Br. J. Pharmac. (1985), **86**, 141–150

Differential blocking actions of idazoxan against the inhibitory effects of 6-fluoronoradrenaline and clonidine in the rat vas deferens

P.E. Hicks, S.Z. Langer & A.D. Macrae

Department of Biology, Laboratories d'Etudes et de Recherches Synthélabo, 58 rue de la Glacière, 75013 Paris, France

1 The prejunctional inhibitory effects of clonidine and 6-fluoronoradrenaline (6-FNA) have been evaluated in the isolated prostatic segment of the rat vas deferens, against the twitch response evoked by low frequency (0.1 Hz) field stimulation.

2 The inhibitory potency of 6FNA was significantly increased in the presence of cocaine (1 μM) or pargyline (10 μM), but was not modified in the vas deferens from rats pretreated with reserpine when the endogenous levels of noradrenaline (NA) were decreased by 97%. Clonidine was significantly more potent than 6-FNA as an inhibitory agonist, and the potency of clonidine was not modified after cocaine, pargyline or reserpine.

3 The α_2-adrenoceptor blocking agent idazoxan, was a competitive antagonist against the inhibitory effects of clonidine under all experimental conditions. In contrast, the only antagonism shown by idazoxan against the inhibitory effects of 6-FNA was in the presence of cocaine (1 μM), and this antagonist effect of idazoxan was not concentration-related.

4 Low concentrations of 6-FNA caused concentration-dependent facilitatory effects on the twitch response, which were significantly greater after treatment with idazoxan (1 μM) in reserpine-treated vas deferens. These facilitatory effects of 6-FNA were always observed in the presence of prazosin (300 nM) and also after treatment of the preparations with phenoxybenzamine (10 μM), a concentration which abolished the inhibitory actions of both clonidine and 6-FNA. The facilitatory effects on the twitch response induced by low concentrations of 6-FNA are therefore unlikely to be due to either α_1- or α_2-adrenoceptor stimulation.

5 In conclusion, the failure of idazoxan to block the inhibitory effects of 6-FNA, while exerting a potent competitive antagonism of clonidine-induced inhibitory effects, supports the proposal that α_2-adrenoceptors may in fact be subdivided into two subclasses, involving imidazoline and phenylethylamine recognition sites.

Introduction

Motor transmission in the rat vas deferens is mediated through two distinct mechanisms, a fast 'twitch' response mediated by an as yet unidentified transmitter (Ambache & Aboo Zar, 1971; McGrath, 1978; Brown *et al.*, 1979), and a second slow phase which is noradrenergic (Ambache & Aboo Zar, 1971; Anton *et al.*, 1977; McGrath, 1978; Brown *et al.*, 1979). The anatomical sectioning of the vas into epididymal or prostatic portions further separates these two components (McGrath, 1978; Brown *et al.*, 1979; MacDonald & McGrath, 1980) with the fast 'twitch' response predominating in the prostatic segment.

Stimulation of α-adrenoceptors which are located on prejunctional nerve terminals, by exogenously applied agonists e.g. clonidine, inhibit the 'twitch' response evoked by low frequency electrical stimulation in the prostatic rat vas deferens (Ambache & Aboo Zar, 1971); these receptors are of the α_2-subclass (Drew, 1977; McGrath, 1978; Brown *et al.*, 1979; MacDonald & McGrath, 1980; Vizi *et al.*, 1983). However, a number of reports in the literature suggest that the prejunctional effects of clonidine-like agents and phenylethylamine-like agents (e.g. noradrenaline (NA)) are different both in the vas deferens preparation (Mottram, 1982; Langer & Shepperson, 1982; Vizi *et al.*, 1983) and in other preparations such as brain

slices (Pelayo *et al.*, 1980), cat spleen (Langer & Dubocovich, 1981), guinea-pig aorta and ileum (Ruffolo *et al.*, 1983), as hypotensive agents in the CNS (Bousquet *et al.*, 1984), and on ^{3}H-transmitter release in dog saphenous vein (Baker *et al.*, 1984). In a number of these studies, the preferential α_2-adrenoceptor antagonist yohimbine was more potent against clonidine-induced than the corresponding phenylethylamine-induced effects (Mottram, 1982; Langer & Shepperson, 1982; Vizi *et al.*, 1983).

In the present study, we have compared the presynaptic inhibitory effects of the α_2-adrenoceptor agonist clonidine with 6-fluoronoradrenaline (6-FNA), (which is reported to be more selective than NA for the α_2-adrenoceptor; Shepperson *et al.*, 1981). We have evaluated the selective α_2-adrenoceptor blocking agent idazoxan (Doxey *et al.*, 1983) as an antagonist of clonidine or 6-FNA-induced inhibitory responses of the prostatic end of the rat vas deferens, using low frequency stimulation. These effects have been evaluated with pargyline, cocaine, or both drugs present in the Krebs medium.

A preliminary report of this work has been presented to the British Pharmacological Society (Hicks *et al.*, 1984).

Methods

The prostatic ends of vasa deferentia from male Sprague-Dawley rats of 300 g (Charles River) were removed, desheathed, cut open longitudinally and mounted in 5 ml organ baths in a magnesium-free Krebs bicarbonate medium containing (in mM): NaCl 112, KCl 4.7, $CaCl_2$ 2.6, NaH_2PO_4 1.0, $NaHCO_3$ 25.0, glucose 11.1 and ascorbic acid 0.11. The preparations were maintained at 37°C and bubbled with 95% O_2 and 5% CO_2. Tissues were placed under an initial resting tension of 5 g, and were washed repeatedly over a period of 30 min.

Propranolol (1 μM) and prazosin (300 nM) were present in the medium throughout the experiment to block β- and α_1-adrenoceptors respectively. The Krebs medium also contained, where indicated, cocaine (1 μM), pargyline (10 μM), or a combination of both drugs.

Tissues were continuously field-stimulated via two platinum electrodes mounted on either side of the preparation by use of a Grass model S88 stimulator. A square wave pulse of 2 ms duration and supramaximal voltage (40 V) at a frequency of 0.1 Hz, resulted in constant isometric tension changes recorded as single 'twitches' of the vas on a Grass model 7D polygraph recorder.

Experimental protocol

Concentration-response curves for the inhibition of the stimulated 'twitch' response of the vas deferens were obtained using the agonists 6-FNA or clonidine, added cumulatively to the organ bath. Only one agonist was evaluated in each preparation. After each agonist-response curve, the tissues were washed in normal Krebs over 20 min (no stimulation). Stable 'twitch' responses were then re-established to electrical stimulation before incubating the tissues with the α_2-adrenoceptor antagonist, idazoxan, for 20 min, a time sufficient to obtain equilibrium conditions at α_2-receptors. Concentration-response curves to 6-FNA or clonidine were then repeated in the presence of antagonist.

A second protocol used naive preparations, which had not previously been exposed to an agonist, and which were treated with antagonist (20 min) before the determination of concentration inhibitory-response curves to the agonist. The rationale for the use of these two different protocols was based on the failure of idazoxan to reverse fully the inhibited twitch-response of the vas after 6-FNA (see Results).

For studies with phenoxybenzamine, tissues were incubated for 30 min with the drug followed by repeated washing of the preparations over 1 h prior to the determination of inhibitory concentration-response curves to the agonist. In some experiments, rats were pretreated with reserpine phosphate (2.5 mg kg^{-1}, s.c.) 24 h before the experiments. Vasa deferentia from control (distilled water) or reserpine pretreated rats were also taken for biochemical determination of tissue noradrenaline content, estimated by a fluorimetric assay (Westerink & Korf, 1977).

Calculation of results

Agonist-induced effects were calculated as the % change in the twitch response (g tension) relative to the starting (before drug) tension values. The concentration of agonist causing 50% inhibition (IC_{50}) of the twitch and the slope of the agonist concentration-response curve were calculated using linear regression analysis. In experiments where the agonist concentration-response curves were evaluated only after treatment with antagonist, the concentration-ratio was calculated as the IC_{50} value for the agonist in the presence of antagonist, relative to the mean IC_{50} (control) for the agonist obtained in separate experiments.

The antagonist potencies of idazoxan (0.1, 1 and 10 μM) were calculated as the apparent dissociation constant (K_B) by the methods of Furchgott (1972), thus

$$K_B = \frac{[B]}{\text{concentration-ratio} - 1}$$

where [B] = antagonist concentration, and concentra-

tion-ratio is the ratio of equi-effective concentrations of agonist in the presence or absence of antagonist.

The method of calculating the concentration-ratio, based on IC_{50} values obtained in different preparations appears valid because: (a) the variation in IC_{50} values for the agonist in control tissues was very small, and (b) the K_B for idazoxan against clonidine was not significantly changed when calculated from data obtained by either method.

Where possible antagonist potency was also calculated as pA_2 (Arunlakshana & Schild 1959), together with the slope of the plot log concentration ratio − 1, against log molar concentration of antagonist.

Statistical analysis

Results are expressed as the means ± s.e.mean. Unpaired Student's t test was used to assess the significance of the experimental results. Significance was accepted when $P < 0.05$.

Drugs

The following were used: ± 6-fluoronoradrenaline HCl (synthesized by the Chemistry Department; Synthélabo, Paris); clonidine HCl (Boehringer Ingelheim); prazosin HCl (Pfizer, Kent); (±)-propranolol HCl (ICI, Cheshire); cocaine HCl (Coop. Pharmaceutique France); pargyline HCl (Sigma); idazoxan HCl (Reckitt and Colman, Hull); reserpine phosphate (Synthélabo); phenoxybenzamine HCl (SKF) was solubilised in 95% ethanol; all other drugs were dissolved in distilled water.

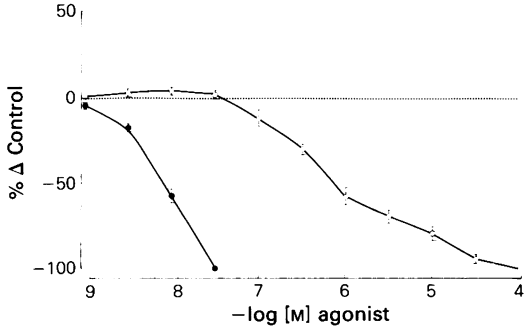

Figure 1 Concentration-response curves for the inhibition of the twitch response of the prostatic vas deferens of the rat induced by clonidine (●), or 6-fluoronoradrenaline (6-FNA) (○). Abscissae: − log molar concentration of agonists. Ordinates: % change from control (before drug) values of g tension developed. Control starting tensions evoked at 0.1 Hz were 1.2 ± 0.1 g, 1.15 ± 0.12 g for the groups exposed to clonidine and 6-FNA respectively. Data are mean of 8 preparations; vertical lines show s.e.mean

Table 1 Inhibitory potencies of 6-fluoronoradrenaline (6-FNA) or clonidine on the twitch response of the rat vas deferens stimulated at 0.1 Hz expressed as pD_2 values

	pD_2	
Drug treatment	*6-FNA*	*Clonidine*
No cocaine No pargyline	4.61 ± 0.2 (n = 13)	8.21 ± 0.04 (n = 4)
Pargyline (10 μM)	4.90 ± 0.05 (n = 6)	8.22 ± 0.03 (n = 8)
Cocaine (1 μM)	5.76 ± 0.07** (n = 8)	8.3 ± 0.04 (n = 8)
Pargyline (10μM) + cocaine (1μM)	5.14 ± 0.06* (n = 4)	8.29 ± 0.05 (n = 4)
Reserpine pretreatment + pargyline	4.96 ± 0.04 (n = 7)	8.46 ± 0.03 (n = 4)

Values are mean ± s.e.mean. pD_2 = log molar IC_{50}. (n) = number of experiments *$P < 0.05$ significantly different from pargyline-treated group; **$P < 0.01$ significantly different from other 6-FNA groups.

Results

Low frequency (0.1 Hz) field stimulation of the prostatic end of the rat vas deferens caused constant reproducible 'twitch' responses. Under these conditions of stimulation, the contraction of the vas was not modified by the α_1-adrenoceptor antagonist prazosin (300 nM), nor by the selective α_2-adrenoceptor antagonist idazoxan (0.1–10 μM).

Reserpine pretreatment (2.5 mg kg^{-1}, s.c., 24 h) reduced by approx. 97% the NA content of the vas deferens from control values of 6.8 ± 0.3 μg g^{-1} tissue (n = 10) to 0.2 ± 0.04 μg g^{-1} tissue (n = 16). The isometric tension generated in response to field stimulation was 1.24 ± 0.19 g (n = 10) in control, and 1.15 ± 0.16 g (n = 10) in reserpine-treated vasa deferentia, respectively. None of the other antagonists used in this study modified the stimulated 'twitch' response of the vas, at the concentrations studied.

Effects of 6-FNA and clonidine on the 'twitch' response

The preferential α_2-adrenoceptor agonists 6-FNA and clonidine, both fully inhibited the twitch response of the vas. Clonidine was markedly more potent than 6-FNA as an inhibitory agent (Figure 1, Table 1), and the slope of the inhibitory curve for clonidine was steeper (75.1) than for 6-FNA (37.2).

The pD_2 values (log M IC_{50}) for inhibition of the twitch response by 6-FNA and clonidine are shown in Table 1, under conditions where cocaine (1 μM),

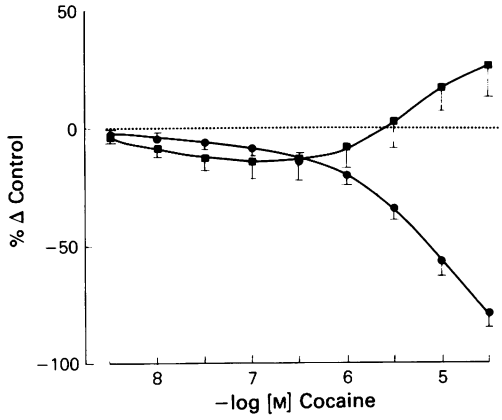

Figure 2 Concentration-response curves for the inhibition of the twitch response of the prostatic vas deferens of the rat elicited by cocaine, in preparations from vehicle (distilled water), (●), or reserpine (2.5 mg kg^{-1} s.c., 24 h)-treated rats (■). Abscissae; − log molar concentration of cocaine. Ordinate: % change from control (before drug), g tension developed. Control resting tension evoked at 0.1 Hz in vehicle- or reserpine-treated vas deferens were 1.3 ± 0.2 g and 1.14 ± 0.09 g respectively. Data are mean of 4 preparations; vertical lines show s.e.mean.

pargyline (10 μM), or a combination of both drugs was present in the Krebs medium. Reserpine pretreatment did not modify the inhibitory potencies of either 6-FNA or clonidine (Table 1). The inhibitory potency of clonidine was not modified by the presence or absence of pargyline or cocaine; however, the inhibitory potency of 6-FNA was significantly increased by cocaine (1 μM, Table 1).

In the present study, cocaine was employed routinely at a concentration of 1 μM: a concentration sufficient to increase the inhibitory potency of 6-FNA, but below the reported IC_{50} (4.2 μM) for neuronal uptake-inhibition of NA in the vas (Langer *et al.*, 1984). Figure 2 shows the inhibitory properties of cocaine on the twitch response in control or reserpine-treated vas deferens stimulated at 0.1 Hz. Exposure to cocaine progressively inhibited the twitch response of the vas in control preparations (IC_{50}: 8 ± 0.2 μM). These inhibitory effects of cocaine were absent in the reserpinised vas deferens (Figure 2).

Antagonist effects of idazoxan on the inhibitory effects of 6-FNA and clonidine

The selective α_2-adrenoceptor blocking agent idazoxan was employed at concentrations of 0.1–10 μM as an antagonist of clonidine or 6-FNA-mediated inhibition of the twitch response.

In preliminary experiments it was observed that idazoxan (0.1–10 μM) failed to reverse completely the inhibitory effects of 6-FNA (from full inhibition). However, the inhibitory effects of clonidine were rapidly and completely reversed by idazoxan 0.1–1 μM. As a result of these experiments, the antagonist potency of idazoxan was only evaluated in naive preparations (see Methods).

Table 2 shows the K_B values for idazoxan (0.1–10 μM) against 6-FNA or clonidine in the pargyline or cocaine models. The graphs of log concentration-ratio − 1, versus molar concentration of idazoxan against these agonists are also shown in Figure 3.

Idazoxan was a competitive antagonist of the clonidine-mediated inhibition of the twitch response (Table 2, Figure 3) with a pA_2 of 7.8 and a slope of 0.9

Table 2 Antagonist potency of idazoxan against 6-fluoronoradrenaline (6-FNA) or clonidine-induced inhibition of the twitch response of the rat vas deferens at 0.1 Hz

	Pargyline (10 μM)		*Cocaine* (1 μM)	
6-FNA	*n*	K_B	*n*	K_B
Idazoxan (0.1 μM)	4	6.5 ± 0.08*	6	8.0 ± 0.04
Idazoxan (1 μM)	4	6.3 ± 0.06*	4	7.0 ± 0.04
Idazoxan (10 μM)	4	5.2 ± 0.08*	4	6.7 ± 0.09
Clonidine				
Idazoxan (0.1 μM)	4	7.8 ± 0.04$^+$	8	7.8 ± 0.13
Idazoxan (1 μM)	4	8.0 ± 0.04$^+$	4	8.2 ± 0.13$^+$
Idazoxan (10 μM)	4	8.2 ± 0.04$^+$	4	8.5 ± 0.02$^+$

Values shown are mean ± s.e.mean.
*$P < 0.05$ significantly different from idazoxan in cocaine alone group; $^+P < 0.05$ significantly different from corresponding 6-FNA data.
K_B: antagonist dissociation constant: K_B = [B]/CR − 1; where [B] = antagonist concentration and the concentration ratio (CR) is the ratio of equieffective (IC_{50}) concentrations of agonist in the presence or absence of antagonist.

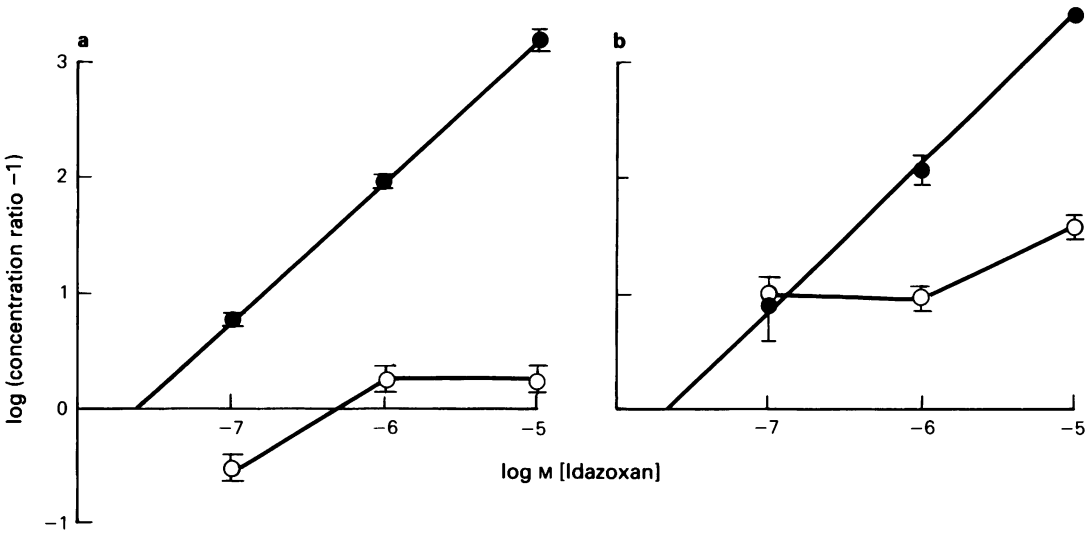

Figure 3 Antagonist effects of idazoxan on the inhibitory effects of clonidine (●) or 6-fluoronoradrenaline (6-FNA) (○) in the prostatic vas deferens of the rat stimulated at 0.1 Hz in the presence of pargyline (10 μM, a) or cocaine (1 μM, b). Abscissae: log molar concentration of idazoxan. Ordinates: log (concentration ratio − 1). Data are mean of at least 4 experiments; vertical lines show s.e.mean. The least squares regression for idazoxan against clonidine in the presence of pargyline has a slope of 1.26, $r = 0.99$, $n = 12$ with a pA_2 of 7.63, and was not significantly modified in the presence of cocaine (1 μM). Slope = 1.21, $r = 0.99$, $n = 20$ with a pA_2 of 7.8.

($n = 12$, $r = 0.92$) in the presence of cocaine (1 μM). In the presence of pargyline (10 μM), the pA_2 was 7.6 and the slope 0.9 ($n = 20$, $r = 0.9$). Furthermore, the K_B for idazoxan against clonidine did not significantly change as a function of any of the conditions used in this study (Tables 2, 3).

In the presence of cocaine (1 μM) alone, idazoxan (0.1 μM) caused a significant blockade of 6-FNA-mediated responses (Figure 3, Table 2), but this antagonist effect of idazoxan was not proportionally increased with increasing concentrations of idazoxan (Figure 3, Table 2). In these, and the other experimental models (pargyline + cocaine; no pargyline, no cocaine; reserpine + pargyline), idazoxan always proved significantly ($P < 0.05$) less potent against 6-FNA than clonidine (Tables 2, 3) with the exception of idazoxan (0.1 μM) in the cocaine model (Table 2, Figure 3).

Table 3 Antagonist potency of idazoxan against 6-fluoronoradrenaline (6-FNA) or clonidine-induced inhibition of the twitch response of the rat vas deferens at 0.1 Hz

6-FNA	*n*	*No pargyline, no cocaine* K_B	*n*	*Pargyline* (10 μM) + *cocaine* (1 μM) K_B	*n*	*Reserpine + pargyline* (10 μM) K_B
Idazoxan (1 μM)	4	$5.8 \pm 0.153^{+}$	4	6.5 ± 0.04	4	6.2 ± 0.03
Clonidine						
Idazoxan (1 μM)	4	$8.0 \pm 0.03^{++}$	4	$7.8 \pm 0.02^{++}$	4	$7.9 \pm 0.03^{++}$

Values shown are mean ± s.e.mean.

$^{+}P < 0.05$ significantly different from idazoxan (1 μM) in cocaine + pargyline group; $^{++}P < 0.05$ significantly different from corresponding 6-FNA data.

K_B: antagonist dissociation constant: $K_B = [B]/CR - 1$; where [B] = antagonist concentration and the concentration ratio (CR) is the ratio of equieffective (IC_{50}) concentrations of agonist in the presence or absence of antagonist. Note that the K_B for idazoxan (1 μM) against 6-FNA in reserpine + pargyline-treated group was also significantly different from idazoxan (1 μM) in cocaine treated group ($P < 0.05$; Table 2).

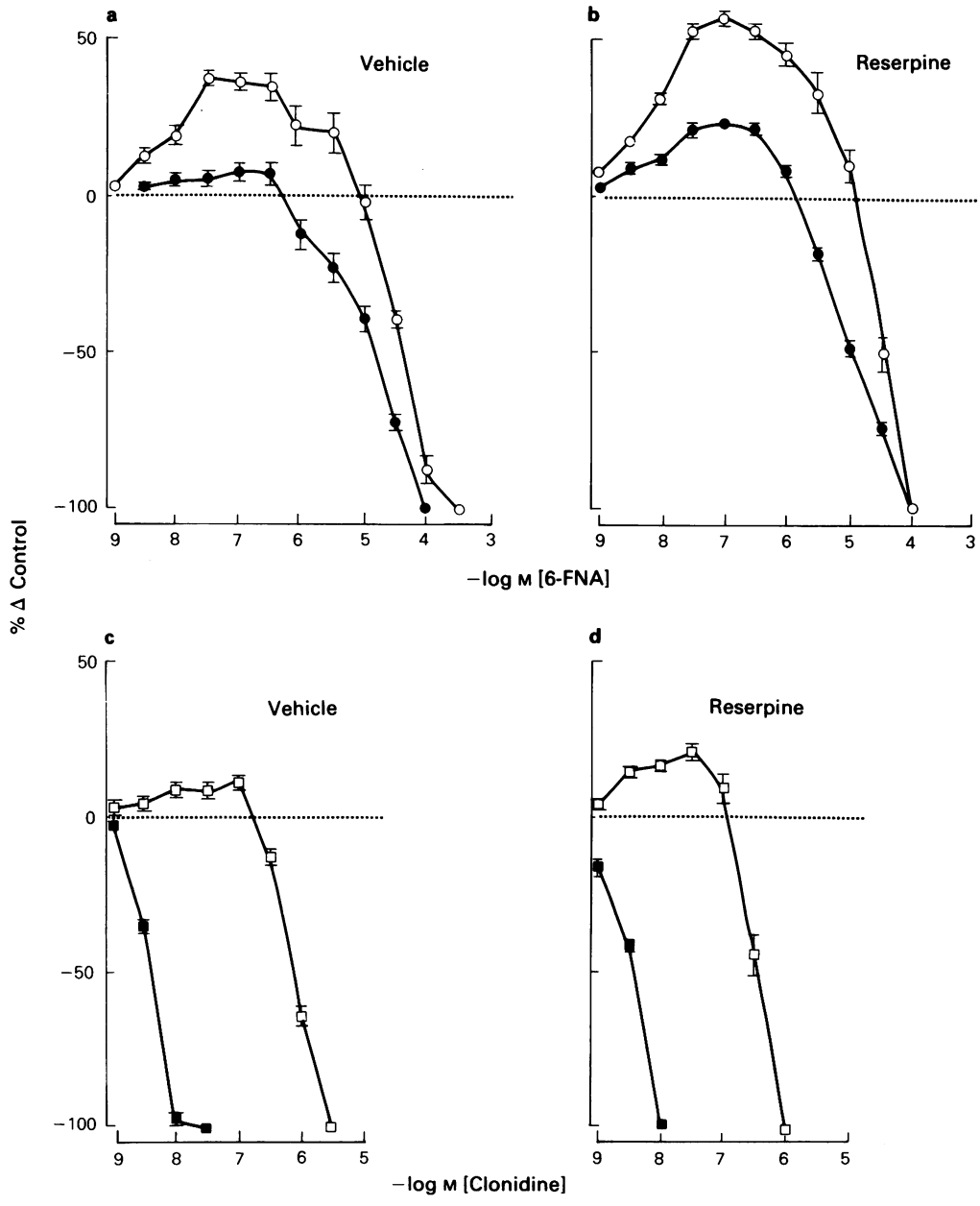

Figure 4 Concentration-response curves for the inhibition of the twitch response of the prostatic vas deferens of the rat elicited by 6-fluoronoradrenaline (6-FNA) (a,b) or clonidine (c,d) in vehicle-treated preparations (a,c) or reserpinised preparations (b,d). Abscissae: − log molar concentration of agonist. Ordinates: % change from control (before drug) values of g tension developed. Inhibitory response curves to 6-FNA (○) or clonidine (□) in the presence of idazoxan (1 μM) were made in naive preparations for both vehicle or reserpinised tissues. The respective control inhibition curves to 6-FNA (●) or clonidine (■) were determined in separate experiments. Data are mean of 4–6 preparations; vertical lines show s.e.mean. Control values of resting tension in 6-FNA experiments were 0.98 ± 0.2 g (●,a) and 1.1 ± 0.1 g (●,b) for vehicle-treated groups, and 0.98 ± 0.2 g (○,a), and 1.2 ± 0.2 g (○,b) for reserpine-treated groups. Control-values of resting tension in clonidine-experiments were 0.9 ± 0.2 g (■,c) and 0.98 ± 0.2 g (□,c) in vehicle-treated groups and 0.94 ± 0.1 g (■,d) and 0.9 ± 0.2 g (□,d) in reserpine-treated groups.

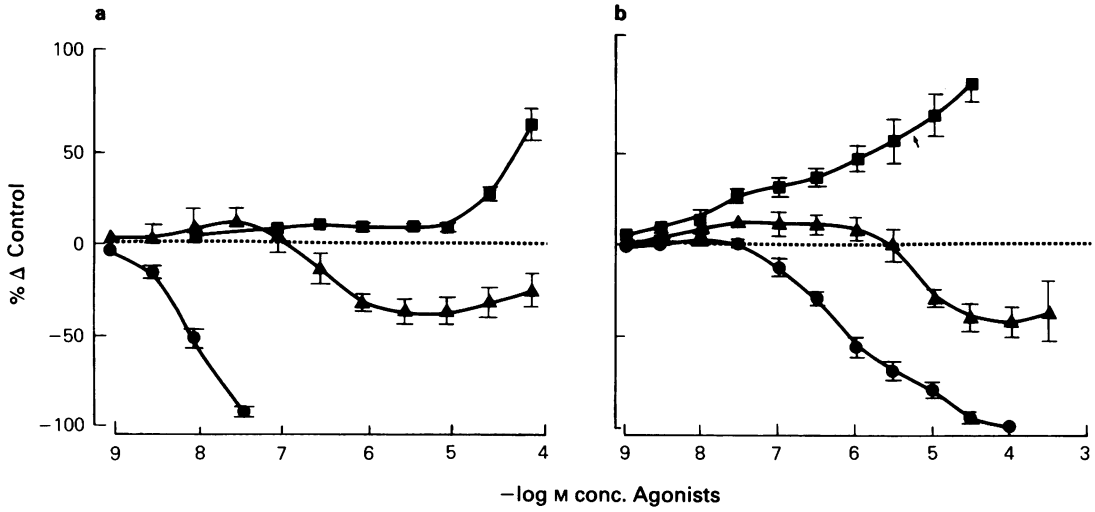

Figure 5 Effects of phenoxybenzamine on the concentration-response curves for the inhibition of the twitch response of the prostatic vas deferens of the rat elicited by clonidine (a) or 6-fluoronoradrenaline (6-FNA) (b). Abscissae: − log molar concentration of agonists. Ordinates: % change from control (before drug) values in g tension developed. Inhibitory-response curves obtained in control preparations (●), after phenoxybenzamine (1 μM), 30 min incubation followed by 1 h washing (▲) or after phenoxybenzamine 10 μM, 30 min incubation followed by 1 h washing (■). Data are mean of 4–6 preparations; vertical lines show s.e.mean.

Facilitatory effects of 6-FNA and clonidine on the twitch response

In the presence of idazoxan (1 μM), exposure to low concentrations of 6-FNA (1–100 nM) invariably caused a facilitation of the twitch response of the vas (Figure 4) prior to its inhibitory effects. This facilitatory effect evoked by 6-FNA on the twitch response, was always more pronounced in the presence of idazoxan. In preparations from rats pretreated with reserpine, the facilitatory effects of 6-FNA were significantly enhanced both in the presence or in the absence of idazoxan (1 μM). Nevertheless, the blocking action of idazoxan against 6-FNA-mediated inhibition of the twitch response observed at higher concentrations of the agonist was not modifed after pretreatment with reserpine (Figure 4, Table 2). In contrast to 6-FNA, the inhibitory effects of clonidine were not preceded by a facilitation of the twitch in control tissues, although a small (10–20%) facilitatory effect of clonidine was seen in reserpinised preparations and after idazoxan (Figure 4). The antagonist potency of idazoxan against clonidine-mediated inhibition of the twitch response was not modified in the vas deferens from rats pretreated with reserpine (Table 2).

In tissues that had been treated with phenoxybenzamine (1 μM) followed by washing for 1 h in normal Krebs, the inhibitory concentration-response curves to 6-FNA and clonidine were significantly antagonized (Figure 5) and showed the typical rightward displacements of the concentration-response curves with reductions in the maximum inhibitory effects of both agonists.

After exposure to 10 μM phenoxybenzamine (30 min, followed by washing for 1 h), 6-FNA only caused concentration-related facilitation of the twitch (Figure 5) and these effects were observed at low concentrations of 6-FNA (10–1000 nM). After phenoxybenzamine (10 μM), the clonidine-induced inhibitory effects were totally blocked (Figure 5), and the small facilitatory effects of the twitch evoked by clonidine in this model were significantly less than for 6-FNA, and occurred at rather high concentrations of clonidine (Figure 5).

Discussion

It is now well established that the fast 'twitch' response of the rat vas deferens to low frequency electrical stimulation is not noradrenergically mediated, despite a rich sympathetic innervation of the tissue (Ambache & Aboo Zar, 1971; McGrath, 1978; Brown *et al.*, 1979; MacDonald & McGrath, 1980). However, as in the vas deferens of other species, notably the guinea-pig and in the cat nictitating membrane (Langer & Pinto, 1976; Duval *et al.*, 1985), it is possible that the

transmitter responsible for the twitch response of the rat vas deferens is ATP (Burnstock, 1982; Sneddon & Westfall, 1984; Meldrum & Burnstock, 1983; Sneddon & Burnstock, 1984).

Our results support the view that NA is not the transmitter responsible for the contractile response of the prostatic end of the rat vas deferens stimulated at low frequencies, since in reserpinised rats, where the NA-content of the vas was decreased by 97%, the twitch response remained unaffected.

Inhibitory α-adrenoceptors are present in the vas, and do modulate motor transmission (Ambache & Aboo Zar, 1971); these receptors are of the α_2-adrenoceptor subtype (Drew, 1977; McGrath, 1978; Brown *et al.*, 1979, Vizi *et al.*, 1983). It is also known that α_2-adrenoceptor agonists of the imidazoline and azepine series are more potent than phenylethylamine-like agonists as inhibitors of the twitch and are also competitively antagonized by yohimbine (Mottram, 1982; Langer & Shepperson, 1982; Vizi *et al.*, 1983). Phenylethylamine-like agonists are, however, relatively resistant to blockade by α_2-adrenoceptor antagonists in the vas deferens. The present results using 6-FNA as a phenylethylamine agonist support the view that there might be two recognition sites for α_2-adrenoceptors in the rat vas deferens, which mediate inhibition of the twitch response. While 6-FNA was always less potent than clonidine at inhibiting the twitch response, both drugs were 'full agonists' although the slopes of the inhibitory response curves were markedly different. Unlike clonidine which was not modified by cocaine, the potency of 6-FNA was significantly increased in the presence of low concentrations of cocaine, confirming that 6-FNA is a substrate for neuronal uptake, as previously demonstrated (Shepperson *et al.*, 1981). It is unlikely that cocaine (1 μM) was fully effective at inhibiting neuronal uptake in these experiments, since the IC_{50} for neuronal uptake blockade of [^{3}H]-NA in the rat vas deferens was 4.2 μM (Langer *et al.*, 1984). However, it was not possible to increase routinely the concentration of cocaine in the present study, since cocaine on its own inhibited the twitch response in a concentration-dependent manner, an effect most likely due to an inhibitory action of endogenous NA on the twitch resulting from the progressive blockade of neuronal uptake of NA in the range of the concentrations of cocaine tested. This effect of cocaine was not observed in reserpinised vas deferens, supporting the view that the role of endogenously released NA in the prostatic vas deferens is predominantly inhibitory (by reducing the release of the excitatory transmitter). Doxey *et al.* (1984) have also demonstrated that cocaine inhibits the twitch response of the rat vas deferens and that these effects are antagonized by idazoxan.

The selective α_2-adrenoceptor antagonist idazoxan (Doxey *et al.*, 1983; 1984) was a competitive antagonist against clonidine-induced inhibition of the twitch in the vas. The pA_2 values reported here are less than those found by Doxey *et al.* (1983, 1984), but are in close agreement with pA_2 values obtained by other workers (Caroon *et al.*, 1982; Dabire *et al.*, 1983). In contrast to the competitive antagonism shown by idazoxan against clonidine, the profile of antagonism by idazoxan against 6-FNA was complex. We observed more than a ten fold shift to the right of the 6-FNA-inhibitory curve, but only in the presence of cocaine. However, this antagonism by idazoxan was not concentration-dependent. In the presence of pargyline, after pretreatment with reserpine, or when neither cocaine nor pargyline were present, idazoxan even at 10 μM produced only marginal antagonism of the 6-FNA-mediated inhibitory responses. These results readily support the findings of Mottram (1982) using α-methylnoradrenaline, and of Langer & Shepperson (1982) and Vizi *et al.* (1983) using NA, suggesting that imidazoline-like and phenylethylamine-like inhibitory receptors in the vas may be different. It is difficult to explain why idazoxan was only an antagonist of 6-FNA in the presence of cocaine. However it is likely that 6-FNA does have weak affinity for the clonidine site, which may be more easily observed when the agonist curve is shifted to the left, as occurs in the presence of cocaine. It may also be possible that the inhibitory effects of 6-FNA, particularly at very high concentrations, are not receptor-mediated events, although such an interpretation would also be applicable to NA, α-methylnoradrenaline or dopamine, all of which are resistant to antagonism by idazoxan and yohimbine in the vas deferens.

Interestingly, the aminotetralin TL99 which is also an α_2-adrenoceptor agonist (Hicks & Cannon, 1981) is competitively antagonized by idazoxan in this model, (pA_2 7.8; slope 0.9), and TL99 is only 8–10 times more potent than 6-FNA as an inhibitory agent, (IC_{50} = 300 nM (Hicks & Langer, unpublished observations).

The facilitatory effects of 6-FNA on the twitch response were mainly observed in preparations treated with idazoxan or in reserpinised tissues. This facilitation is unlikely to be due to the α_1-adrenoceptor agonist properties of 6-FNA, since these effects were observed in the presence of the α_1-adrenoceptor antagonist prazosin, and were clearly present after irreversible blockade of all α-receptor subtypes with phenoxybenzamine. A facilitatory effect on the twitch response of the vas has been observed with other α_1-adrenoceptor agonists, like cirazoline or amidephrine (Docherty & McGrath, 1984), and also with the phenylethylamines, methoxamine, NA or dopamine (Hicks & Langer, unpublished observations) in preparations where α- and β-adrenoceptor subtypes were blocked (prazosin, idazoxan, propranolol). It

remains to be determined whether this facilitatory effect of 6-FNA is receptor mediated, or involves non-receptor mediated depolarization of nerve terminals and/or the facilitation of the release of the excitatory transmitter in the vas. Recently Hyland *et al.* (1984) have shown that the dihydropyridine Ca^{2+}-agonist Bay K8644 induces a similar effect, which is not blocked by α-adrenoceptor antagonists, and which may involve increased Ca^{2+} flux mechanisms in the facilitation of the twitch.

In conclusion, both clonidine and 6-FNA fully inhibit the twitch response of the rat vas deferens to low frequency electrical stimulation. These agonists demonstrated markedly different inhibitory potencies, but only the effects of 6-FNA were potentiated by cocaine or cocaine and pargyline. The selective α_2-adrenoceptor blocking agent, idazoxan, was a competitive antagonist against clonidine under all of the experimental conditions used in this study; however, against the inhibitory effects of 6-FNA, idazoxan failed to block, or was particularly weak in the presence of pargyline, pargyline and cocaine, or when neither drug was present. An initial antagonist effect of idazoxan could be demonstrated in the presence of cocaine alone, but this blockade was not further increased by increasing the concentration of idazoxan. These results further support the general view that the α_2-adrenoceptor may be subdivided into imidazoline- and phenylethylamine-sensitive subclasses (Pelayo *et al.*, 1980; Langer & Dubocovich, 1981; Mottram, 1982; Langer & Shepperson, 1982; Ruffolo *et al.*, 1983; Vizi *et al.*, 1983; Baker *et al.*, 1984).

The facilitatory effect on the twitch response of the vas elicited by 6-FNA and perhaps other agonists of this class is unlikely to be due to α_1-adrenoceptor of β-adrenoceptor stimulation. At present, the facilitation of the twitch response obtained in the presence of α_1-, α_2-, β_1- and β_2-adrenoceptor antagonists, appears to be related to a direct effect on the nerve terminals to enhance transmitter release, which is unrelated to the stimulation of α_1-, α_2-, β_1- or β_2-adrenoceptors.

The authors wish to thank Miss K. Grandet for excellent technical assistance, and Dr J. Dedek for the fluorimetric assay of NA. The financial assistance for A.D.M. from the Physiological Society and University of Glasgow is gratefully acknowledged. We are also grateful to Colette Villebeuf for typing the manuscript.

References

AMBACHE, N. & ABOO ZAR, M. (1971). Evidence against adrenergic motor transmission in the guinea-pig vas deferens. *J. Physiol.*, **216**, 359–389.

ANTON, P.Q., DUNCAN, M.E. & MCGRATH, J.C. (1977). An analysis of the anatomical basis for the mechanical response to motor nerve stimulation of the rat vas deferens. *J. Physiol.*, **273**, 23–43.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

BAKER, D.J., DREW, G.M. & HILDTITCH, A. (1984). Presynaptic α-adrenoceptors: do exogenous and neuronally released noradrenaline act at different sites? *Br. J. Pharmac.*, **81**, 457–464.

BOUSQUET, P., FELDMAN, J. & SCHWARTZ, J. (1984). Central cardiovascular effects of alpha-adrenergic drugs: Differences between catecholamines and imidazolines. *J. Pharmac. exp. Ther.*, **230**, 232–236.

BROWN, C.M., MCGRATH, J.C. & SUMMERS, R.J. (1979). The effects of alpha-adrenoceptor agonists and antagonists on responses of transmurally stimulated prostatic and epididymal portions of the isolated vas deferens of the rat. *Br. J. Pharmac.*, **66**, 553–564.

BURNSTOCK, G. (1982). The cotransmitter hypothesis with special reference to the storage and release of ATP with noradrenaline and acetylcholine. In *Cotransmission*. ed. Cuello, A.C. pp. 151–163. London: Macmillan Press.

CAROON, J.M., CLARK, R.D., KLUGE, A.F., OLAH, R., REPKE, D.B., UNGER, S.H., MICHEL, A.D. & WHITING, R.L. (1982). Structural activity relationships for 2-substituted imidazoles as alpha$_2$-adrenoceptor antagonists. *J. med. Chem.*, **25**, 666–674.

DABIRE, H., DAUSSE, J.P., MOUILLE, P., SCHMITT, H. & MEYER, P. (1983). In vitro studies with (imidazolinyl-2)-2-benzodioxane-1-4(±)-170-150, a new potent alpha$_2$-adrenoceptor blocking agent. *Eur. J. Pharmac.*, **86**, 87–90.

DOCHERTY, J.R. & MCGRATH, J.C. (1984). The actions of cirazoline on the rat vas deferens. *Br. J. Pharmac.*, **82**, 9–14.

DOXEY, J.C., ROACH, A.G. & SMITH, C.F.E. (1983). Studies on RX 781094, a selective specific antagonist of alpha$_2$-adrenoceptor. *Br. J. Pharmac.*, **78**, 489–506.

DOXEY, J.C., LANE, A.C., ROACH, A.G. & VIRDEE, N.K. (1984). Comparison of the alpha-adrenoceptor antagonist profiles of idazoxan (RX 781094), yohimbine, rauwolscine and corynanthine. *Naunyn-Schmiedebergs Arch. Pharmac.*, **325**, 136–144.

DREW, G.M. (1977). Pharmacological characterization of the presynaptic alpha-adrenoceptor in the rat vas deferens. *Eur. J. Pharmac.*, **42**, 123–130.

DUVAL, N., HICKS, P.E. & LANGER, S.Z. (1985). Inhibitory effects of α,β-methylene-ATP on nerve mediated contractions of the nictitating membrane in reserpinised cats. *Eur. J. Pharmac.*, **110**, 373–377.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors) and evaluation from the stand point of receptor theory. In *Catecholamines*, ed. Blaschko, H. & Muscholl, E. pp. 283–335. Berlin: Springer-Verlag.

HICKS, P.E. & CANNON, J.G. (1981). Cardiovascular effects of 2-(N,N-dimethyl)-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene in the pithed rat: Differential antagonism by yohimbine and prazosin. *J. Pharm. Pharmac.*, **32**, 786–788.

HICKS, P.E., LANGER, S.Z. & MACRAE, A.D. (1984). Complex

inhibitory effects of 6-fluoronoradrenaline on the twitch response of the rat vas deferens at different frequencies. *Br. J. Pharmac.*, **81**, 17P.

HYLAND, L., WARNOCK, P. & DOCHERTY, J.R. (1984). An investigation of the calcium entry facilitator BAY K8644 on the rat vas deferens. *Eur. J. Pharmac.*, **104**, 363–367.

LANGER, S.Z. & PINTO, J.E.B. (1976). Possible involvement of a transmitter different from norepinephrine in the residual responses to nerve stimulation of the cat nictitating membrane after pretreatment with reserpine. *J. Pharmac. exp. Ther.*, **196**, 697–713.

LANGER, S.Z. & DUBOCOVICH, M.L. (1981). Cocaine and amphetamine antagonize the decrease of noradrenergic neurotransmission elicited by oxymetazoline but potentiate the inhibition by alpha-methyl norepinephrine in the perfused cat spleen. *J. Pharmac. exp. Ther.*, **216**, 162–171.

LANGER, S.Z. & SHEPPERSON, N.B. (1982). Differences between noradrenaline and clonidine on the presynaptic alpha$_2$-adrenoceptor mediated inhibition of the twitch response of the rat vas deferens. *Br. J. Pharmac.*, **76**, 319P.

LANGER, S.Z., TAHRAOUI, L., RAISMAN, R., ARBILLA, S., NAJAR, M. & DEDEK, J. (1984). ^{3}H-Desipramine labels a site associated with the neuronal uptake of noradrenaline in the peripheral and central nervous system. In *Neuronal and Extraneuronal Events in Autonomic Pharmacology*. ed. Fleming, W., Langer, S.Z., Graeze, K.H. & Weiner, N. pp. 37–49. New York: Raven Press.

MACDONALD, A. & MCGRATH, J.C. (1980). The distribution of adrenoceptors and other drug receptors between the two ends of the rat vas deferens as revealed by selective agonists and antagonists. *Br. J. Pharmac.*, **71**, 445–458.

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

MELDRUM, L.A. & BURNSTOCK, Q. (1983). Evidence that ATP acts as a co-transmitter with noradrenaline in sympathetic nerves supplying the guinea-pig vas deferens. *Eur. J. Pharmac.*, **92**, 161–163.

MOTTRAM, D.R. (1982). Pharmacological evidence for high and low affinity sites on prejunctional alpha$_2$-adrenoceptors. *Br. J. Pharmac.*, **77**, 534P.

PELAYO, F., DUBOCOVICH, M.L. & LANGER, S.Z. (1980). Inhibition of neuronal uptake reduces the presynaptic effect of clonidine but not of alpha-methylnoradrenaline from rat occipital cortex slices. *Eur. J. Pharmac.*, **64**, 143–155.

RUFFOLO, J.R.R., RICE, P.J., PATIL, P.N., HAMADA, A. & MILLER, D.D. (1983). Differences in the applicability of the Easson-Stedman hypothesis to the alpha$_1$ and alpha$_2$ adrenergic effects of phenylethylamines and imidazolines. *Eur. J. Pharmac.*, **86**, 471–475.

SHEPPERSON, N.B., PURCELL, T., MASSINGHAM, R. & LANGER, S.Z. (1981). In vitro studies on 6-fluoronoradrenaline at several peripheral sympathetic neuroeffector junctions. *Naunyn-Schmiedebergs Arch. Pharmac.*, **317**, 1–4.

SNEDDON, P. & BURNSTOCK, G. (1984). Inhibition of excitatory junction potentials in guinea-pig vas deferens by α-β-methylene-ATP: further evidence for ATP and noradrenaline as cotransmitters. *Eur. J. Pharmac.*, **100**, 85–90.

SNEDDON, P. & WESTFALL, D.P. (1984). Pharmacological evidence that adenosine triphosphate and noradrenaline are co-transmitters in guinea-pig vas deferens. *J. Physiol.*, **347**, 561–565.

VIZI, E.Z., LUDVIG, N., RONAI, A.Z. & FOLLY, G. (1983). Dissociation of presynaptic adrenoceptors following prazosin administration: presynaptic effect of prazosin. *Eur. J. Pharmac.*, **95**, 287–290.

WESTERINK, B.H.C. & KORF, J. (1977). Rapid concurrent automated fluorimetric assay of noradrenaline dopamine ^{3}H-dihydroxyphenylacetic acid and 3-methoxytyramine in milligram amounts of nervous tissue after isolation on sephadex G-10. *J. Neurochem.*, **29**, 697–705.

(*Received February 5, 1985.*
Revised April 8, 1985.
Accepted April 26, 1985.)