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Analysis of the α-adrenoceptor-mediated, and other, components in the sympathetic vasopressor responses of the pithed rat

N.A. Flavahan[1], T.L. Grant, J. Greig & J.C. McGrath

Institute of Physiology, University of Glasgow, Glasgow G12 8QQ

1 The vascular receptors activated following sympatho-adrenal stimulation were determined by analysing the effects of 'selective' antagonists on the vasopressor response to spinal sympathetic nerve activation in the pithed rat.

2 The net vascular response to adrenal stimulation was a balance between α-adrenoceptor-mediated vasoconstriction and β-adrenoceptor-mediated vasodepression. Part of the α-adrenoceptor-mediated response was 'prazosin-sensitive' (α_1) and the remainder was abolished by rauwolscine (α_2).

3 As with adrenal stimulation, direct sympathetic nerve stimulation of the vasculature evoked pressor responses which were partly resistant to prazosin. Rauwolscine only partly blocked the prazosin-sensitive component. Reserpine pretreatment led to smaller responses than prazosin plus rauwolscine. Thus, the response resistant to α-adrenoceptor antagonists could be mediated, in part, by adrenoceptors distinct from α-adrenoceptors, as currently defined.

4 α, β-Methylene ATP reduced the nerve-mediated pressor response after α-adrenoceptor blockade or reserpine pretreatment but not in drug-free controls.

5 The results suggest that stimulation of the adrenal medulla can produce a vasopressor response which consists of summating α_1- and α_2-adrenoceptor-mediated components, and is identical to the effect of injected adrenaline. In contrast, the response to vasopressor nerve stimulation appears to be essentially mediated by α_1-adrenoceptors, with a facilitatory influence from α_2-adrenoceptors. A further response obtained after α-adrenoceptor blockade may contain a purinergic component and another which is adrenergic but not mediated by stimulation of α-adrenoceptors.

Introduction

Circulating catecholamines constrict vascular resistance vessels by activating postjunctional α_1- and α_2-adrenoceptors (Flavahan & McGrath, 1980; Willfert *et al.*, 1982; McGrath *et al.*, 1982). It seems also that more than one receptor is stimulated by nerve-released noradrenaline (McGrath, 1982). Initial observations suggested that postganglionic sympathetic nerves innervated preferentially the α_1-site (Docherty & McGrath, 1980, a,b; Yamaguchi & Kopin, 1980; Langer *et al.*, 1980; Willfert *et al.*, 1982; Langer & Shepperson, 1982), but that an α_2-adrenoceptor-mediated component could be demonstrated in some preparations (Madjar *et al.*, 1980; McGrath *et al.*, 1982). More recently, it has been proposed that α_1- and α_2-adrenoceptors are both located extra-junctionally and that the sympathetic transmitter activates only postjunctional γ-adrenoceptors (Neild & Zelcer, 1982). In addition, it has been proposed that co-transmission involving adrenergic and purinergic elements may occur in some blood vessels (Burnstock & Sneddon, 1984).

Previous studies have failed to demonstrate significant vascular effects following stimulation of the adrenal medulla (Celander, 1954; Bell & Kushinsky, 1978; Yamaguchi & Kopin, 1979; Docherty, 1979), suggesting that extra-synaptic or 'hormonal' (Ariens & Simonis, 1976) receptor sites may have a limited role in cardiovascular regulation (see also, Neil, 1975; Lewis, 1975). The aim of the present study was to investigate the vascular effects evoked by sympatho-adrenal stimulation in the pithed rat in order to determine which populations of postjunctional adrenoceptors might be activated, under physiological conditions, by the sympathetic nervous system and whether these could account for all of the response.

[1]Present address: Department of Physiology and Biophysics, Mayo Clinic, Rochester, Minnesota 55901, U.S.A.

Methods

Male Wistar rats (250–275 g) were pithed by the method of Gillespie *et al.* (1970) and ventilated with 100% O_2 at a rate of 60 min^{-1} and a stroke volume sufficient to maintain arterial P_{CO_2} and pH within the normal range (Flavahan & McGrath, 1981a). Carotid arterial pressure and heart rate were monitored continuously. The right jugular vein was cannulated for drug injections.

Sympathetic nerve stimulation

Vasopressor responses were evoked by stimulation of the spinal sympathetic outflow (10 mm electrode, T6–T8, 0.05 ms pulses) at 5 Hz with increasing train lengths (1, 2, 5 or 10 pulses). With this short pulse duration, neuromuscular blockade was not necessary because skeletal muscle twitching was acceptably small. The analysis of sympathetic responses was, therefore, free from the autonomic side-effects associated with muscle relaxants (Docherty & McGrath, 1978). Vascular reactivity was assessed as changes in diastolic blood pressure.

Reproducible control responses were obtained at each train length before the administration of adrenoceptor antagonists. The effects of these agents, which were administered sequentially in the doses and orders described below, were determined 5 min after their intravenous injection. The order of stimulus trains of different duration (elicited at 90 s intervals) was varied between experiments to rule out error due to a time-dependent decline in the effects of the antagonists. In practice, no such effect was found within the time scale employed.

Sympathectomized or adrenalectomized rats

Chemical sympathectomy was achieved by pretreating rats with 6-hydroxydopamine (6-OHDA) before pithing: intraperitoneal injections, 2 × 50 mg kg^{-1} on day 1, 2 × 100 mg kg^{-1} on day 4, rats pithed on day 5 or 6 (Thoenen & Tranzer, 1968; Docherty & McGrath, 1978) 6-OHDA was dissolved in de-oxygenated 0.9% w/v NaCl solution (saline) containing ascorbic acid (1 mg kg^{-1}).

Acute adrenalectomy was performed in some rats after pithing, via lateral incisions above each kidney. Following adrenalectomy, pithed rats were allowed 15 min to recover before continuing the experiment. No significant differences were observed between the effects of vasopressor nerve stimulation in control and sham-operated pithed rats.

Reserpine pretreatment

Reserpine was dissolved in 2% (w/v) ascorbic acid and, when appropriate, was administered intraperitoneally (3 mg kg^{-1}) 18 h before killing the rat. This schedule reduces the noradrenaline content of the rat heart, anococcygeus and vas deferens by more than 98% (Gillespie & McGrath, 1974).

Administration of α,β-methylene adenosine 5'-triphosphate (α,β-Me ATP)

A similar protocol was followed in all experiments testing the effects of α, β-Me ATP. Control responses were obtained to nerve stimulation or noradrenaline, propranolol (1 mg kg^{-1}) was given and responses repeated. α,β-Me ATP was then given cumulatively

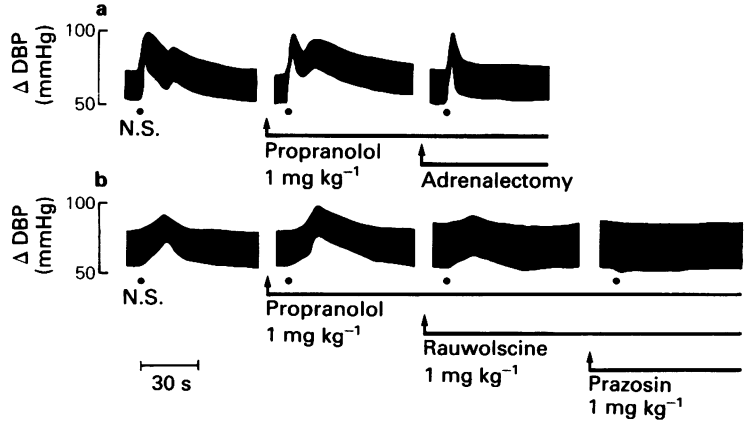

Figure 1 (a) Under control conditions, spinal sympathetic nerve stimulation (N.S.) (T6–T8, 5 Hz, 10 pulses) evokes a biphasic pressor response (change in diastolic blood pressure, Δ DBP). The secondary component, but not the initial transient response, is increased following β-adrenoceptor blockade with propranolol and abolished after adrenalectomy. (b) Following chemical sympathectomy, sympathetic stimulation (N.S.) (T6–T8, 5 Hz, 10 pulses) evokes a monophasic adrenal response which can be abolished by adrenoceptor antagonists. The results of typical experiments are shown.

with responses repeated between doses. Each dose was injected slowly to minimize the pressor responses which followed injection of α,β-Me ATP since these produced rapid deterioration of the preparations.

Drugs

α,β-Methylene ATP (Sigma), 6-hydroxydopamine (Sigma), guanethidine sulphate (CIBA), hexamethonium bromide (Koch-Light), propranolol hydrochloride (Sigma), prazosin hydrochloride (Pfizer), reserpine base (Koch-Light) and rauwolscine base (Inverni della beffa) were used.

Results

Sympatho-adrenal stimulation

Stimulation of the spinal sympathetic outflow (T6–T8, 5 Hz, 1–10 pulses) evoked a biphasic pressor response (Figure 1a). An initial transient component was unaffected by adrenalectomy but was abolished following chemical sympathectomy (Figure 1a and b, respectively), hexamethonium (5 mg kg^{-1}) (n = 15) or guanethidine (1 mg kg^{-1}) (n = 40), indicating that it was mediated by postganglionic sympathetic nerve stimulation. A slower secondary response, which became more prominent following propranolol (1 mg kg^{-1}) (Figures 1a and 2), was unaffected by sympathectomy but was abolished following adrenalectomy (Figure 1b and a, respectively), indicating that it was mediated by stimulation of the adrenal medulla. We have also demonstrated, in recovery experiments, that this response, but not the early component, is abolished by either adrenalectomy or adrenal demedullation (data not shown).

Effects of adrenoceptor antagonists on the adrenal component

In the absence of propranolol, the adrenal component of the vasopressor response to sympathetic stimulation (5 Hz, 10 pulses) was reversed to a depressor effect by prazosin (0.1 mg kg^{-1}) or rauwolscine (1 mg kg^{-1}). At the doses used, prazosin and rauwolscine are 'selective' antagonists of α_1- and α_2-adrenoceptors respectively, according to their effects against phenylephrine and xylazine (Flavahan & McGrath, 1980; 1981b,c). As with 'adrenaline reversal' (Flavahan & McGrath, 1980), this 'endogenous adrenaline reversal' was abolished by propranolol (1 mg kg^{-1}).

Following propranolol (1 mg kg^{-1}), the adrenal pressor responses evoked by sympathetic stimulation (5 or 10 pulses, 5 Hz) were increased significantly ($P < 0.01$, Figure 2). After β-receptor blockade, prazosin (0.1 mg kg^{-1}) or rauwolscine (1 mg kg^{-1}) produced significant attenuation of the adrenal response to each train length ($P < 0.05$, Figure 2a and b respectively). Following prazosin (0.1 mg kg^{-1}), a larger dose of the α_1-adrenoceptor antagonist (1 mg kg^{-1}) produced no further decrease in the pressor responses (Figure 2a). However, these 'prazosin-resistant' responses (Flavahan & McGrath, 1980) were abolished at each train length by rauwolscine (1 mg kg^{-1}) (Figure 2a).

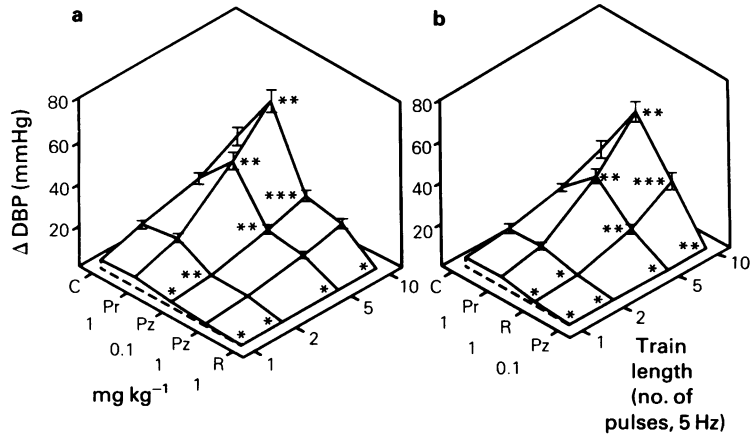

Figure 2 The effects of sequential administration of adrenoceptor antagonists on the adrenal component of the diastolic pressor response (Δ DBP) to sympathetic nerve stimulation (T6–T8, 5 Hz, 1, 2, 5 or 10 pulses). The sequence and doses were varied in two groups of experiments (a) and (b). Control responses (C) and those obtained 5 min after propranolol (Pr), prazosin (Pz) and rauwolscine (R) are shown sequentially from left to right. Following antagonists, the response to each train length was compared to the previous response to the same stimulus by Student's paired t test: *$P < 0.05$, **$0.01 > P > 0.001$, ***$P < 0.001$. n = 5 in each group. Vertical lines represent s.e.mean except where this is within the width of the line.

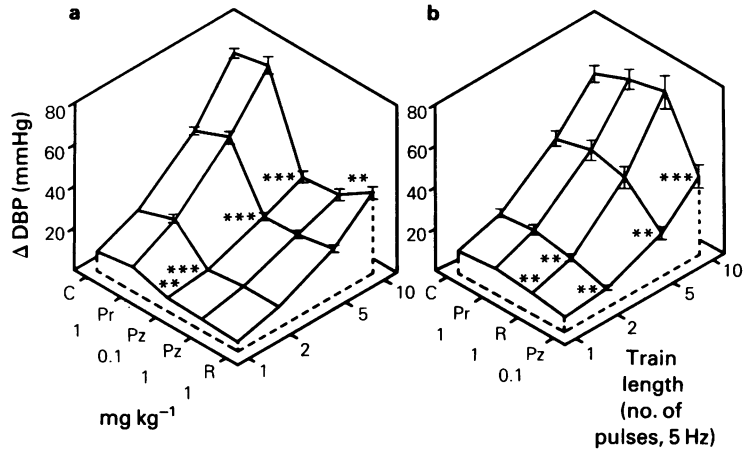

Figure 3 The effects of sequential administration of adrenoceptor antagonists on the direct component of the diastolic pressor response (Δ DBP) to sympathetic nerve stimulation (T6–T8, 5 Hz, 1, 2, 5 or 10 pulses). The sequence and doses were varied in two groups of experiments (a) and (b). Control responses (C) and those obtained 5 min after propranolol (Pr), prazosin (Pz) and rauwolscine (R) are shown sequentially from left to right. Following antagonists, the response to each train length was compared to the previous response to the same stimulus by Student's paired t test: $*P<0.05$, $**0.01>P>0.001$, $***P<0.001$. $n=5$ in each group. Vertical lines represent s.e.mean except where this is within the width of the line.

After rauwolscine (1 mg kg^{-1}), prazosin (0.1 mg kg^{-1}), which previously produced only partial attenuation of the adrenal responses, abolished the remaining responses to each train length (Figure 2b).

Effects of adrenoceptor antagonists on the direct component

In contrast to the adrenal component, β-adrenoceptor activation could not be demonstrated following direct sympathetic nerve stimulation of the vasculature. No 'reversal' response was observed following α-adrenoceptor antagonists and propranolol (1 mg kg^{-1}) did not augment the direct pressor responses evoked by nerve stimulation (1–10 pulses, 5 Hz, Figure 3).

Following propranolol (1 mg kg^{-1}), prazosin (0.1 mg kg^{-1}) reduced significantly the direct pressor responses to each train length ($P<0.01$, Figure 3a). As with the adrenal component, a higher dose of prazosin (1 mg kg^{-1}) produced no further significant decrease in the pressor responses (Figure 3a). These 'prazosin-resistant' pressor responses were not antagonized by rauwolscine (1 mg kg^{-1}): the responses evoked by low train lengths (1, 2 or 5 pulses) were unaffected, whereas that evoked by a longer train length (10 pulses) was significantly increased ($P<0.01$, Figure 3a).

In the absence of prazosin, rauwolscine (1 mg kg^{-1}) decreased significantly the direct effects evoked by low train lengths of nerve stimulation (5 Hz, 1 or 2 pulses; $P<0.01$, Figure 3b) but did not affect those to higher train lengths (5 or 10 pulses; Figure 3b).

Following rauwolscine (1 mg kg^{-1}), prazosin (0.1 mg kg^{-1}) reduced significantly the remaining pressor responses to each train length ($P<0.01$) except that to a single pulse (Figure 3b).

The direct responses to sympathetic nerve stimulation (5 Hz, 1–10 pulses) remaining after prazosin plus rauwolscine, were not significantly different, irrespective of the order of drug administration (c.f. Figure 3a and b). The time course of this residual response after α-adrenoceptor blockade was of very short duration compared with that of the control response (Figure 4).

Reserpine pretreatment

In a preliminary series of experiments (results not shown), vasopressor responses to stimulation via the pithing rod where examined at different times after reserpine (18 h–14 days). Responses showed a maintained depression up to 6 days, after which there was some recovery, particularly of the adrenal component. The effects of α,β-methylene ATP were tested against the responses at the various times and the effects showed no marked variation with time. Consequently, the subsequent experiments were carried out 18 h after reserpine (1 mg kg^{-1}, i.v.). It is difficult to determine the noradrenaline content of resistant blood vessels but the noradrenaline content of the heart, whose sympathetic nerves are also tonically active, is max-

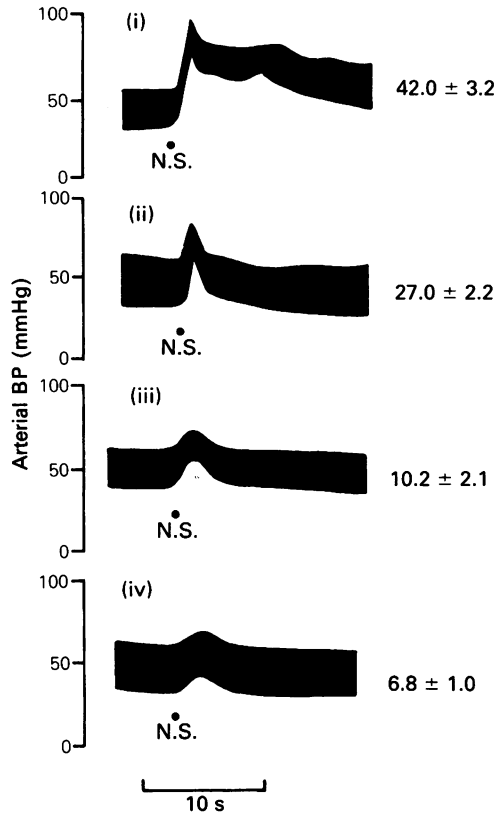

Figure 4 Shows typical arterial pressure responses (mmHg) to stimulation of the spinal sympathetic outflow (T6–T8, 0.05 ms pulse width, 20 Hz, 1 s duration) in the pithed rat. Dots indicate time of stimulus. (i), (ii) and (iii) are sequential responses in one rat. (i) Control, (ii) the effects of a combination of prazosin and rauwolscine (both 1 mg kg^{-1}), (iii) the effects of α,β-methylene ATP (0.05 mg kg^{-1}) in the presence of prazosin and rauwolscine (both 1 mg kg^{-1}). A typical pressor response to nerve stimulation after reserpine pretreatment (1 mg kg^{-1}, 18 h) is shown in (iv). Both groups of rats were pretreated with propranolol (1 mg kg^{-1}, i.v.) after pithing to prevent any β-adrenoceptor-mediated effects. The figures on the right indicate the mean Δ DBP under the four conditions (± s.e.mean) from groups of 5 rats.

imally depressed within 6–12 h and tissues did not show substantial recovery until after 10 to 21 days (McGrath, 1973; Gillespie & McGrath, 1974).

Reserpine pretreatment reduced the pressor responses to electrical stimulation both in height and in time course. The response was reduced to a transient rise, shorter even than the original 'direct response', with little sign of an adrenal component. The responses were significantly smaller than those found in control rats which had been given prazosin plus rauwolscine (each 1 mg kg^{-1}) (Figure 4). Responses to noradrenaline (0.15–0.5 μg kg^{-1}) were similar to those in controls.

α,β-*Methylene ATP*

α,β-Methylene ATP raised the blood pressure of the pithed rat in a dose-dependent manner and, if a large dose (> 25 μg kg^{-1}) was given as a single intravenous bolus, the heart stopped. If the substance was administered in divided doses a viable preparation could be maintained. Each dose produced a pressor response which reached a peak within a minute and returned to baseline almost as quickly. This is similar to the transient contraction of the smooth muscle of rat vas deferens which is produced by α,β-methylene ATP

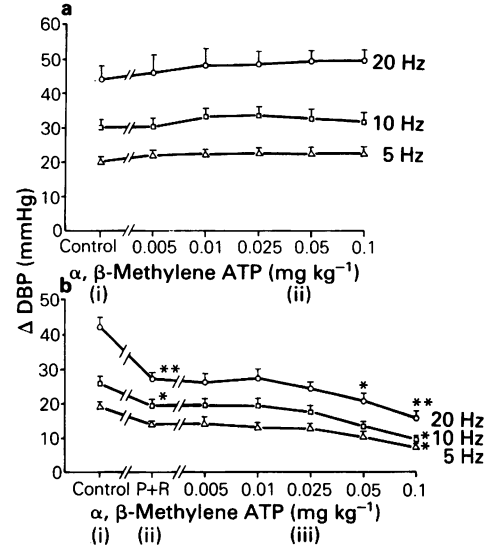

Figure 5 The effects of sequential administration of α,β-methylene ATP on pressor responses in pithed rats to stimulation of the spinal sympathetic outflow (T6–T8, 0.05 ms pulse width, 1 s duration) at 5 Hz (Δ), 10 Hz (□) and 20 Hz (○). (a) Cumulative administration of α,β-methylene ATP (0.005–0.1 mg kg^{-1}) in the absence of α-receptor blockade. (b) Cumulative administration of α,β-methylene ATP (0.005–0.1 mg kg^{-1}) following prazosin (P) and rauwolscine (R) (1 mg kg^{-1} each). All rats were pretreated with propranolol (1 mg kg^{-1}) after pithing. Pressor responses were expressed as peak changes in diastolic pressure. In (a), responses to nerve stimulation after α,β-methylene ATP (ii) were compared with the control responses (i). In (b), responses after α-blockers (ii) were compared with the controls before α-receptor blockade (i). Responses to nerve stimulation after α,β-methylene ATP (iii) were compared with those after α-blockers alone (ii).
*$P < 0.05$; **$0.01 > P > 0.001$ (Student's paired t test). $n = 5$ in each group (a and b). Vertical lines represent s.e.mean.

(T.C. Cunnane & J.C. McGrath, unpublished observations). The heart rate was not affected (except when it stopped).

Despite this apparent tachyphylaxis of its own response upon basal blood pressure, α,β-methylene ATP did not affect the pressor responses to noradrenaline, indicating specificity of the tachyphylaxis. (Noradrenaline, 0.15, 0.35 and 0.5 $\mu g\,kg^{-1}$ i.v., produced dose-related increases in diastolic blood pressure across the range 15 to 40 mm Hg, i.e. of similar height to nerve-induced responses: α,β-methylene ATP 0.005 to 0.1 $mg\,kg^{-1}$ had no significant effect on these responses.)

α,β-Methylene ATP did not significantly affect the pressor responses to electrical stimulation in the absence of other forms of blockade (Figure 5a).

The small responses to stimulation, which remained after reserpine, were reduced by α,β-methylene ATP. For example, responses in rats pretreated with reserpine to pressor nerve stimulation for 1 s, before and after α,β-methylene ATP, 0.05 $mg\,kg^{-1}$, 5 Hz, 3.9 ± 0.6, 1.0 ± 0.6, $n = 6$, $P < 0.05$ (compared using paired t test); 10 Hz, 6.4 ± 1.5, 3.0 ± 0.3, $P < 0.05$, $n = 5$; 20 Hz, 10.2 ± 2.1, 6.8 ± 1.0, not significant, $n = 5$. It should be noted that these responses are very small and approach the limit of accurate measurement.

After blockade of α-adrenoceptors, with the combination of prazosin and rauwolscine, α,β-Me ATP reduced the remaining response in a dose-related manner (Figure 5). This reduction was significant at doses of 0.05 $mg\,kg^{-1}$ (at 20 Hz) or 0.1 $mg\,kg^{-1}$ (at 5 and 10 Hz).

Discussion

The results confirm that both nerve-released noradrenaline and blood-borne catecholamines from the adrenals activate vascular, post-junctional α-adrenoceptors but only the adrenals activate vascular β-adrenoceptors. The sympathetic nerve-mediated response is mainly α-adrenoceptor-mediated but, after elimination of the main response, some other processes are uncovered.

Adrenals

The vascular effects produced by stimulation of the adrenal gland were similar to those evoked by exogenous adrenaline (Flavahan & McGrath, 1980); the major catecholamine secretion in the pithed rat (Yamaguchi & Kopin, 1979). As with adrenaline, the net vascular response to adrenal stimulation appears to be a balance between two α-adrenoceptor-mediated pressor effects (α_1 and α_2) and a β-adrenoceptor-mediated depressor response. Blockade of either α-subtype allows the β-receptor-mediated response to overcome the remaining excitatory influence, causing 'endogenous adrenaline reversal' (Barnett *et al.*, 1980). Antagonism of the β-receptor-mediated depressor effect increases the magnitude of the adrenal responses and enables the sequential removal of the two α-receptor-mediated effects. The postjunctional α_1-receptor-mediated pressor response was characterized by its antagonism by prazosin (Drew & Whiting, 1979; McGrath, 1982; Timmermans & van Zwieten, 1982), a 'selective' α-receptor-antagonist (Cambridge *et al.*, 1977). However, part of the pressor response evoked by adrenal stimulation was 'prazosin-resistant': prazosin (0.1 $mg\,kg^{-1}$) produced maximal blockade of the pressor effects and a 10 times increment in dose (1 $mg\,kg^{-1}$) caused no further attenuation. These 'prazosin-resistant' pressor responses were abolished by rauwolscine, a 'selective' α_2-adrenoceptor antagonist (Tanaka *et al.*, 1978), suggesting that they were mediated by α_2-adrenoceptors. Due to its limited 'selectivity' (McGrath, 1982), higher doses of rauwolscine produce further attenuation of adrenal pressor responses and no 'rauwolscine-resistant' component can be observed. However, after rauwolscine (1 $mg\,kg^{-1}$), prazosin (0.1 $mg\,kg^{-1}$) abolished the remaining pressor effect, indicating that this dose of rauwolscine, while abolishing the α_2-component, had left a substantial α_1-component.

No evidence for postjunctional β-receptor activation following direct sympathetic stimulation of the vasculature was found. This could result from the absence of these receptor sites from junctional areas (Osswald & Guimaraes, 1983) or from the inability of the sympathetic transmitter, noradrenaline, to activate β_2-adrenoceptors (Lands *et al.*, 1967). Certainly the pressor response to intravenous noradrenaline is unaffected by propranolol, supporting the latter hypothesis (Barnett *et al.*, 1980).

Previous workers failed to observe significant vascular effects following activation of the adrenal medulla (Celander, 1954; Bell & Kushinsky, 1978; Yamaguchi & Kopin, 1979; Docherty, 1979). However, the present study demonstrates that the excitatory effects of adrenal stimulation can be observed more clearly following β-receptor blockade. When this is done, adrenal-mediated vasoconstriction can be evoked whilst using very low stimulation parameters, including a single pulse. It is likely that such vasoconstriction occurs in localized areas even without β-receptor blockade but the effect on systemic pressure is masked by vasodilatation elsewhere. Blood-borne catecholamines, released from the adrenal medulla, have the capacity to play an important physiological role in cardiovascular regulation (see also, Clutter *et al.*, 1980; Bevan *et al.*, 1980). Following elevation of the levels of circulating catecholamines, these blood-borne agonists will act on different parts

of the vascular system to produce direct vasoconstriction, direct vasodilatation or modulation of transmitter output. The net effect will depend on the pre-existing degree of neurogenic tone and the local conditions. The ability of circulating agonists and neurogenic transmitters to activate distinct receptor populations which are affected differently by the environment might mean that under certain conditions, e.g. acidosis (Flavahan & McGrath, 1981a, McGrath *et al.*, 1982), the neurogenic response is attenuated while vasoconstriction mediated by blood-borne agents is permitted.

α-Adrenoceptors in direct sympathetic nerve-mediated response

As with the adrenal response, part of the pressor response to direct stimulation can be considered 'prazosin-sensitive' and part 'prazosin-resistant'. There is a complication in interpreting the effects of rauwolscine since it is possible that prejunctional augmentation of noradrenaline release might overcome postjunctional α_2-receptor blockade by rauwolscine. Antagonism of prejunctional α_2-adrenoceptors can increase the stimulation-induced release of noradrenaline (Gillespie, 1980; Starke, 1981). However, α_2-receptor-mediated feedback of neurotransmitter release probably does not occur at the lower train lengths used in this study, especially a single pulse (Rand *et al.*, 1982). In the absence of prazosin, rauwolscine reduced the pressor effects evoked by low train lengths of stimulation (1 or 2 pulses) without affecting those to longer train lengths (5 or 10 pulses, Figure 3). Since rauwolscine does not add to the blockade produced by prazosin, at low frequencies of stimulation, this suggests that rauwolscine antagonizes part of the 'prazosin-sensitive' response. This inhibitory effect may be obscured at longer train lengths or higher frequency by prejunctional facilitation of neurotransmission. The simplest intepretation is that the prazosin-sensitive component is activated primarily via α_1-adrenoceptors and that the postjunctional blockade by rauwolscine is mediated by α_1-receptors, reflecting its lack of selectivity. However, the ineffectiveness of rauwolscine ($1\ mg\ kg^{-1}$) against pressor responses to amidephrine and low doses of phenylephrine, makes this unlikely (Flavahan & McGrath, 1981a; Flavahan, 1983).

It is possible that the response, being susceptible to either an α_1- or an α_2-receptor antagonist, might have contributions from both types of receptor. There is currently interest in the co-transmission hypothesis, particularly release of the classical autonomic transmitters together with neuropeptides (Lundberg & Hökfelt, 1983), which suggests that two agents released from the sympathetic nerve terminal might act in concert to initiate and sustain a response. By analogy, so might one agent, noradrenaline, act through two or more receptors, each taking a different part in the excitation-contraction coupling process to produce an integrated response whose net effect might vary with the state of the tissue. For example, α_2-receptor activation might not produce vasoconstriction on its own but might facilitate transmission initiated by other receptors (this would explain why rauwolscine blocks on its own but not after prazosin). This adjustment of the α_1-receptor mediated response might occur only in specific local circumstances and would represent an extra layer of control.

The prazosin-resistant component is clearly not mediated by α_2-adrenoceptors. By a process of elimination, a response which is 'prazosin-resistant' might be due to stimulation of α_2-adrenoceptors (e.g. Drew, 1981). However, these 'prazosin-resistant' effects were not antagonized by rauwolscine. Indeed, after prazosin, rauwolscine increased the vasopressor response to sympathetic nerve stimulation when the train was of sufficient length for α-receptor-mediated feedback (5 Hz, 10 pulses, Figure 3). Since this dose of rauwolscine is the highest which is practicable to use and retain 'selectivity', the 'prazosin-resistant' responses evoked by sympathetic nerve stimulation may be considered also to be 'rauwolscine-resistant' and thus, neither α_2- nor α_1-receptor mediated: this response constitutes approximately half of the height of the control e.g. single pulse, 52%; 10 pulses, 47%.

Electrophysiological evidence from guinea-pig mesenteric arterioles supports the concept of a population of vascular adrenoceptors, distinct from α-receptors, which can mediate vasoconstriction (Hirst & Neild, 1980). These have been termed γ-adrenoceptors. They are proposed to be located at 'junctional' areas and to be activated by high concentrations of transmitter attained intra-junctionally (Neild & Zelcer, 1982). It is possible that the 'prazosin- and rauwolscine-resistant' neuronal response observed in the present study results from stimulation of a similar population of receptors or, at least, indicates a similar phenomenon. Hirst & Neild (1981) and Neild & Zelcer (1982) proposed that postjunctional α-adrenoceptors are found only in extra-junctional areas and that antagonism of neuronal responses by prazosin is due to non-specific depression of smooth muscle function rather than α_1-receptor blockade. In the present study, the effective doses of prazosin are sufficiently low that the 'prazosin-sensitive' component of the pressor response to sympathetic nerve stimulation does appear to result from α_1-receptor stimulation. Maximal attenuation by prazosin of the neuronal response occurred at a dose which also produces maximal antagonism of α_1-receptor mediated effects of circulating agonists without significant inhibition of the pressor responses to xylazine or guanabenz (α_2) or 5-hydroxytryptamine (Flavahan & McGrath, 1980; Bar-

nett et al., 1980). Further increments in the dose of prazosin produced no further attenuation of the neuronal responses. Therefore, we conclude that transmitter from sympathetic nerves activates postjunctional α_1- and α_2-adrenoceptors and has the capacity to produce some further activation, which is different from that activated by circulating catecholamines.

Previous studies in the pithed rat, employing (i) stimulation of the entire sympathetic outflow (Yamaguchi & Kopin, 1980), (ii) DMPP-induced release of transmitter (Willfert et al, 1982) or (iii) stimulation of the spinal outflow between T2–T6 (Docherty & McGrath, 1980a,b) have led to the conclusion that nerve-released noradrenaline activated mainly, if not exclusively, postjunctional α_1-receptors. On the basis of the greater potency of prazosin against nerve stimulation, an extrajunctional location for α_2-receptors has been suggested to occur in the resistance vessels of the pithed rat and in other vascular systems (cat spleen, Langer & Shepperson, 1982; dog hind-limb, Langer et al., 1980). However, this is not universal for vascular smooth muscle. Innervated α_2-receptors are found in the pithed rabbit (McGrath et al., 1982), in human and dog saphenous vein (Docherty & Hyland, 1985; Flavahan et al., 1984), in dog hind limb (Gardiner & Peters, 1982) and in the autoperfused hind-limb of the rabbit (Madjar et al., 1980). The present study adds to the latter group and shows that a role for postjunctional α_2-receptors is more likely to be detected at low frequencies and short trains of nerve stimulation.

The case for additional mechanisms

A critical question is whether the α-receptor antagonist-resistant pressor response is mediated by noradrenaline. Our evidence suggests that part of it is and part of it is not. Reserpine produces a greater attenuation of the response than does the combination of α-receptor antagonists. If depletion of peripheral catecholamine stores was the only action of reserpine, we would be justified in suggesting that the additional blockade by reserpine was due to the removal of an action of noradrenaline at adrenoceptors which were not α-receptors. However, this is not the case. Reserpine has many other actions, principally through depletion of amines in the CNS. Most critical among these is likely to be its influence on aminergic transmission in the hypothalamus with consequent imbalances of a large number of circulating hormones. Since it is becoming apparent that peripheral neurotransmission and excitation-contraction mechanisms are influenced by many blood-borne factors, particularly steroid hormones and angiotensin II (Timmermans & Van Zwieten, 1982), it would be rash to put too much stress on the quantitative comparison between these groups of acute α-receptor blocked or reserpine pretreated rats. If the effect of reserpine is caused only by depletion of peripheral catecholamines, of course, this supports the concept of non-α-receptor-mediated, but, nevertheless, adrenergic, transmission, e.g. through γ-adrenoceptors. At least part of this system is, however, susceptible to α,β-methylene ATP.

The question of whether the pressor response resistant to α-receptor blockade is mediated by sympathetic nerves can be answered with more confidence. The response is abolished by hexamethonium or guanethidine or by pretreatment with 6-OHDA. This indicates that electrical stimulation via the pithing rod activates pre-ganglionic autonomic nerves and that transmission can be stopped at the level of the post-ganglionic sympathetic nerve terminals by preventing their depolarization or by destroying them. The response seems, therefore, to be mediated by a transmitter or transmitters from the post-ganglionic sympathetic nerve terminals and, since the actions of guanethidine and 6-OHDA are selective for terminals which take up and store phenylethanolamines, these are likely to be the 'classical' vascular noradrenergic terminals.

The possibility of purinergic co-transmission from sympathetic terminals was tested with α,β-methylene ATP. In the absence of α-adrenoceptor blockade or reserpine, it failed to block responses to nerves or to noradrenaline. Attenuation was found only with the very short-lived response which survived α-receptor blockade or reserpine. This suggests that the α,β-methylene ATP-sensitive component does not contribute significantly to the vasopressor nerve response under normal circumstances. However, in the absence of the major transmitter or with blockade of the receptors through which it usually acts a transient response is found, which may be due to ATP or to other co-transmitters released from the nerves. Apparently this substance(s) does not normally summate with or influence the main α-adrenoceptor-mediated response. This contrasts with the vas deferens, in which the transient, reserpine-resistant, α-blocker-resistant, α,β-methylene ATP-sensitive component of sympathetic transmission produces a contractile response sufficiently large and rapid that it can be seen in the absence of any antagonists (Brown et al., 1983; Cunnane & McGrath, unpublished). This suggests that ATP or adenosine, released from the sympathetic nerves, can influence vascular smooth muscle but apparently not as a primary transmitter, or even as a facilitator, in this particular preparation of the pithed rat. It is quite possible that such a mechanism does operate in beds or vessels which do not contribute greatly to this systemic, arterial, 'resistance' response, e.g. skeletal muscle (essentially vasoconstricted in this preparation), the pulmonary bed or veins (which could provide transient responses via increased venous return).

Since it is now known that, in the vas deferens, both nifedipine and α,β-methylene ATP, acting by quite separate mechanisms, block both excitatory junction potential (e.j.ps) and the non-adrenergic contractile component to sympathetic nerve stimulation, but do not influence the simultaneous adrenoceptor-mediated contractile response (Blakeley *et al.*, 1981; Sneddon & Burnstock, 1984), it is difficult to escape the conclusion that the e.j.p. is not directly involved in adrenergic motor transmission or, by implication, with smooth muscle contraction mediated by α-adrenoceptors.

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