphate is a specific adenosine triphosphate antagonist. Science. 208, 1273-1275.

Holck, M.I. & Marks, B.H. (1978). Purine nucleotide and nucleoside interactions on normal subsensitive alpha-adrenoceptor responsiveness in guinea-pig vas deferens. J. Pharmacol. exp. Ther.205, 104-117.

Holgate, S. T., Cushley, M. J., Mann, J. S., Hughes, P. & Church M. K. (1986). The action of purines on human airways. Arch. int. Pharmacodyn. 280, Suppl., 240-252.

Holton, P. (1959). The liberation of adenosine triphosphate on antidromic stimulation of sensory nerves. J. Physiol. (London.) 145, 494-504.

Holton, F.A. & Holton, P. (1954). The capillary dilator sunstances in dry powders of spinal roots; a possible role of adenosine triphosphate in chemical transmission from nerve endings. J. Physiol. (London.) 126, 124-140.

Hooper, M., Spedding, M. Sweetman, A.J. & Weetman D.F. (1974). 2-2'pyridylisatogen tosylate: an antagonist of the inhibitory effects of ATP on smooth muscle. Br. J. Pharmac. 50, 458-459.

Hulten, L. (1969). Extrinsic nervous contol of colonic motility and blood flow. Acta. Physiol. Scand. Suppl.335.

Irvin, C. G., Boileau, R., Tremblay, J., Martin, R. R. & Macklem, P. T. (1980). Bronchodilation: noncholinergic, nonadrenergic mediation demonstrated in vivo in the cat. Science 207, 791-792.

Irvin, C. G., Martin, R. R. & Macklem, P. T. (1982). Nonpurinergic nature and efficacy of nonadrenergic bronchodilatation. J. Appl. Physiol. 52, 562-569.

Jauernig, R.A., Moulds, R.F.W. & Shaw, J. (1978). The action of prazosin in human vascular preparations Arch. Int. Pharmacodyn.,231, 81-89.

Kamikawa, Y. & Shimo, Y. (1976). Mediation of prostaglandin E_2 in the biphasic response to ATP of the isolated tracheal muscle of guinea-pigs. J. Pharm. Pharmacol. 28, 294-297.

Karlsson, J. A. & Persson C. G. A. (1984). Neither vasoactive intestinal peptide (VIP) nor purine derivatives may mediate non-adrenergic tracheal inhibition. Acta. Physiol. Scand. 122, 589-598.

Kasakov, L. & Burnstock, G. (1983). The use of the slowly degradable analogue , -methylene ATP, to produce desensitisation of the P_2-purinoceptor: effect on non-adrenergic, non-cholinergic responses of the guinea pig urinary bladder Eur. J. Pharmac.86, 291-294.

Kazic, T. & Milosavljevic, D. (1980). Interaction between adenosine triphosphate & noradrenaline in the isolated vas deferens of the guinea-pig. Br. J. Pharmac. 71, 93-98.

Kerr, D.I.B. & Krantis, A. (1979). A new class of ATP antagonist. Proc. Austr. Physiol. Pharm. Soc.10, 156P.

Kobinger, W. & Pichler, L. (1980). Investigation into different types of post- and pre-synaptic alpha-adrenoceptors at cardiovascular sites in rats. Eur. J. Pharmacol. 65, 393-402.

Laitinen, A., Partanen, M., Heruonen, A., Peto-Huikko, M. & Laitinen, L.A. (1985). VIP-like immunoreactive nerves in the human respiratory tract. Light and electron microscopy study. Histochemistry, 82, 313-319.

Langer, S. Z. (1973). The regulation of transmitter release elicited by nerve stimulation through a presynaptic feedback mechanism. In : Frontiers in cathecholamine research, 543- 549, Eds., Usdin, E. & Snyder, S. H., Pergamon Press, New York.

Langer, S. Z. (1974). Commentary: Presynaptic regulation of catecholamine release. Biochem. Pharmacol., 23, 1793-1800.

Langer, S.Z. & Pinto, J.E.B. (1976). Possible involvement of a transmitter different from norepinephrine in residual responses to nerve stimualtion of cat nictitating membrane after pretreatment with reserpine. J. Pharmacol. exp. Ther. 196, 697-713.

Langercrantz, H. (1976). On the composition and function of large dense-cored vesicles in sympathetic nerves. Neuroscience.1, 81-92.

Langley, J. N. (1905). On the reaction of cells and of nerve endings to certain poisons, chiefly as regards the reaction of striated muscle to nicotine and to curare. J. Physiol.,33, 374-413.

Langley, J.N. & Anderson, H.K. (1895). The innervation of the pelvic and adjoining viscera. J. Physiol.19, 71-84.

Lembeck, F. & Skofitsch, G. (1982). Visceral pain reflex after pretreatment with capsaicin and morphine. Naunyn Schmiedebergs Arch. Pharmacol.321, 116-122.

Loewi, O. (1921). Uber Humorale Ubertragbarkeit der Herznerrenwirkung I Milteilung. Pfluger's Archives, 189, 239-242.

Londos, C., Cooper, D.M.F. & Wolff, J. (1980). Subclasses of external adenosine receptors. Proc. Natl. Acad. Sci. U.S.A.77, 2551-2554.

Londos, C. & Wolff, J. (1977). Two distinct adenosine-sensitive sites on adenylate cyclase.Proc. Natl. Acad. Sci. U.S.A.74, 5482-5486.

Luchelli-Fortis, M.A., Fredholm, B.B. & Langer, S.Z. (1979). Release of radioactive purines from cat nictitating membrane labelled with [^{3}H] adenosine. Eur. J. Pharmacol.58, 389-398.

Lundberg, J.M. Hokfelt, T., Martling, C-R., Saria, A. & Cuello, C. (1984). Substance P-immunoreactive sensory nerves in the lower respiratory tract of various mammals including man. Cell Tissue Res.235, 251-261.

Madjar, H. Docherty, J. R., Starke, K. (1980). An examination of pre and postsynaptic alpha-adrenoceptors in the autoperfused rabbit hindlimb. J. Cardiovasc. Pharmacol.,2, 619-627.

Maguire, M. H. & Satchell, D. G. (1981). Purinergic receptors in visceral smooth muscle. In: Receptors & Recognition, Series B. Vol.12.pp49-88.

Manery, J.F. & Dryden, E.E. (1979). Ecto-enzymes concerned with nucleotide metabolism. In: Physiological and regulatory functions of adenosine and adenine nucleotides. Ed. Baer, H.P. & Drummond, G.I. pp323-339. Raven Press. New York.

McGrath, J. C. (1973). The inhibitory and motor innervation of the anococcygeus muscle. Ph.D. Thesis, University of Glasgow.

McGrath,J. C. (1978). Adrenergic and non-adrenergic components in the contractile response of the vas deferens to a single indirect stimulus. J. Physiol. (Lond.)283, 23-29.

McGrath, J. C. (1982a). Commentary: Evidence for more than one type of postjunctional alpha-adrenoceptor. Biochem. Pharmacol., 31, (4), 467-484.

McGrath, J.C., Flavahan, N.A. & McKean, C.E. (1982). Alpha$_1$- and alpha$_2$-adrenoceptor-mediated pressor and chronotropic effects in the rat and rabbit. J.Cardiovasc.Pharmac. 4: S101-S107

McGrath, J.C. & McKean, C.E. (1981). Alpha$_2$-adrenoceptors in the rabbit vasculature. Br. J. Pharmac. 74: 846P-847P

McGrath, J.C. & McKean, C.E. (1982). A study with 'selective' alpha$_2$-adrenoceptor antagonists in the rabbit vasculature. Br. J. Pharmac. 76: 261P

McGrath, J.C. & McKenzie, J. E. (1977). The effects of intravenous anaesthetics on the cardiovascular system of the rabbit. Br. J. Pharmac. 61,199-212.

McGrath, J.C. & O'Brien, J.W. (1987). Blockade by nifedipine of responses to intravenous bolus injection or infusion of alpha$_1$- and alpha$_2$-adrenoceptor agonists in the pithed rat. Br. J. Pharmac.91, 355-365.

Meldrum, L.A. (1984). Purinoceptors in neurotransmission neurotransmission, co-transmission and neuromodulation. Ph.D. Thesis, University of London.

Michel, A. D. & Whiting, R. L. (1981). 2-(2-imidazolyl methyl)- 1,4-benzodioxans, a series of selective alpha$_2$-adrenoceptor antagonists. Br. J. Pharmac. 74: 255P-256P.

Milner-White, E.J. & Rycroft, D.S. (1983). The , -methylene analogues of ADP and ATP act as substrates for Creatine Kinase. Eur. J. Biochem.133, 169-172.

Moir, T. W. & Downs, T.D. (1972). Myocardial reactive hyperemia: Comparative effects of adenosine, ATP, ADP, & AMP. Am. J. Physiol.222, 1386-1390.

Moulds, R.F.W. & Jauernig, R.A. (1977). Mechanism of prazosin collapse. Lancet1, 200-201.

Mustafa, S.J. (1980). Cellular and molecular mechanisms of coronary flow regulation by adenosine. J. Mol. Cell. Biochem.31, 67-87.

Nahorski, S.R., Barnett, D.B. & Cheung, Y.D. (1985). Alpha- adrenoceptor-effector coupling: affinity states or heterogeneity of the alpha$_2$-adrenoceptor? Clinical Science 68 Suppl. 10: 39s-42s

Nakanishi, H. & Takeda, H. (1973). The possibility that adenosine triphosphate is an excitatory transmitter in guinea-pig seminal vesicle. Jap.J. Pharmacol. 22, 269-270.

Oliver, G. & Schafer, E.A. (1895). The physiological effects of extracts from suprarenal capsules. J. Physiol.18,230-237.

Paciorek, P. M. & Shepperson, N. B. (1983). Alpha$_1$-adrenoceptor agonist activity of alpha$_2$-adrenoceptor antagonists in the pithed rat preparation. Br. J. Pharmac. 79: 12-14.

Paddle, B.M. & Burnstock, G. (1974). Release of ATP from perfused heart during coronary vasodilation. Blood Vessels. 11, 110-119.

Palmar, J.B., Cuss, F.M.C. & Barnes, P.J. (1986). VIP and PHM and their role in non-adrenergic inhibitory responses in isolated human airways. J. Appl. Physiol.61, 1322-1328.

Paton, D.M. (1981). Structure-activity relations for pre-synaptic inhibition of non-adrenergic and cholinergic transmission by adenosine: evidence for action on A_1 receptors. J. Auton. Pharmacol.1, 287-290.

Paton, W.D.M. & Vane, J.R. (1963). An analysis of the responses of the isolated stomach to electrical stimulation and to drugs. J. Physiol.165,10-46.

Pauwels, R. & Van Der Straeten, M. (1986). The bronchial effects of adenosine in the rat. Arch. int. Pharmacodyn. 280, Suppl., 229-239.

Polak, J. M. & Bloom, S. R. (1986). Regulatory peptides of the gastrointestinal and respiratory tracts. International Archives of Pharmacology. 280 (2), 16-50.

Ribeiro, J. A. (1979). Purinergic modulation of transmitter release. J. Theor. Biol.80, 259-270.

Ribeiro, J. A. & Domaniguez, M.L. (1978). Mechanisms of depression of neuromuscular transmission by ATP and adenosine. J. Physiol. (Paris). 74, 491-496.

Ribeiro, J. A. & Walker, J. (1975). The effects of adenosine triphosphate and adenosine diphosphate on transmission at the rat and frog neuromuscular junction. Br. J. Pharmac.54,213-218.

Rice, W.R. & Singleton, F.M. (1986). P_2-purinoceptors regulate surfactant secretion from rat isolated alveolar Type II cells. Br.J. Pharmac. 89, 485-491.

Robinson, P. M., McLean, J.R. & Burnstock, G. (1971). Ultrastructural identification of non-adrenergic nerve fibres. J. Pharmac. exp. Ther.179, 149-160.

Ruffolo, R. R., Jr. & Waddell, J. E. (1982). Receptor interactions of imidazolines: alpha-adrenoceptors of rat and rabbit aortae differentiated by relative potencies, affinities and efficacies of imidazoline agonists. Br. J. Pharmac. 77: 169-176

Rubio, R., Berne, R.M., Bockman, E.L. & Curnish, R. (1975). Relationship between adenosine concentration and oxygen supply in rat brain. Am. J. Physiol. 228, 1896-1902.

Satchell, D.G., Burnstock, G. & Dann, P. (1973). Antagonism of the effects of purinergic nerve stimulation and exogenously applied ATP on the guinea pig taenia coli by 2-substituted imidazolines & related compounds. Eur. J. Pharmacol. 23, 264-269.

Satchell, D.G. & Burnstock, G. (1975). Comparison of the inhibitory effects on the guinea pig taenia coli of adenine nucleotides and adenosine in the presence and absence of dipyridamole. Eur. J. Pharmacol.32, 324-328.

Schumann, H & Lues, I (1983). Postjunctional alpha-adrenoceptors in the isolated saphenous vein of the rabbit. Characterisation and influence of angiotensin. Naunyn Schmiedebergs Arch. Pharmacol., 323, 328-334.

Schwieler, G. H. (1966). The effect of vagal stimulation on the bronchial tree in the cat. Acta. Physiol. Scand.66, 495-500.

Smellie, F.W., Davis, C.W., Daly, J.W. & Wells, J.N. (1979). Alkylxanthines: inhibition of adenosine-elicited accumulation of cyclic AMP in brain slices and of brain phosphodiesterase activity. Life Sci.24, 2475.

Smith, A.D. (1972). Subcellular localisation of noradrenaline in sympathetic neurones. Pharmacol. Rev.24, 435-467.

Sjarne, L., Hedqvist, P. & Langercrantz, H. (1970). Catecholamines and adenine nucleotide material in effluent from stimulated adrenal medulla. Biochem. Pharmacol.19, 1147-1158.

Sneddon, P., Westfall, D.P. & Fedan, J.S. (1982). Investigations of relaxations of the rabbit anococcygeus muscle by nerve stimulation and ATP using the ATP antagonist ANAPP$_3$. Eur. J. Pharmacol. 80, 93-98.

Sollevi, A. & Fredholm, B.B. (1981). Role of adenosine in adipose tissue circulation. Acta. Physiol. Scand. 112, 292-298.

Starke, K. (1972). Alpha sympathomimetic inhibition of adrenergic and cholinergic transmission in the rabbit heart. Naunyn Schmiedebergs Arch. Pharmacol. 274, 18-45.

Starke, K. (1972). Influence of extracellular noradrenaline on the stimulation-evoked secretion of noradrenaline from sympathetic nerves: Evidence for an alpha-receptor mediated feedback inhibition of noradrenaline release. Naunyn Schmiedebergs Arch. Pharmacol.275, 11-23.

Starke, K. (1977). Regulation of noradrenaline release by presynaptic receptor systems Rev. Physiol. Biochem. Pharmacol.,77, 1-124.

Starke, K. (1981). Alpha-adrenoceptor subclassification.

Rev. Physiol. Biochem. Pharmacol. 88, 199-236.

Stevens, P., Robinson, R.L., Van Dyke, K. & Stitzel, R. (1972). Studies of the synthesis and release of adenosine 5'-triphosphate-8-^{3}H in the isolated perfused cat adrenal gland. J. Pharmacol. exp. Ther. 181, 463-471.

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

Stolz, F. (1904). Uber adrenalin und alkylaminoacetobrentzcatechin. Ber. dtsch. chem. Ges.,37, 4149-4154.

Su, C. (1975) Neurogenic release of purine compounds in blood vessels.J. Pharmacol. exp. Ther.195, 159-166.

Su, C. (1978). Purinergic inhibition of adrenergic transmission in rabbit blood vessels. J. Pharmacol. exp. Ther.205, 351-357.

Su, C., Bevan, J.A. & Burnstock, G. (1971). [^{3}H]-Adenosine triphosphate: release during stimulation of enteric nerves. Science. 173, 351-357.

Sugiyama, K. (1971). Calcium-dependent histamine release with degranulation from isolated rat mast cells by adenosine 5'-triphosphate. Jpn. J. Pharmacol., 21, 209-226.

Sugiyama, K. & Yamasaki, H. (1969). Calcium dependent histamine release by ATP from isolated rat mast cells. Jpn. J. Pharmacol. 19, 175- 176.

Takamine, J. (1901). The isolation of the active principal of the suprarenal gland. J. Physiol., 27, 29P-30P.

Theobald, R. J. (1983) Arylazido aminopropionyl ATP ($ANAPP_3$) antagonism of cat urinary bladder contractions. J. Auton. Pharmacol. 3, 175-179.

Timmermans, P.S.M.W.M., Kum, H.Y. & van Zwieten, D.A. (1979). Possible subdivision of postsynaptic alpha-adrenoceptors mediating pressor responses in the pithed rat. Naunyn Schmiedebergs Arch. Pharmacol., 310, 189-193.

Timmermans, P.S.M.W.M. & van Zwieten, D.A. (1980). Postsynaptic $alpha_1$ and $alpha_2$-adreneoceptors in the circulatory system of the pithed rat: selective stimulation of the $alpha_2$ type by BHT 933. Eur. J. Pharmacol., 63, 199-202.

Tomita, T. & Watanabe, H. (1973). Factors controlling myogenic activity in smooth muscle. Phil. Trans. R. Soc. 265, 73-85.

Van Calkner, D., Muller, M. & Hamprecht, B. (1979). Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured braon cells. J. Neurochem. 33,999-1005.

Vladmirova, I.A. & Shuba, M.F. (1978). Strychnine, hydrastine and apamin effect on synaptic transmission in smooth muscle cells. Neurophysiology. 10, 295-299.

Vizi, E.S. (1979). Presynaptic modulation of neurochemical transmission. Prog. Neurobiol. 12, 181-290.

Vizi, E.S. & Knoll, J. (1976). The inhibitory effect of adenosine and related nucleotides on the release of acetylcholine. Neuroscience. 1, 391-398.

Waterfall, J.F., Rhodes, K.F. & Lattimer, N. (1985). Studies of alpha$_2$-adrenoceptor antagonist potency in vitro: comparisons in tissues from rats, rabbits, dogs and humans. Clinical Science 68 Suppl. 10 : 21s-24s

Webber, A. & Herz, R. (1968). The relationship between caffeine contracture of intact muscle and the effect of caffeine on reticulum. J. Gen. Physiol. 52, (5), 750-759.

Welton, A.F. & Simko, B.A. (1980). Regulatory role of adenosine in antigen induced histamine release from the lung tissue of actively sensitised guinea pigs. Biochem. Pharmacol. 29, 1085-1092.

Westfall, D.P. (1977). Local regulation of adrenergic transmission. Physiol. Rev. 57, 659-728.

Westfall, D.P., Hogaboom, G.K., O'Donnell, J.P., Colby, J. & Fedan, J.S. (1980). Effect of arylazido aminopropionyl ATP (ANAPP$_3$), an ATP antagonist, on the response to agonists and transmural electrical stimulation in the guinea-pig bladder. Pharmacologist 22, 165.

Westfall, D.P., Colby, J., Fedan, J.S., Hogaboom, G.K. & O'Donnell, J.P. (1981). Effect of arylazido aminopropionyl ATP (ANAPP$_3$), an ATP antagonist, on response to agonists and transmural electrical stimulation of the guinea-pig taenia coli. Fed. Proc. 40, 709.

Widdicombe, J.G. (1979). The parasympathetic nervous system in airway disease. Scand. J. Respir. Dis. (Suppl.), 103, 38-43.

Wolff, M.M. & Berne, R.M. (1956). Coronary vasodilator properties of purine and pyrimidine derivatives. Circulatory Res. 4, 343-348.