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# An investigation of presynaptic α-adrenoceptor subtypes in the pithed rat heart and in the rat isolated vas deferens

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**1** The presynaptic cardio-inhibitory effects of the α-adrenoceptor agonists xylazine, cirazoline and amidephrine and their interaction with the antagonists yohimbine and prazosin were investigated in the pithed rat.

**2** The presynaptic inhibitory effects of the $\alpha_2$-selective agonist xylazine were antagonized by the $\alpha_2$-antagonist yohimbine but not by the $\alpha_1$-antagonist prazosin, thus demonstrating the lack of $\alpha_2$-adrenoceptor antagonism by prazosin.

**3** The presynaptic inhibitory effects of cirazoline were antagonized equally by yohimbine and prazosin, and the presynaptic inhibitory effects of the selective $\alpha_1$-agonist amidephrine were antagonized by prazosin more potently than by yohimbine.

**4** In the nifedipine-treated isolated epididymal portion of the rat vas deferens, both xylazine and amidephrine produced concentration-dependent inhibition of the isometric contraction to single pulse electrical stimulation. The $\alpha_2$-antagonist rauwolscine antagonized the inhibitory effects of xylazine but not of amidephrine.

**5** It is concluded that inhibitory $\alpha_1$-adrenoceptors, as well as the already established $\alpha_2$-receptors, are present presynaptically in the pithed rat heart and in the rat vas deferens.

## Introduction

Peripheral α-adrenoceptors were initially subclassified into $\alpha_1$-postsynaptic and $\alpha_2$-presynaptic adrenoceptors (Langer, 1974). It is now known that $\alpha_2$-adrenoceptors are not restricted to nerve terminals but are also present on vascular smooth muscle cells (Drew & Whiting, 1979; Docherty *et al.*, 1979), and these and other findings have brought about the adoption of a subclassification of α-adrenoceptors independent of receptor location (Berthelsen & Pettinger, 1977; Starke & Langer, 1979). However, presynaptic adrenoceptors have until recently been assumed to be exclusively of the $\alpha_2$-type, although recent reports (Kobinger & Pichler, 1989; 1982; Docherty, 1983a, b) suggest that $\alpha_1$-adrenoceptors may also be present presynaptically in the pithed rat heart, and these, like the $\alpha_2$-receptors, mediate inhibition of neurotransmitter release (see Langer, 1974; Starke, 1977).

The purpose of the present investigation is to examine further the presynaptic α-adrenoceptors of the pithed rat heart, and to extend the work to an *in vitro* preparation in which presynaptic receptors can be more carefully studied, namely the rat vas deferens. Agonist and antagonist drugs were chosen on the basis of $\alpha_1$- or $\alpha_2$-adrenoceptor selectivity: prazosin, selective $\alpha_1$-antagonist (Cambridge *et al.*, 1977); yohimbine and rauwolscine, selective $\alpha_2$-antagonists (Weitzell *et al.*, 1979); xylazine, selective $\alpha_2$-agonist (Docherty & McGrath, 1980a); cirazoline and amidephrine, relatively selective $\alpha_1$-agonists (Roach *et al.*, 1979; Flavahan & McGrath, 1980).

Some of this work has been published in abstract and shortened form (Docherty, 1983a, b).

## Methods

Male Wistar rats (225–275 g) were used.

### *Pithed rats*

Rats were pithed by the method of Gillespie *et al.* (1970) and respired with 100% $O_2$, 1 ml 100 $g^{-1}$ $beat^{-1}$, at a rate of 60 $min^{-1}$. Heart rate

was extracted from carotid arterial pressure, and the right jugular vein was used for drug injections.

Cardioaccelerator responses were obtained to electrical stimulation (0.05 ms pulses, supramaximal voltage) of the sympathetic outflow at $T_1$ employing either single pulses every 2 min or intermittent trains of 10 pulses at a frequency of 1 Hz. Cumulative dose-response curves were obtained to each agonist, with 1 log unit increases) When comparing relative pre- and postsynaptic potencies of agonists, presynaptic effects were assessed as the inhibition of the cardioacceleration to a single stimulus pulse given approximately 1 min after drug injection; postsynaptic effects were assessed as the pressor response to the agonist at the time of the stimulus pulse. In interaction experiments, the antagonist was injected intravenously 10 min before beginning the agonist dose-response curve. Agonist $ID_{50}$ values (dose producing 50% inhibition of the cardioacceleration to a single stimulus pulse) and $ED_{50}$ values (dose producing a rise in diastolic blood pressure of approximately 50% of maximum for that agonist) were obtained from each individual experiment. Since it was not always possible to obtain a maximum pressor response to the agonist in experiments in the presence of antagonists, arbitrary values of $ED_{50}$ for each agonist were chosen from control experiments: 45 mmHg for xylazine ($\alpha_2$-mediated response) and 60 mmHg for cirazoline and amidephrine ($\alpha_1$-mediated response) (see Figure 2).

In experiments examining cardioaccelerator responses to a train of 10 pulses at 1 Hz, presynaptic effects of agonists were assessed as the percentage inhibition of the cardioacceleration to stimulation 5 min after drug injection. In interaction experiments, the antagonist was injected intravenously 10 min before beginning the agonist dose-response curve.

*Vas deferens*

Vasa deferentia were bisected into prostatic and epididymal portions (McGrath, 1979), and epididymal portions were used. Tissues were placed between platinum electrodes in organ baths (50 ml), and bathed at 37°C in Krebs-Henseleit solution of the following composition (mM): NaCl 119, $NaHCO_3$ 25, d-glucose 11.1, KCl 4.7, $CaCl_2$ 2.5, $KH_2PO_4$ 1.2, $MgSO_4$ 1.0. The tissues were attached to myograph transducers for recording of isometric tension. Responses to single pulse field stimulation (supramaximal voltage, 0.5 ms) were obtained at intervals of 5 min. When consistent control responses had been obtained, the effects of drugs were assessed on nerve-stimulation evoked responses)

Experiments were carried out in the presence of the calcium entry blocker nifedipine ($10^{-5}$ M), and the effects of cumulative concentrations of agonist were assessed on nerve-stimulation evoked contractions, either in the absence of any prior drug treatment or in the presence of rauwolscine or xylazine. In all experiments consistent control responses (in absence or presence of prior drug treatment) were obtained before beginning concentration-response curves and one nerve-evoked response was obtained in the presence of a given concentration of drug before the next concentration was added (0.5 log unit increases). Agonist $IC_{50}$ values (concentration producing 50% inhibition of the contraction to a single pulse) were obtained from each individual experiment.

Drugs used were (±)-amidephrine hydrochloride (gift: Mead Johnstone) cirazoline hydrochloride (gift: Synthelabo); nifedipine (gift: Bayer); prazosin hydrochloride (gift: Pfizer); rauwolscine hydrochloride (Roth); yohimbine hydrochloride (Sigma); xylazine hydrochloride (gift:sBayer). For *in vitro* experiments, drug stocks were prepared in distilled water, except for nifedipine (100% ethanol), and drug dilutions were administered in distilled water (nifedipine administered in 10% ethanol). For pithed rat experiments, drug stocks were prepared in distilled water and drug dilutions were administered in 0.9% w/v NaCl solution (saline), except for prazosin (distilled water).

*Statistics*

Differences in $ID_{50}$, $IC_{50}$ or $ED_{50}$ values between groups were compared by Student's $t$ test for unpaired data.

## Results

*Pithed rat*

In pithed rats basal heart rate was in the range of 270–330 $min^{-1}$, and resting diastolic blood pressure was 30–40 mmHg. High concentrations of agonists ($> 1\ mg\ kg^{-1}$) produced marked falls in heart rate, and in some animals cirazoline or amidephrine ($1\ mg\ kg^{-1}$) proved fatal. Low doses ($1-100\ \mu g\ kg^{-1}$) of cirazoline or amidephrine but not xylazine produced small rises in heart rate (e.g. a rise of $14.0 \pm 3.5\ min^{-1}$ by cirazoline $10\ \mu g\ kg^{-1}$) and the tachycardia was prevented by prazosin ($100\ \mu g\ kg^{-1}$) but not by yohimbine ($1\ mg\ kg^{-1}$). Single pulse stimulation produced a cardioacceleration of $24 \pm 1.4\ min^{-1}$ ($n = 28$). Yohimbine and prazosin caused an initial large inhibition of the cardioacceleration, but the response had recovered to (not significantly) less than the pre-antagonist level by 1 min before agonist injection.

**Table 1** Relative potencies of test drugs at pre- and postsynaptic α-adrenoceptors in the pithed rat assessed at the time of the stimulus 1 min (a) or 5 min (b) after drug injection

| | *Pressor effect* ($ED_{50}$) | *Cardiac inhibition* ($ID_{50}$) | *Dose ratio* ($ED_{50}/ID_{50}$) |
|---|---|---|---|
| (a) *1 min* | | | |
| Xylazine | 219 | 4.8 | 45.6 |
| Cirazoline | 7.9 | 2.6 | 3.0 |
| Amidephrine | 32.4 | 75.8 | 0.43 |
| (b) *5 min* | | | |
| Xylazine | 785 | 23.0 | 34.1 |
| Cirazoline | 41.5 | 5.5 | 7.6 |
| Amidephrine | 36.0 | 77.6 | 0.46 |

Values are mean of $ED_{50}$ and $ID_{50}$ values ($\mu g\,kg^{-1}$) obtained from each individual experiment.

Xylazine, cirazoline and amidephrine produced dose-dependent pressor responses and dose-dependent inhibition of the cardioacceleration to a single pulse. Presynaptic inhibition can only be assessed during nerve stimulation, approximately 1, 3 and 5 min after drug injection, and postsynaptic pressor responses should be measured at the same point in time (Docherty & McGrath, 1980c). The advantage of measuring responses 5 min after drug injection is that agonist-induced changes in basal heart rate are much reduced, but the disadvantage is that higher drug doses are required to produce a given effect at 5 min than at 1 min (Table 1). However, since the relative potencies of agonists at pre- and postsynaptic α-receptors are similar at 1 min as at 5 min after injection (Table 1) the data of Figures 1 and 2 and

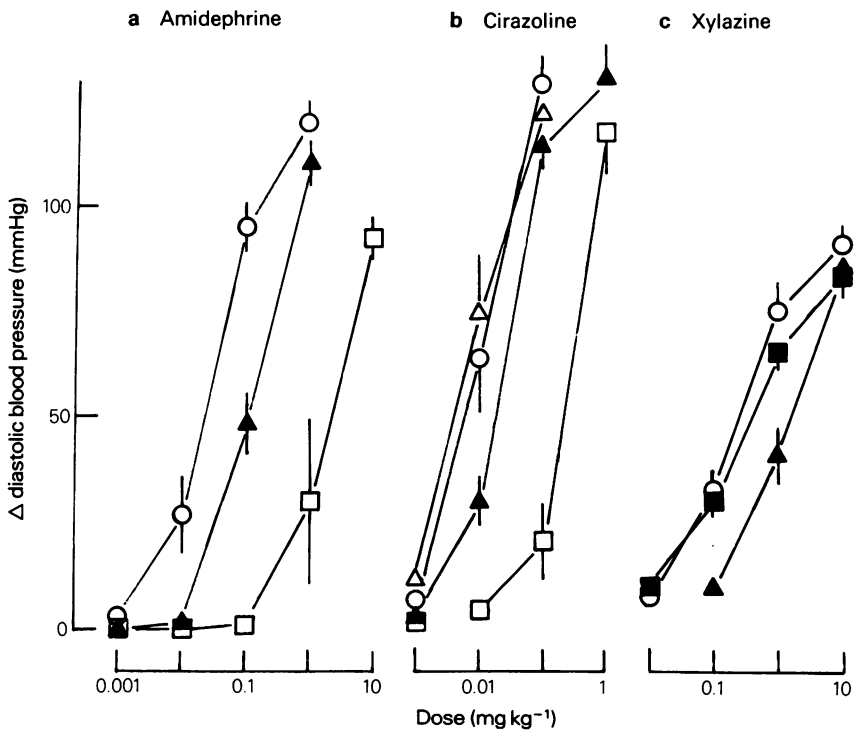


**Figure 1** Pressor responses to (a) amidephrine, (b) cirazoline and (c) xylazine. Control responses (○); responses in the presence of prazosin ($100\,\mu g\,kg^{-1}$) (□) or $1\,mg\,kg^{-1}$ (■); responses in the presence of yohimbine $100\,\mu g\,kg^{-1}$ (△) or $1\,mg\,kg^{-1}$ (▲). Responses were measured at the time of the first stimulus pulse after the drug injection (approximately 1 min after injection). Vertical bars represent s.e.mean, $n = 4-7$.

**Table 2** Pressor and cardio-inhibitory responses to xylazine, cirazoline and amidephrine and the interaction with prazosin and yohimbine

| | *Postsynaptic $ED_{50}$* | | | *Presynaptic $ID_{50}$* | | |
|---|---|---|---|---|---|---|
| | *Xylazine* | *Cirazoline* | *Amidephrine* | *Xylazine* | *Cirazoline* | *Amidephrine* |
| No antagonist | 219 (117–407) | 7.9 (2.9–21.9) | 32.4 (20.9–50.1) | 4.8 (3.2–8.9) | 2.6 (1.7–3.9) | 75.8 (44.7–135) |
| Prazosin ($1\ mg\ kg^{-1}$) | 282 (178–447) | 851*** (457–1580) | > 1000*** | 8.7 (3.6–20.9) | 38.0*** (21.9–66.1) | 616** (155–2450) |
| Yohimbine ($1\ mg\ kg^{-1}$) | 1110** (400–3020) | 21.4* (15.5–29.5) | 129* (56.2–295) | 302*** (110–832) | 37.2*** (15.8–87.1) | 263* (95.5–724) |

Effects of agonists are expressed as dose ($\mu g\ kg^{-1}$) producing a rise in diastolic blood pressure of 45 mmHg (xylazine) or 60 mmHg (cirazoline and amidephrine) (postsynaptic $ED_{50}$) or 50% inhibition of the cardio-acceleration to a single stimulus pulse (presynaptic $ID_{50}$); effects were measured at the stimulus pulse 1 min after injection of the agonist. Values are the geometric mean and 95% confidence limits, obtained from linear regression analysis of individual dose-response curves, and are the mean of at least 4 experiments.
Asterisks denote response to agonist significantly different from response to agonist in absence of antagonist (Student's *t* test: $*P<0.05$; $**P<0.01$; $***P<0.001$).

Table 2 were assessed at the stimulus pulse approximately 1 min after drug injection.

Pressor dose-response curves were constructed for amidephrine, cirazoline and xylazine and their interaction with antagonist drugs (Figure 1). Prazosin ($100\ \mu g\ kg^{-1}$ and $1\ mg\ kg^{-1}$) produced large shifts in the pressor dose-response curves of amidephrine and cirazoline, with significant shifts in the $ED_{50}$ values (Table 2), while even prazosin ($1\ mg\ kg^{-1}$) had no effect on the pressor dose-response curve of xylazine. Yohimbine ($100\ \mu g\ kg^{-1}$) had no effect on the pressor dose-response curve of cirazoline, but yohimbine ($1\ mg\ kg^{-1}$) produced significant shifts in the $ED_{50}$ of all 3 agonists (Table 2). Yohimbine was less potent

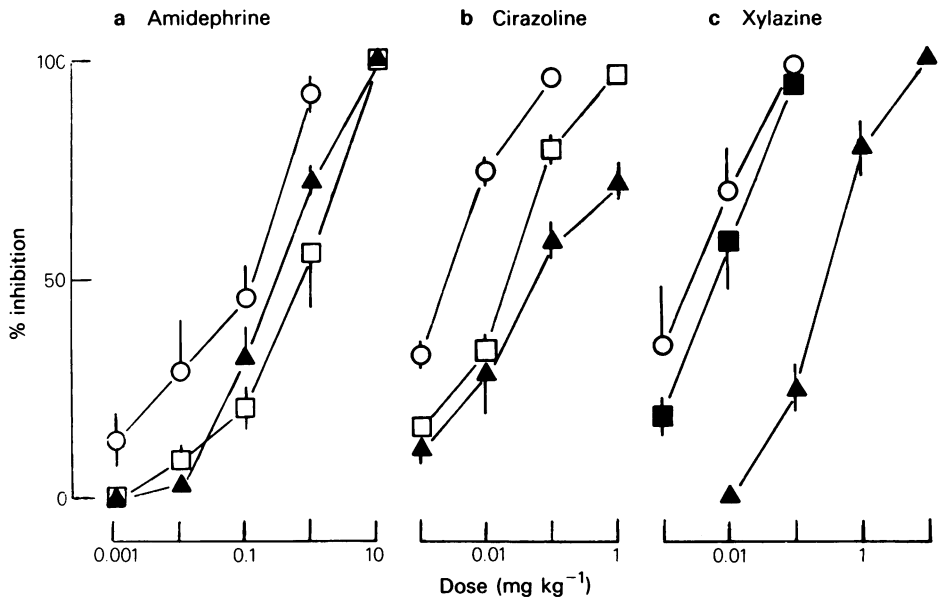


**Figure 2** Presynaptic cardio-inhibitory effects of (a) amidephrine, (b) cirazoline and (c) xylazine. Control responses (○); responses in the presence of prazosin $100\ \mu g\ kg^{-1}$ (□) or $1\ mg\ kg^{-1}$ (■); responses in the presence of yohimbine $1\ mg\ kg^{-1}$ (▲). Responses were measured as inhibition of the first stimulus pulse after drug injection (approximately 1 min after injection). Vertical bars represent s.e.mean, $n = 4–8$.

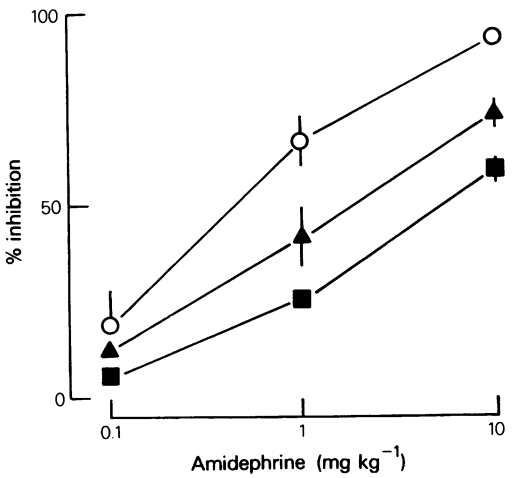


**Figure 3** The inhibition by amidephrine of the cardioacceleration to 10 pulses at 1 Hz, measured 5 min after drug injection. Control response (○); response in the presence of prazosin 1 mg kg$^{-1}$ (■); response in the presence of yohimbine 1 mg kg$^{-1}$ (▲). Vertical bars represent s.e.mean, $n = 4-5$.

than prazosin at antagonizing the pressor effects of cirazoline and amidephrine.

Dose-response curves were constructed for the presynaptic cardio-inhibitory effects of the three agonists and their interaction with the antagonists prazosin and yohimbine, assessed against the response to a single stimulus pulse (Figure 2). Prazosin (100 μg kg$^{-1}$) was more effective than yohimbine (1 mg kg$^{-1}$) at shifting the inhibitory dose-response curve of amidephrine, both were equi-effective against cirazoline, but even prazosin (1 mg kg$^{-1}$) failed to shift the inhibitory dose-response curve of xylazine. In contrast, yohimbine (1 mg kg$^{-1}$) produced a large shift to the right of the cardio-inhibitory dose-response curve of xylazine (Figure 2 and Table 2).

However, since amidephrine and cirazoline produced small rises in basal heart rate, and this rise could be prevented by prazosin, further experiments were carried out employing stimulation with 10 pulses at a frequency of 1 Hz, producing a cardioacceleration of 82 ± 8.6 min$^{-1}$, a response large enough to minimize the effects of small changes in basal heart rate. Amidephrine produced a dose-dependent inhibition of the cardioacceleration (Figure 3), with an

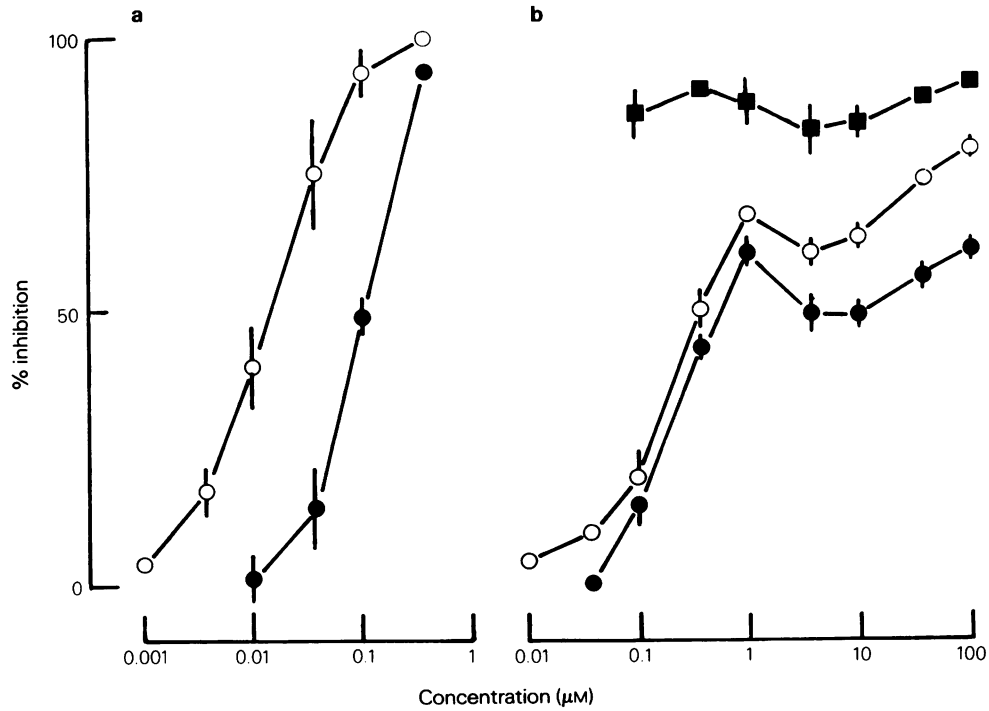


**Figure 4** Concentration-response curves for the effects of (a) xylazine and (b) amidephrine on the isometric contraction to single pulse field stimulation of epididymal portions of rat vas deferens in the presence of nifedipine (10 μM). Control responses (○); responses in the presence of rauwolscine 100 nM (●). In (b) only, responses to amidephrine in presence of xylazine 30 nM (■). Vertical bars represent s.e.mean, $n = 4-6$.

$ID_{50}$ of 428 $\mu g\,kg^{-1}$. Yohimbine and prazosin (1 $mg\,kg^{-1}$) both produced significant shifts in the inhibitory dose-response curve of amidephrine, prazosin being most potent. The $ID_{50}$ of amidephrine was shifted to 5.5 and 2.0 $mg\,kg^{-1}$ in the presence of prazosin and yohimbine, respectively ($P<0.05$).

*Vas deferens*

Epididymal portions of the rat vas deferens were employed, and were given nifedipine (10 $\mu$M) at the beginning of the experiment. This treatment abolishes the early non-adrenergic component of the contractile response to a single pulse, leaving only the adrenergic component so that the response becomes monophasic (see Blakely *et al.*, 1981). Under these conditions, xylazine produced a concentration-dependent inhibition of the isometric contraction to a single stimulus pulse (Figure 4). In the presence of rauwolscine (100 nM), a concentration which did not significantly alter the contractile response, the concentration-response curve of xylazine was shifted to the right (Figure 4), and there was a significant reduction in the $IC_{50}$ of xylazine (Table 3). Amidephrine produced a concentration-dependent inhibition of the contractile response over the range 10 nM–1 $\mu$M, but with higher concentrations there was little further inhibition and even some reversal of the inhibition (Figure 4). In the presence of rauwolscine (100 nM), there was no shift in the $IC_{50}$ of amidephrine (Table 3) but the reversal of the inhibition which occurs at higher concentrations was more pronounced (Figure 4). Higher concentrations of rauwolscine and all effective concentrations of prazosin reduced the contractile response to stimulation.

**Table 3** Presynaptic $IC_{50}$ values (concentration producing 50% inhibition) for the effects of xylazine and amidephrine on the isometric contraction to single pulse field stimulation of epididymal portions of rat vas deferens in the presence of nifedipine (10 $\mu$M)

| | *Control* | *Rauwolscine* (100 nM) |
|---|---|---|
| Xylazine | 7.76 (7.54–7.98) | 7.04*** (6.92–7.16) |
| Amidephrine | 6.41 (6.25–6.57) | 6.38 (6.24–6.52) |

Values are geometric mean (−logM) and 95% confidence limits, obtained from linear regression analysis of individual concentration-response curves, and are the mean of at least 4 experiments. Asterisks denote response to agonist in the presence of rauwolscine significantly different from control response to agonist (Student's *t* test: ***$P<0.001$).

**Table 4** Relative potencies of xylazine and amidephrine at inhibiting the nerve-mediated response to a single pulse in the pithed rat heart ($ID_{50}$) and rat vas deferens ($IC_{50}$)

| | *Pithed rat heart* | | *Rat vas deferens* | |
|---|---|---|---|---|
| | *$ID_{50}$* ($nmol\,kg^{-1}$) | *Relative potency* | *$IC_{50}$* (nM) | *Relative potency* |
| Xylazine | 18.7 | 1 | 17.4 | 1 |
| Amidephrine | 270 | 14.4 | 389 | 22.4 |

In further experiments, the time course of the inhibitory action of xylazine (30 nM) was examined. With continued exposure to xylazine there was some further inhibition at the second and third stimuli (10 and 15 min after administration of xylazine), but thereafter there was no further increase in the inhibitory effect of xylazine (not shown). When a concentration-response curve to amidephrine was carried out in the presence of xylazine (30 nM), amidephrine (1–10 $\mu$M) produced some reversal of the inhibitory effects of xylazine but higher concentrations of amidephrine again reduced the response (Figure 4).

Table 4 shows the relative potencies of xylazine at inhibiting the nerve mediated response to a single stimulus pulse:in the pithed rat heart xylazine was 14 times more potent than amidephrine, and 22 times more potent in the rat vas deferens.

## Discussion

It is now well established that pressor responses to exogenous agonists in the pithed rat are mediated by both $\alpha_1$- and $\alpha_2$-adrenoceptors (Drew & Whiting, 1979; Docherty *et al.*, 1979; Timmermans *et al.*, 1979). In the present experiments, the pressor responses to the $\alpha_2$-agonist xylazine were antagonized by yohimbine but not by prazosin, and the pressor responses to the $\alpha_1$-selective agonists cirazoline and amidephrine were antagonized markedly by prazosin but much less so by yohimbine. However, whereas the presynaptic inhibitory effects of xylazine were antagonized by yohimbine but not by prazosin, the presynaptic inhibitory effects of cirazoline were antagonized by yohimbine and prazosin to a similar extent, and the presynaptic inhibitory effects of amidephrine were antagonized more by prazosin than yohimbine. These results are explicable when subtype specificity of both agonists and antagonists are considered. It is clear that prazosin and xylazine do not interact at either pre- or postsynaptic recep-

tors, thus further confirming the high degree of selectivity as $\alpha_1$-antagonist and $\alpha_2$-agonist, respectively. This selectivity of prazosin allows us to conclude that the predominant presynaptic effects of amidephrine are by an action at $\alpha_1$-adrenoceptors. The combination of yohimbine and prazosin is more potent than either alone at antagonizing the presynaptic inhibitory effects of cirazoline (Docherty, 1983b), demonstrating that cirazoline has both presynaptic $\alpha_1$- and $\alpha_2$-mediated actions. Although yohimbine did produce some degree of antagonism of the presynaptic effects of amidephrine, this effect of yohimbine may have been $\alpha_1$-mediated since yohimbine does not show absolute selectivity for $\alpha_2$-receptors. Alternatively, amidephrine may have some small action at presynaptic $\alpha_2$-receptors in addition to its predominant action at $\alpha_1$-receptors.

α-Adrenoceptors were initially subclassified on the basis of pre- and postsynaptic potencies of a series of agonists and antagonists, assuming that homogeneous populations of different receptors were present at each site (see Starke & Docherty, 1980 for references). It is interesting to note that despite the discovery of postsynaptic $\alpha_2$- and presynaptic $\alpha_1$-adrenoceptors, selectivity of an agonist can still be assessed based on the ratio of postsynaptic potency to the presynaptic potency (see Table 1 and Docherty & McGrath, 1980b). The order of agonist selectivity obtained in Table 1 does confirm the high $\alpha_2$-receptor selectivity of xylazine and $\alpha_1$-receptor selectivity of amidephrine, with cirazoline somewhere in between. The order of selectivity for $\alpha_1$ or $\alpha_2$-receptors can only agree with relative post- to presynaptic potency if $\alpha_1$-receptors still predominate postsynaptically and/or $\alpha_2$ predominate presynaptically: this is confirmed by the fact that xylazine is approximately 20 times more potent than amidephrine presynaptically, and by the fact that yohimbine and prazosin are equipotent against cirazoline presynaptically whereas prazosin is much more potent postsynaptically.

$\alpha_1$-Adrenoceptor agonists produce prazosin-sensitive rises in basal heart rate in the pithed rat (Flavahan & McGrath, 1982). Since prevention of this $\alpha_1$-mediated tachycardia by prazosin may in itself influence the apparent presynaptic potency of the $\alpha_1$-agonist, evidence must be presented that the agonist-induced inhibition of the cardioaccelerator response to stimulation is not by a postsynaptic action affecting basal heart rate. Three pieces of evidence can be presented to suggest a presynaptic site of action. Firstly, the degree of inhibition of the cardioaccelerator response to a single stimulus did not correlate well with the rise in basal heart rate (author, unpublished observations). Secondly, in experiments employing stimulation with 10 pulses at 1 Hz, so that rises in basal heart rate are small compared with the stimulation-induced cardioacceleration, $\alpha_1$-adrenoceptor mediated inhibition was still demonstrable. Thirdly, as discussed below, inhibition of the isometric contraction to single pulse nerve stimulation by an $\alpha_1$-adrenoceptor agonist could be shown in the epididymal portion of the rat vas deferens *in vitro*.

The isometric contraction obtained to single pulse field stimulation of the rat vas deferens is biphasic: the first (presumed non-adrenergic) component is dominant in the prostatic portion whereas the second (adrenergic) component dominates in the epididymal portion (see McGrath, 1978). $\alpha_2$-Adrenoceptor agonists produce concentration-dependent inhibition of nerve-evoked contractions in both prostatic and epididymal portions, whereas $\alpha_1$-adrenoceptor agonists produce a concentration-dependent potentiation of nerve-evoked contractions by an action at postsynaptic receptors (Docherty *et al.a* 1979; MacDonald & McGrath, 1980; Docherty & McGrath, unpublished observations). The calcium entry blocker nifedipine abolishes the non-adrenergic component of the contraction to nerve stimulation (Blakely *et al.*, 1981), and greatly attenuates the postsynaptically-mediated potentiation of nerve mediated contractions by $\alpha_1$-receptor agonists (Butler & Jenkinson, 1978; Docherty & McGrath, unpublished observations), while leaving intact the adrenergic component of the contraction to nerve stimulation. The nifedipine-treated epididymal portion is thus suitable for investigation of the presynaptic effects of $\alpha_1$-receptor agonists in the absence of complicating postsynaptic effects.

In the presence of nifedipine (10 $\mu$M), xylazine produced a concentration-dependent inhibition of the isometric contraction to a single stimulus pulse, and this inhibition could be antagonized by rauwolscine. The $\alpha_1$-receptor agonist amidephrine had two effects on the isometric contraction to a single stimulus pulse: a concentration-dependent inhibition over the range 0.01–1 $\mu$M, and a residual postsynaptic potentiation of nerve-mediated contractions at higher concentrations. In the presence of rauwolscine (100 $\mu$M) the inhibitory effects of amidephrine (0.01–1 $\mu$M) and the $IC_{50}$ values were unaltered, showing that this effect is not mediated by $\alpha_2$-adrenoceptors. It was not possible to demonstrate conclusively that this action was mediated by $\alpha_1$-receptors since prazosin reduced the isometric contraction to single pulse stimulation at any effective concentration. However, the additional evidence obtained from pithed rat experiments allows us to conclude tentatively that this presynaptic inhibitory action of amidephrine is mediated by $\alpha_1$-adrenoceptors, especially since the relative potencies of xywazine to amidephrine were so similar in the pithed rat and vas deferens (see Table 4). In the presence of rauwolscine (100 nM) the residual postsynaptic potentiation of nerve-evoked contractions by amidephrine was

accentuated. The reason for this is unclear, unless high concentrations of amidephrine have an $\alpha_2$-mediated inhibitory effect to counteract partially the postsynaptic potentiation in that same concentration range.

Although the data presented here demonstrate the existence of presynaptic $\alpha_1$-adrenoceptors (see also Kobinger & Pilcher, 1980; 1982), they shed very little light on the possible physiological importance of these receptors. If these presynaptic $\alpha_1$-receptors are targets for the endogenous neurotransmitter noradrenaline then blockade of these receptors with prazosin should result in increased transmitter release. In rabbit aorta and portal vein, *in vitro*, yohimbine (0.01 $\mu$M) significantly increased transmitter overflow but prazosin (0.01 $\mu$M) did not (Docherty & Starke, 1980); likewise in the pithed rat, under conditions in which yohimbine potentiated nerve-induced cardio-acceleration prazosin failed to do so (Docherty & McGrath, 1980a). These results argue against a role for $\alpha_1$-adrenoceptors as autoreceptors at least under conditions when $\alpha_2$-autoreceptors are functional. Moreover, the present results confirm that the predominant presynaptic receptor is $\alpha_2$, so that, for the moment, the presynaptic $\alpha_1$-adrenoceptors may be considered of pharmacological rather than physiological interest.

In conclusion, the present results confirm the existence of an $\alpha_1$-receptor-mediated inhibition of cardioaccelerator responses in the pithed rat, and demonstrate a similar $\alpha_1$-mediated inhibition of nerve mediated responses in rat vas deferens. The possible physiological role, if any, of these receptors remains to be established.

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