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THE DISTRIBUTION OF ADRENOCEPTORS AND OTHER DRUG RECEPTORS BETWEEN THE TWO ENDS OF THE RAT VAS DEFERENS AS REVEALED BY SELECTIVE AGONISTS AND ANTAGONISTS

A. MacDONALD & J.C. McGRATH*

Department of Biological Sciences, Glasgow College of Technology, Cowcaddens Road, Glasgow G4 0BA and
*Institute of Physiology, University of Glasgow, Glasgow G12 8QQ

1 The effects of adrenoceptor agonists and other agonists on the contractile responses of the prostatic and epididymal portions of the rat isolated vas deferens to single pulse field stimulation were investigated.

2 α-Adrenoceptor agonists produced prejunctional α_2-mediated inhibition and post-junctional α_1-mediated potentiation of the nerve-induced responses. Guanabenz and xylazine produced mainly inhibitory effects, xylazine being 10 times less potent. Clonidine and oxymetazoline produced inhibition with similar potency to guanabenz but at higher concentrations excitatory effects were present, particularly in the epididymal portion. Phenylephrine produced only potentiation of the nerve-induced response in both portions. Potentiation of nerve-induced responses was a more sensitive and quantitative index of excitation than was direct contraction of the tissue.

3 Isoprenaline and salbutamol both gave β_2-mediated inhibition of the nerve-induced responses in both portions of tissue. At least part of the effect was post-junctional since phenylephrine contractions were inhibited. Isoprenaline also produced a post-junctional α_1-mediated excitation.

4 Noradrenaline produced effects qualitatively similar to those of the other α-adrenoceptor agonists, inhibition and excitation predominating in the prostatic and epididymal ends respectively.

5 Morphine produced inhibition in the mouse but not in the rat vas deferens. In rat vas, however, enkephalin analogues produced pre-junctional inhibition of responses in both portions which could be partly reversed by naloxone; restoration of the adrenergic component was more complete. Rat anococcygeus showed no equivalent effect.

6 Adenosine 5'-triphosphate (ATP) inhibited nerve-induced responses in each portion with a greater effect on the prostatic portion.

Introduction

The effects of drugs which modify adrenergic transmission are different in the two ends of rat vas deferens. In the epididymal end, contractile responses to nerve stimulation are mainly adrenergic since they are reduced by reserpine pretreatment or acute administration of post-junctional α-adrenoceptor antagonists and are potentiated by blockers of the neuronal uptake of noradrenaline. In contrast, the main part of the response in the prostatic end is resistant to these types of drugs (Anton, Duncan & McGrath, 1977; McGrath, 1978; Brown, McGrath & Summers, 1979).

The present study was undertaken to investigate possible differences in the effects of agonist drugs between the two ends. Effects on the muscle and against neurotransmission were both examined, first for adrenoceptor agonists (α and β) and then for drugs known to act at other types of receptor in this organ, i.e. 'opioids' and adenosine 5'-triphosphate (ATP) (Henderson, Hughes & Kosterlitz, 1972; Clanachan, Johns & Paton, 1977).

Field stimulation of the intramural nerve fibres was applied only as single pulses to avoid eliciting endogenous inhibition by noradrenaline acting at prejunctional α_2-adrenoceptors (Brown *et al.*, 1979). Earlier work on whole vasa had demonstrated that these prejunctional receptors could be activated by the agonists clonidine, oxymetazoline and xylazine (but not by phenylephrine) and could be antagonized by yohimbine (but not by prazosin) (Drew, 1977; Doxey, Smith & Walker, 1977). To study the distribution of α-adrenoceptors, therefore, we employed the above drugs plus the clonidine-like drug, guanabenz

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(Baum, Shropshire, Rowles, Van Pelt, Fernandez, Eckfeld & Gluckman, 1970). To examine effects on β-adrenoceptors the agonists, isoprenaline and salbutamol, and the antagonist, sotalol, were employed, the latter lacking much of the local anaesthetic activity of other β-adrenoceptor antagonists (Lish, Weikel & Dungan, 1965).

Each drug was tested for its effect on the resting tension of the preparations and then for its effects on the response to a single pulse of electrical field stimulation. The response to a single stimulus allows separate assessment of potential prejunctional effects of agonists or antagonists against the 'non-adrenergic' response, which predominates in the prostatic portion, and the relatively straighforward 'adrenergic' response, which predominates in the epididymal portion (McGrath, 1978). With these methods it was, therefore, possible to chart the distribution of the different sub-classes of adrenoceptors and other receptors within the vas deferens.

Preliminary communications of part of this work have been published (Docherty, MacDonald & McGrath, 1979; MacDonald, McGrath & Murdoch, 1979).

Methods

Male Wistar rats (250 to 300 g) were killed by a blow on the head and exsanguination. Vasa deferentia were isolated and set up bisected transversely into two portions of equal length through 'ring and hook' Ag:AgCl electrodes in a 30 ml organ bath containing Krebs bicarbonate solution at 37°C and gassed with 95% O_2 and 5% CO_2 (see Anton *et al.*, 1977).

Isometric longitudinal tension was recorded on a Grass Model 7 polygraph, a Devices M2 recorder or a Tektronix D13 storage oscilloscope. Responses were elicited by field stimulation via the electrodes with single supramaximal pulses of 0.5 ms duration. In some experiments rat anococcygeus preparations were set up in a similar manner (see Gillespie, 1972) and in one series of experiments vasa from mice (Parkes strain, 40 to 50 g) were employed.

For analysis of the effects of drugs on the 'non-adrenergic' response in the absence of the adrenergic component, reserpine (3 mg/kg, i.p., 18 h) pretreatment of control rats was used. This treatment reduces the noradrenaline content of the rat vas deferens to less than 1% of control values (Gillespie & McGrath, 1974b) and selectively eliminates the adrenergic component of the contractile response to nerve stimulation (Booth, Connell, Docherty & McGrath, 1978). Reserpine was dissolved in 0.4% w/v ascorbic acid immediately before use.

Drugs were dissolved in saline and added to the organ bath in a maximum dilution of 1 in 100 to give the appropriate molar concentration. When a range of concentrations of one drug was being tested increasing concentrations were added cumulatively. In most experiments field stimuli were applied at 5 min intervals and drugs were added 3 to 4 min before a stimulus was due. In this way the effect of the drug on resting tone could be assessed. Stimuli were continued at 5 min intervals and the response measured when equilibrium was established i.e. consecutive responses were identical, or at 20 min, whichever was the longer. When testing antagonists against the effect of agonists three different procedures were employed as mentioned in Results, according to the properties of the agents involved: (1) addition of the antagonist after addition of the agonist and while the latter was still present, i.e. reversal of the agonists's effect; (2) construction of a concentration-response curve for the agonist, washing to obtain a new control, addition of the antagonist and construction of a concentration-response curve for the agonist in the antagonist's presence. This was only possible if the effects of the highest concentration of agonist were completely reversible; if not, (3) concentration-response curves for the agonist in the presence or in the absence of the antagonist had to be obtained on different tissues.

An unexpected observation which might be noted by those working in this field was that either clonidine or guanabenz, if added to the bath in concentrations of above 10^{-8} M adhered to the glass and/or Araldite of the bath and the electrode assembly resulting in contamination of the Krebs solution in subsequent experiments. This effect might have gone unnoticed but for the distinct change in the time course of the response to a single stimulus in the epididymal portion which is produced by low concentrations of these agents. This observation is also confirmed by the finding that radiolabelled clonidine adheres avidly to glass fibre filter discs (R.J. Summers, personal communication). High concentrations of prazosin presented similar problems; this may be related to the relatively poor solubility of this agent in saline. These problems were overcome by rejecting preliminary experiments in which contamination was suspected and by paying particular attention to decontamination in subsequent experiments.

The Krebs bicarbonate solution employed had the following composition (mM): NaCl 119, KCl 4.7, $MgSO_4$ 1.0, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 11.1.

Drugs used were: adenosine 5'-triphosphate (ATP: Sigma); Bayer 6781 (2-2-methyl-6-ethyl-cyclohexylamino)-2-oxazoline) (Bayer); clonidine hydrochloride (Catapres, Boehringer Ingelheim); guanabenz acetate (Wyeth); (−)-isoprenaline hydrochloride (Sigma); morphine hydrochloride (Macarthys); (Met)-enkephalin (Wellcome); naloxone (Endo); naphazoline hydrochloride (CIBA); noradrenaline bitartrate (Koch-Light); oxymetazoline hydrochloride (Merck);

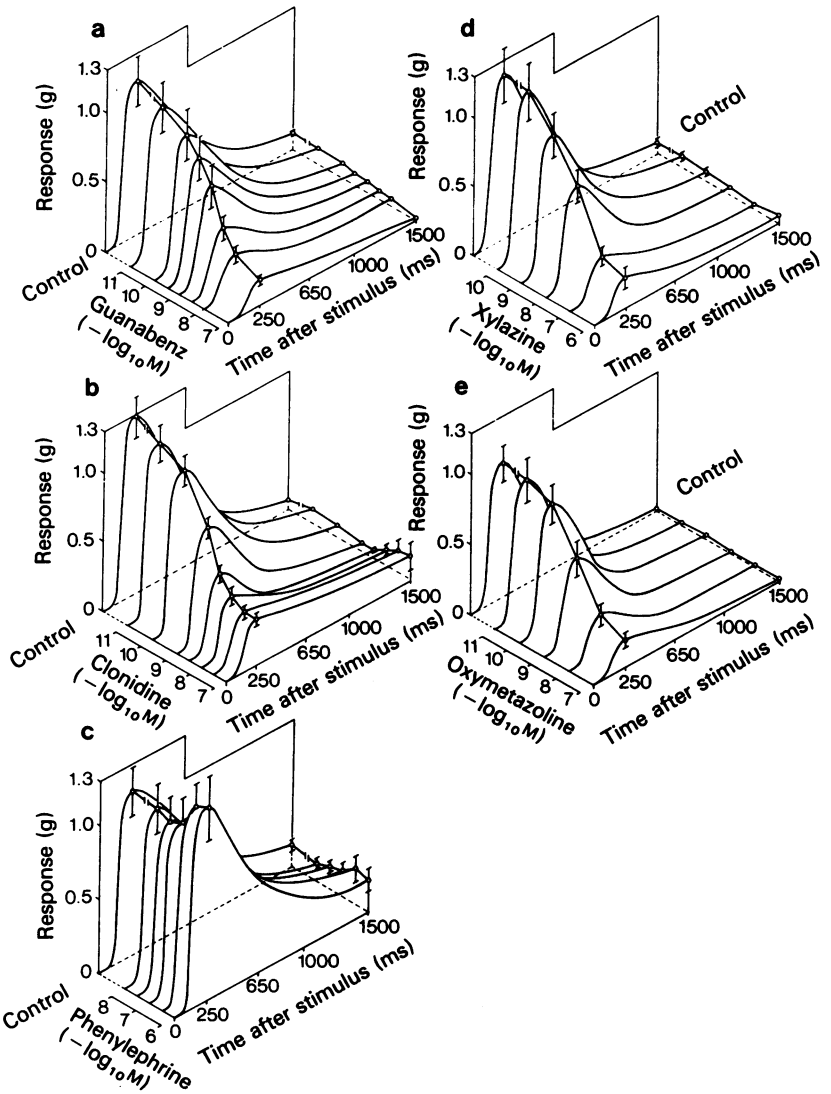

Figure 1 The effects of α-adrenoceptor agonists on responses of prostatic portions of the rat vas deferens to a single transmural impulse (0.5 ms pulse width, supramaximal voltage) ($n \geq 6$). Each graph shows the relationship between the isometric response (expressed as g tension) and the time after the stimulus for increasing concentrations of the drugs: (a) guanabenz; (b) clonidine; (c) phenylephrine; (d) xylazine and (e) oxymetazoline. For clarity, s.e. bars are shown only for points at 250 and 1500 ms after each stimulus.

(−)-phenylephrine hydrochloride (Sigma); prazosin hydrochloride (Pfizer); reserpine, crystalline (Koch-Light); salbutamol (Allen & Hanburys); sotalol hydrochloride (Duncan Flockhart); Tyr-D-Ala-Gly-MePhe-Met(0)-ol (Sandoz FK 33-824); Tyr-D-Ala-Gly-Phe-D-Leu ([D-Ala^2, D-Leu^5]-enkephalin, Wellcome BW 180 C); WB 4101 (2-N([2',6'-dimethoxyphenoxyethyl] aminoethyl) 1,4-benzodioxan hydrochloride, Ward Blenkinsop); xylazine hydrochloride (Bayer); xylometazoline hydrochloride (CIBA) and yohimbine hydrochloride (Sigma).

Where appropriate, statistical analysis was performed by use of Student's t test or of a t test for paired data.

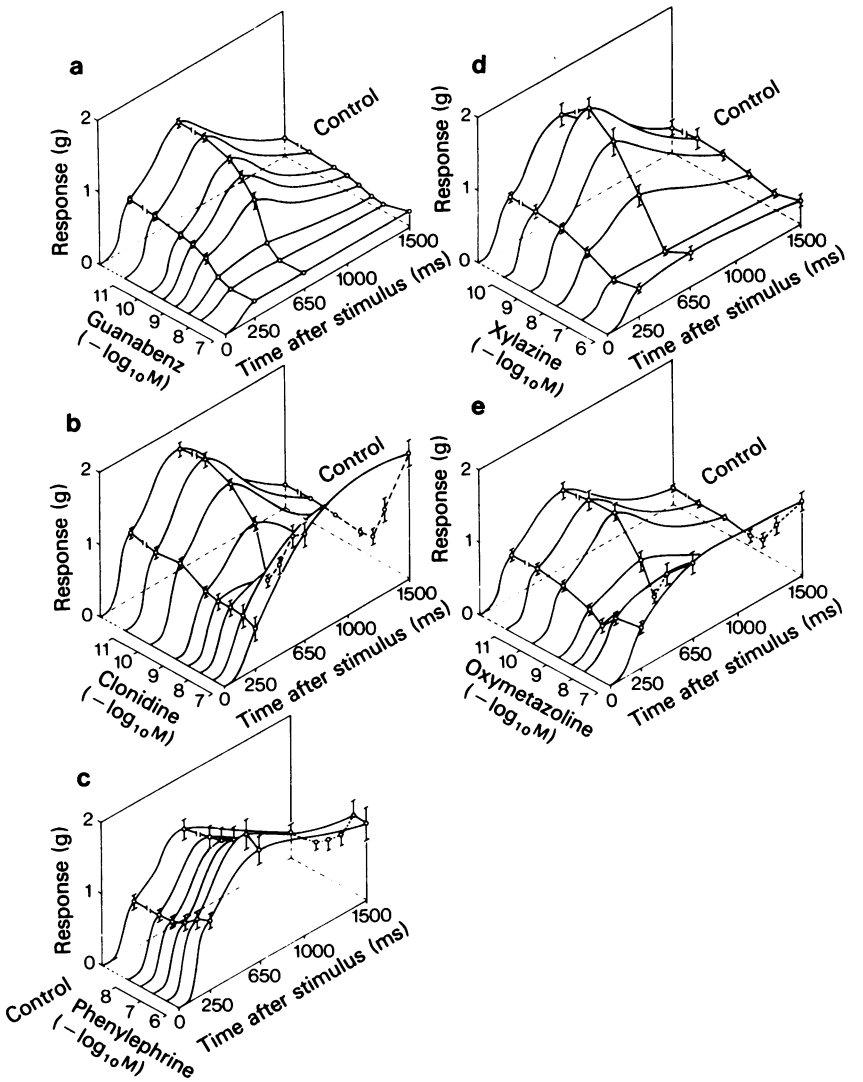

Figure 2 The effects of α-adrenoceptor agonists on responses of epididymal portions of the rat vas deferens to a single transmural impulse (0.5 ms pulse width, supramaximal voltage) ($n \geqslant 6$). Each graph shows the relationship between the isometric response (expressed as g tension) and the time after the stimulus for increasing concentrations of the drugs: (a) guanabenz; (b) clonidine; (c) phenylephrine; (d) xylazine and (e) oxymetazoline. For clarity s.e. bars are shown only for points at 250, 650 and 1500 ms after each stimulus.

Results

Control responses to electrical stimulation consisted of (1) in the prostatic portion, an essentially monophasic contraction reaching a maximum 250 ms after stimulation and which was resistant to α-adrenoceptor antagonists; (2) in the epididymal portion, a biphasic response with a dominant second phase reaching a maximum at 650 ms; this second phase was abolished by α-adrenoceptor antagonists (McGrath 1978). The early peak in the prostatic portion and the late peak in the epididymal portion can thus be taken to represent the effects of the 'non-adrenergic' and the adrenergic transmission respectively.

Adrenoceptor agonists

α-Adrenoceptor agonists Of the drugs tested, each had a distinct profile of activity against resting muscle tone and against nerve-induced responses according to its mixture of pre- and post-junctional actions. The time course of the response to a single stimulus in each portion of the tissue is shown in Figures 1 and 2 and the effects of the agonists are summarized in Table 1. The prolonged responses found after α-agonists sometimes became wave-like as seen previously after cocaine (McGrath, 1978). This is shown most markedly in Figure 3 and partly in Figure 4c.

Guanabenz and xylazine produced mainly inhibition of the nerve-induced responses with a relative increase in the size of responses occurring only at the highest doses (Figure 1a, 1d, 2a and 2d).

Phenylephrine increased both the height and duration of the nerve-induced responses in the epididymal portion and had a similar but less marked effect on the prostatic portion (Figure 1c and 2c). Higher concentrations induced phasic contractile activity in both portions of the vas.

Clonidine and oxymetazoline produced effects which were intermediate between those of guanabenz and phenylephrine. The nerve-induced contraction in the prostatic portion was reduced but in the epididymal portion, after an initial reduction in height with low concentration, the nerve-induced response was increased in height and duration (Figures 1b, 1e, 2b and 2e). Clonidine and oxymetazoline also induced phasic contractions (Table 1). Two further imidazolidines, xylometazoline and naphazoline, and Bayer 6781, an oxazoline with structural similarities to clonidine (Werner, Starke & Schümann, 1972), were found to have effects qualitatively similar to those of oxymetazoline or clonidine (Table 1).

Evidence that the intermediate position of clonidine between guanabenz and phenylephrine is produced by 'physiological antagonism' produced by two distinct pharmacological actions is provided by Figures 3 and 4. These show that the effects of a high concentration of clonidine (3×10^{-8} M), phasic contractions and reduction of height and increase in duration of the nerve-induced response (Figure 3), can be mimicked by phenylephrine (10^{-6} M) plus guanabenz (10^{-8} M) (Figure 4).

The excitatory effects of phenylephrine, clonidine and oxymetazoline were abolished by prazosin (6×10^{-6} M). WB 4101 (10^{-7} M) and yohimbine (6×10^{-6} M) also blocked the excitatory effects of phenylephrine.

Table 1 Threshold concentrations for effects of agonists on the rat vas deferens

	Threshold concentration (M)		
	Inhibition of nerve-induced response (prostatic 250 ms)	*Potentiation of nerve-induced response (epididymal 1500 ms)*	*Contractile activity (epididymal end)*
α-Adrenoceptor agonists			
Bayer 6781	10^{-11}	ND $\leqslant 10^{-9}$	ND $\leqslant 10^{-9}$
Xylazine	10^{-10}	10^{-6}	ND $\leqslant 10^{-6}$
Guanabenz	10^{-10}	10^{-7}	ND $\leqslant 10^{-7}$
Clonidine	10^{-10}	10^{-8}	$3 \times 10^{-8} - 10^{-7}$
Oxymetazoline	10^{-10}	10^{-9}–10^{-8}	10^{-8}–10^{-7}
Xylometazoline	10^{-9}	3×10^{-8}	3×10^{-7}
Naphazoline	10^{-9}	3×10^{-8}	3×10^{-7}
Noradrenaline	10^{-8}–3×10^{-8}	3×10^{-6}	3×10^{-6}
Phenylephrine	ND $\leqslant 10^{-6}$	3×10^{-8}	10^{-7}–3×10^{-7}
β-Adrenoceptor agonists			
Isoprenaline	10^{-8}–10^{-7}	10^{-5}	10^{-5}–3×10^{-5}
Salbutamol	10^{-7}	ND $\leqslant 10^{-4}$	ND $\leqslant 10^{-4}$
Other agonists			
Morphine	ND $\leqslant 10^{-5}$	ND $\leqslant 10^{-5}$	ND $\leqslant 10^{-5}$
D-Ala^2, D-Leu^5-enkephalin	10^{-7}	ND $\leqslant 10^{-6}$	ND $\leqslant 10^{-6}$
FK 33-824	10^{-8}	ND $\leqslant 10^{-6}$	ND $\leqslant 10^{-6}$
ATP	10^{-5}	ND $\leqslant 3 \times 10^{-4}$	10^{-4}

ND: not detected. Nerve-induced responses were induced by single pulse field stimulation. The most sensitive measurements for inhibition and potentiation were the prostatic (250 ms) and epididymal (1500 ms) responses respectively.

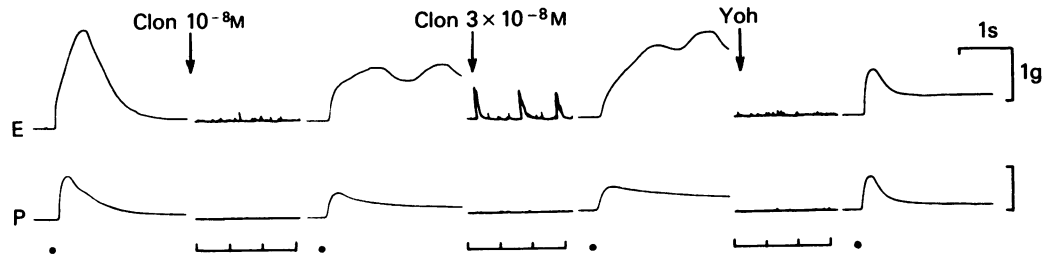

Figure 3 The effects of clonidine and yohimbine on the response of bisected portions of a rat vas deferens to a single stimulus (0.5 ms pulse width, supramaximal voltage). Upper traces represent the isometric tension (g) of the epididymal portion (E) and the lower trace that of the prostatic portion (P). Each gap in the trace is of at least 5 min. Traces were recorded at the speed indicated by the right hand calibration except for the sections following addition of drugs which were recorded at the slower speed indicated by the 1 min calibrations below the lower trace. Drugs were administered between traces as indicated by arrows, i.e. clonidine (Clon) 10^{-8} M, increase of clonidine concentration to 3×10^{-8} M and finally addition of yohimbine (Yoh) 6×10^{-6} M. Electrical stimuli were applied at dots below traces and those after drugs represent the effect of the drug after equilibration, i.e. at least 10 min. Note that yohimbine antagonizes both excitatory and inhibitory effects of clonidine.

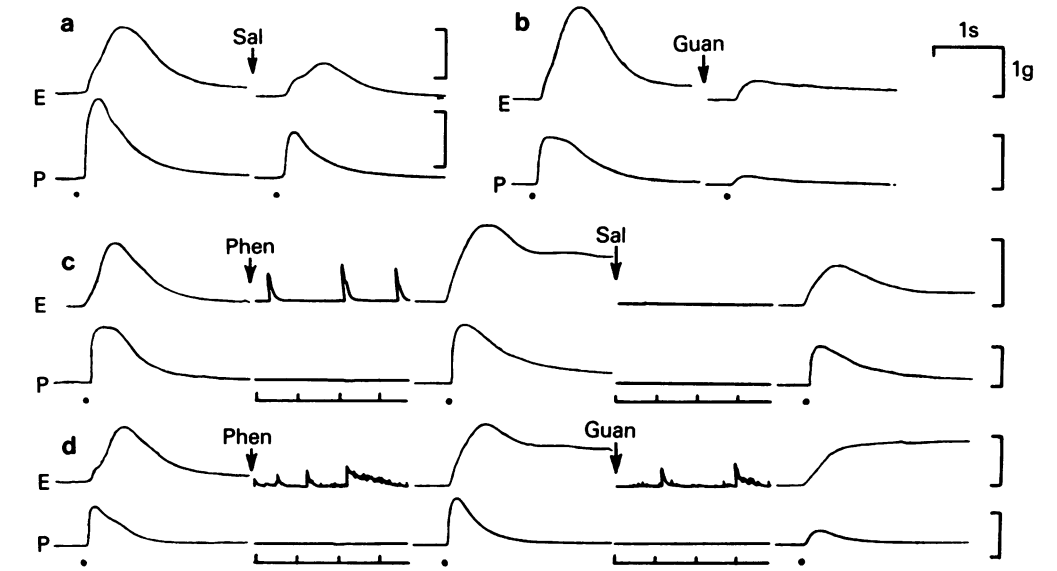

Figure 4 The effects of adrenoceptor agonists on the response of bisected portions of rat vas deferens to a single stimulus (0.5 ms pulse width, supramaximal voltage). Each panel represents a different tissue. Within each panel (a–d) the upper trace represents the isometric tension (g) of the epididymal portion (E) and the lower trace that of the corresponding prostatic portion (P). All traces were recorded at the speed indicated at the top right hand corner except for the periods following administration of drugs in (c) and (d) which were recorded at a slower speed indicated by the 1 min calibrations below the lower traces. Recorder sensitivity varies as indicated by vertical calibrations (all 1 g). Drugs were administered between traces as indicated by arrows. Electrical stimuli were applied at dots below traces and those after drugs represent the effect of the drug after equilibration, i.e. at least 10 min. (a) Salbutamol 10^{-5} M (Sal); (b) guanabenz 10^{-8} M (Guan); (c) phenylephrine (Phen) 10^{-6} M followed by the addition of salbutamol 10^{-5} M; (d) phenylephrine 10^{-6} M followed by the addition of guanabenz 10^{-8} M. Note that guanabenz does not abolish the contractions induced by phenylephrine.

After reserpine pretreatment of the rats (3 mg/kg i.p., 18 h), which removed the adrenergic component of the nerve-induced contraction, in the prostatic portion clonidine and phenylephrine produced, respectively, inhibition and potentiation similar to that in controls: in the epididymal portion both drugs potentiated the residual contraction (peak 250 ms) in both height and duration.

The inhibitory effects of the α-adrenoceptor agonists on the peak (250 ms) component of the prostatic response were alleviated by subsequent addition of yohimbine (6×10^{-6} M). This is shown quantitatively for guanabenz and clonidine in Table 2. In some experiments yohimbine (6×10^{-6} M) alone could reduce the control response and this should be taken into account when assessing the degree of restoration of the response by yohimbine.

A quantitative comparison between the agonists was also made (Figure 5): (a) The prostatic response at 250 ms was inhibited in a concentration-dependent manner by guanabenz, clonidine, oxymetazoline and xylazine. Xylazine was approximately ten times less potent than the others. Phenylephrine produced only potentiation of the nerve-induced response (Figure 5a). (b) The epididymal response at 650 ms was inhibited in a concentration-dependent manner by guanabenz, clonidine, oxymetazoline and xylazine at concentrations lower than the threshold for post-junctional effects (see (c) below). Above the latter threshold, clonidine and oxymetazoline returned responses towards or even beyond control levels, xylazine produced some potentiation relative to the lower doses but guanabenz produced a maintained inhibition throughout the concentration range tested. Phenylephrine produced only potentiation (Figure 5b). (c) Over the 'purely inhibitory' concentration range for each drug, the epididymal response at 1500 ms reflected the reduction found at 650 ms. However, with the exception of guanabenz and xylazine a larger increase in response was found with each agonist at higher concentrations. Even in the case of guanabenz and xylazine, increasing the concentration to 10^{-7} M or 10^{-6} M respectively, which produced only small increases in the responses at 650 ms, gave a significant increase in the response at 1500 ms compared with lower concentrations (Figure 5c).

With every agonist the potentiation at 1500 ms could be reduced by prazosin (6×10^{-6} M) or yohimbine (6×10^{-6} M) leaving only a small, monophasic response qualitatively similar to that which would have been produced after the antagonist alone (Brown *et al.*, 1979) e.g. epididymal response, 1500 ms, as a percent of control; after clonidine (10^{-7} M) 643 ± 80; after clonidine and yohimbine (6×10^{-6} M) 126 ± 21 ($n = 6$, paired t test, $P < 0.0001$); this indicates that some excitatory effect remains, i.e. inhibition is incomplete, since the corresponding value after yohimbine alone (due to the inhibition of the adrenergic component) is 54 ± 4 ($n = 6$).

β-Adrenoceptor agonists Isoprenaline produced a mixture of excitatory and inhibitory effects with net results similar in some respects to those produced by α-adrenoceptor agonists.

In the prostatic portion, isoprenaline inhibited the peak (250 ms) response in a concentration-dependent manner (Figures 5a, 6b and 7a) and this effect was antagonized by the β-adrenoceptor antagonist, sotalol (3×10^{-6} M) (Figure 7a). The concentration-inhibition relationship was shallower than in the case of the α-adrenoceptor agonists (Figure 5a).

In the epididymal portion, isoprenaline produced a concentration-dependent reduction in the nerve-induced response at the lower concentrations with increases in the response at high concentrations (Figure 6e and 7b). At concentrations higher than the threshold for potentiation, isoprenaline caused contraction of the epididymal end (Table 1). These excitatory effects of isoprenaline were absent in the presence of prazosin (3×10^{-7} M). In contrast to the prostatic

Table 2 The effect of yohimbine (6×10^{-6} M) on the inhibition of nerve-induced responses in the rat vas deferens by α-adrenoceptor agonists

Agonist	n	*Prostatic (250 ms) response % control response before yohimbine*	*after yohimbine*	*Significance of difference (paired t test)*
None	6	100	88 ± 6	$P > 0.05$
Guanabenz (10^{-7} M)	4	17 ± 4	75 ± 4	$P < 0.01$
Clonidine (10^{-7} M)	7	25 ± 6	69 ± 7	$P < 0.02$
Noradrenaline (3×10^{-5} M)	4	32 ± 5	—	—
Noradrenaline (3×10^{-5} M) plus WB 4101 (10^{-7} M)*	4	23 ± 3	57 ± 7	$P < 0.05$

* WB 4101 was added in order to antagonize the excitatory effect of noradrenaline and fully reveal the inhibitory effect.

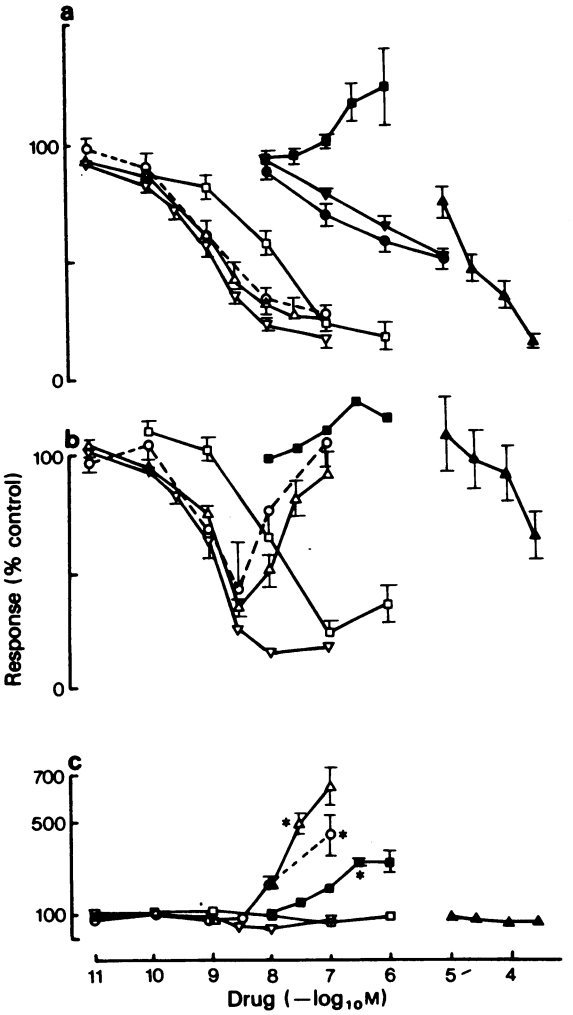

Figure 5 Concentration-response relationship for the effects of drugs on responses of portions of rat vas deferens to a single transmural stimulus (0.5 ms pulse width, supramaximal voltage). Responses are expressed as a % of pre-drug control values ($n \geq 6$). (a) Prostatic portion, measure 250 ms after stimulus; (b) epididymal portion, measured 650 ms after stimulus; (c) epididymal portion, measured 1500 ms after stimulus. Guanabenz (▽), clonidine (△), oxymetazoline (○, broken line), xylazine (□), phenylephrine (■), ATP (▲). Salbutamol (▼) and isoprenaline (●) are shown in (a) only: for epididymal effects see Figure 7. Asterisks in (c) indicate the first concentration at which each drug produces any detectable contraction in over 50% of tissues. Vertical bars represent s.e.

portion, the inhibition of the dominant (650 ms) response in the epididymal end by isoprenaline was not prevented by sotalol (3×10^{-6} M) (Figure 7b).

After reserpine pretreatment of the rats (3 mg/kg, i.p., 18 h), isoprenaline inhibited the prostatic response in a manner similar to that in normal rats and potentiated the small monophasic response remaining in the epididymal end.

Salbutamol, a β_2-adrenoceptor agonist, produced inhibition without the complication of the excitatory effects produced by isoprenaline (Figures 5a, 6a, 6d, 7a and 7b). The inhibitory effects on the prostatic end were considerably attenuated in the presence of sotalol (3×10^{-6} M) (Figure 7a) but in the epididymal end, sotalol significantly antagonized the inhibitory effect only of salbutamol 10^{-7} M (Figure 7b). In vasa from reserpine-treated rats, salbutamol still inhibited the prostatic and epididymal responses.

Phasic contractions induced by phenylephrine (10^{-6} M) were inhibited by salbutamol (10^{-5} M) and restored by sotalol (3×10^{-6} M). This occurred in both portions of the vas and is shown for the epididymal end in Figure 4.

Noradrenaline Noradrenaline produced a mixture of inhibitory and excitatory effects (Figure 6c and f). WB 4101 (10^{-7} M) prevented the post-junctional excitatory effect, and revealed the full inhibitory effect of noradrenaline (Table 2). In the prostatic portion addition of yohimbine (6×10^{-6} M) following WB 4101 (10^{-7} M) brought the response back towards control levels (Table 2). Noradrenaline also induced contractions in both portions of the vas, the threshold being at the same concentration as the threshold for increase of the nerve-induced responses i.e., 3×10^{-6} M (Table 1). WB 4101 (10^{-7} M) inhibited the contractions.

Other drugs

Morphine and enkephalin analogues Morphine did not produce significant inhibition of the nerve-induced response nor did it contract either portion of the rat vas deferens ($\leqslant 10^{-5}$ M) (Table 1). However, in the mouse vas which has a distribution of adrenergic and 'non-adrenergic' responses along the tissue similar to that in the rat (McGrath 1978), morphine or met-enkephalin produced concentration-dependent inhibition which was relatively more marked against the adrenergic component in the epididymal portion (Figure 8). This effect could be reversed by naloxone (10^{-7} M) (Figure 8).

The enkephalin analogue D-Ala^2, D-Leu^5-enkephalin produced concentration-dependent inhibition of responses in both portions of the rat vas deferens (Figures 9a and b, Table 1). In each portion this inhibition could be partly reversed by naloxone but this reversal was more complete for the adrenergic component (Figure 9c and d). D-Ala^2, D-Leu^5-enkephalin (10^{-5} M) did not affect the contractile effects of

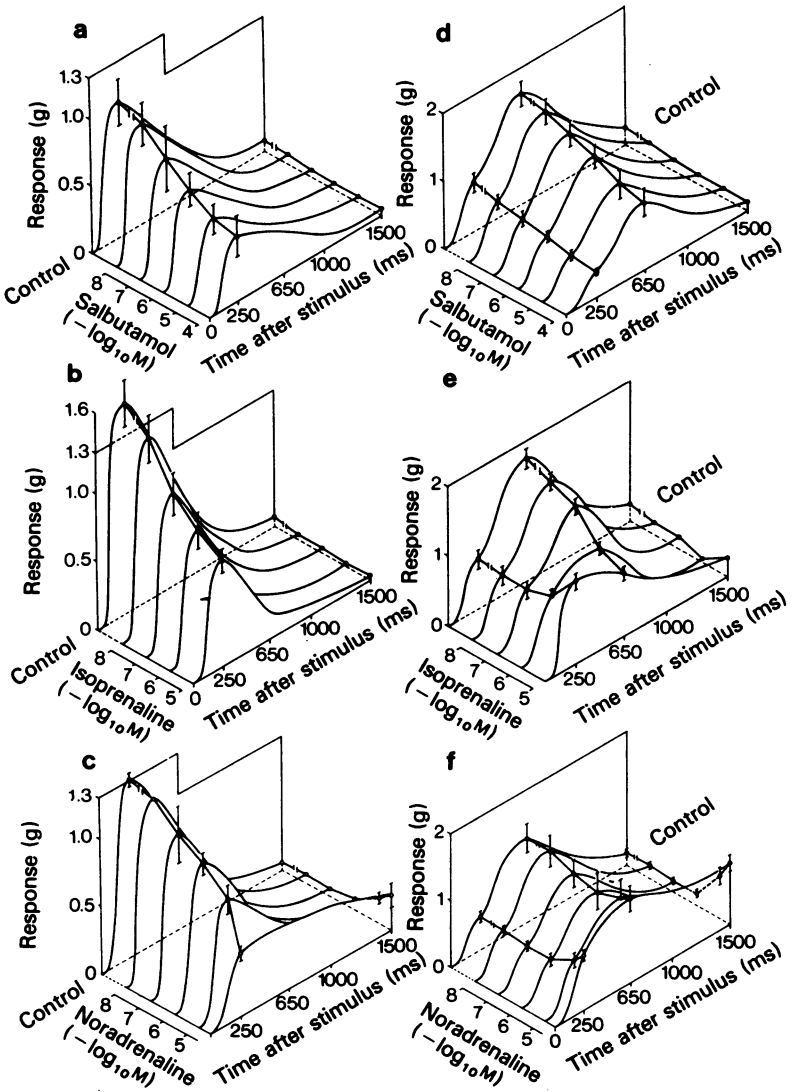

Figure 6 The effects of salbutamol (a and d), isoprenaline (b and e) and noradrenaline (c and f) on responses of prostatic (a–c) and epididymal (d–f) portions of the rat vas deferens to a single transmural impulse (0.5 ms pulse width, supramaximal voltage) ($n \geq 5$). Each graph shows the relationship between the isometric response (expressed as g tension) and the time after the stimulus for increasing concentrations of drug. For clarity s.e. bars are shown only for points at 250 and 1500 ms (a–f) and 650 ms (d–f) after each stimulus.

phenylephrine (10^{-6} M). Naloxone (10^{-7} M) on its own did not affect the tone of the preparations nor alter the nerve-induced responses and did not reverse the inhibitory effects of xylazine (10^{-7} M), salbutamol (10^{-5} M) or ATP (10^{-4} M).

In four separate experiments the contraction of rat anococcygeus to a single field stimulus was not detectably affected by D-Ala^2, D-Leu^5-enkephalin (10^{-8} M to 10^{-5} M).

Another pentapeptide, Tyr-D-Ala-Gly-MePhe-Met(O)-ol (Sandoz FK 33-824) is a potent 'morphinomimetic' drug in several test systems (Roemer, Buescher, Hill, Pless, Bauer, Cardinaux, Closse, Hauser & Huguenin, 1977). FK 33-824 produced concentration-dependent inhibition of nerve-induced responses in both portions of the rat vas and was approximately ten times more potent than D-Ala^2, D-Leu^5-enkephalin (Figure 9a and b, Table 1). The

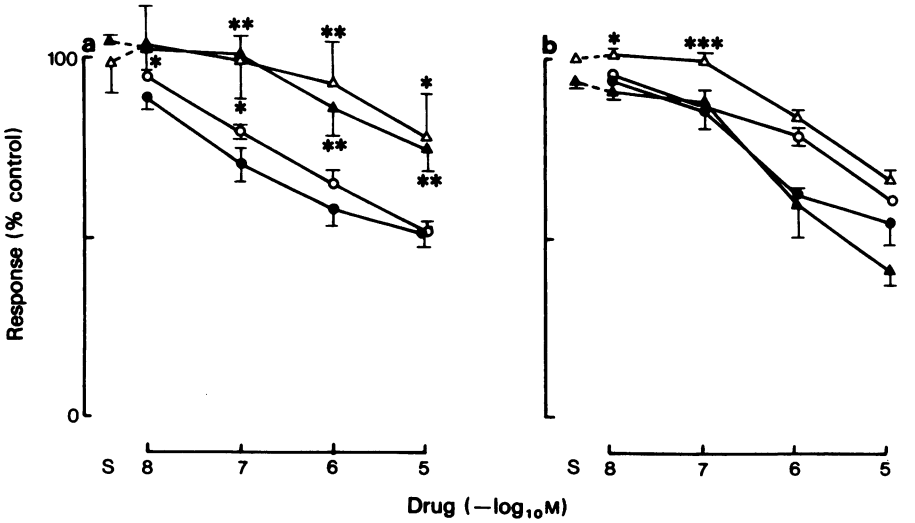

Figure 7 The effects of salbutamol and isoprenaline on responses of portions of rat vas deferens to a single transmural impulse (0.5 ms pulse width, supramaximal voltage). Responses are expressed as a % of pre-drug control values, (a) prostatic portion, measured at 250 ms after stimulus; (b) epidymal portions, measured at 650 ms after stimulus. In (a) and (b), no antagonist present (○); in the presence of sotalol (3×10^{-6} M) (△); salbutamol, open symbols; isoprenaline, filled symbols. Fresh tissues were used for the effect of sotalol; S—indicates the effect of sotalol alone ($n \geqslant 6$). Asterisks denote the significance of the difference between the effects of each concentration of agonist with or without sotalol assessed by Student's t test; *$0.05 > P > 0.01$; **$0.01 > P > 0.001$; ***$P < 0.001$. Vertical bars represent s.e.

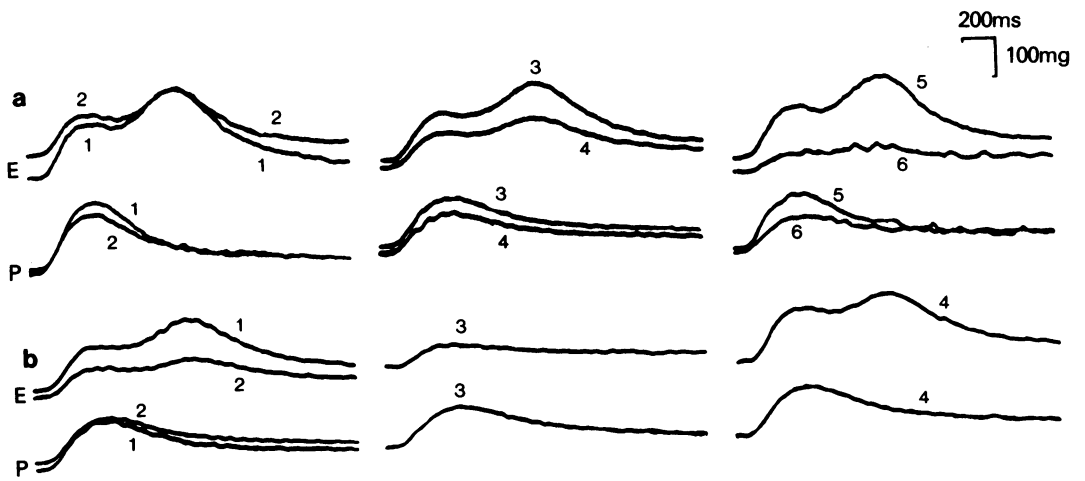

Figure 8 Effects of met-enkephalin or morphine on the responses of isolated portions of mouse (Parkes strain) bisected vas deferens to single supramaximal stimuli (0.3 ms). Epididymal portion (E), prostatic portion (P). (a) Control (1); in the presence of met-enkephalin 10^{-9} M (2); control following wash-out (3); met-enkephalin 10^{-8} M (4); control following wash-out (5); met-enkephalin 10^{-7} M (6). (b) Following on from (a), control following wash-out (1); in the presence of morphine 10^{-7} M (2); morphine 10^{-6} M (3); in the additional presence of naloxone 10^{-7} M (4). Responses following met-enkephalin in (a) were obtained after 5 to 10 min equilibration and represent the optimal effect of these concentrations.

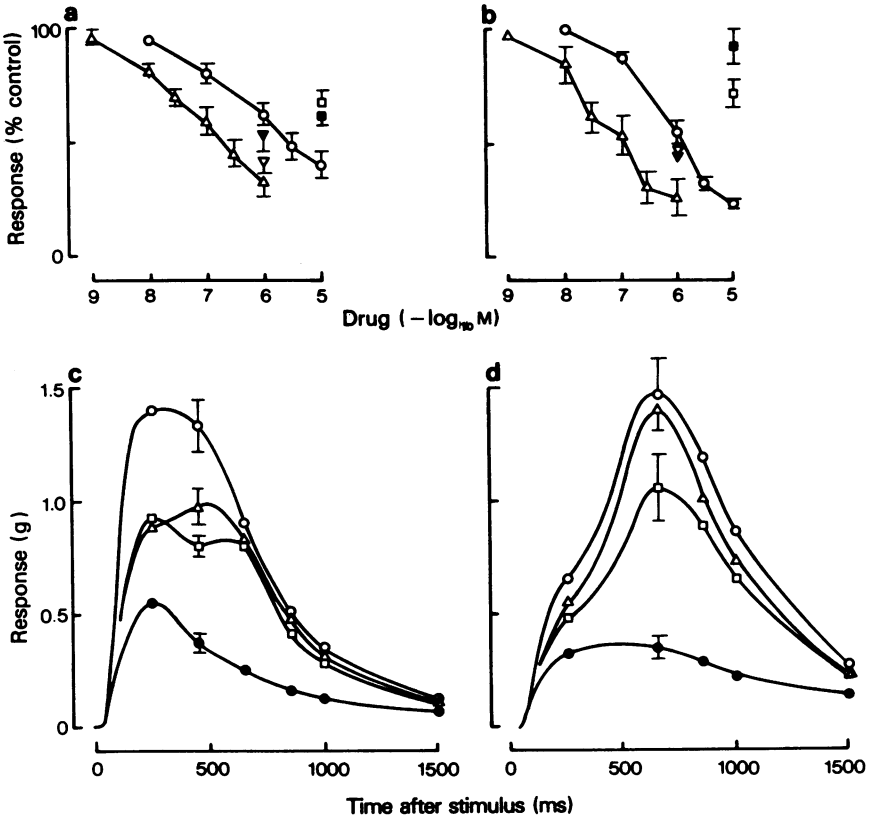

Figure 9 The effects of opioid peptides on the response of bisected portions of rat vas deferens to a single stimulus (0.5 ms pulse width, supramaximal voltage). (a) and (b) show the effects of increasing concentrations of FK 33-824 (△) or D-Ala^2, D-Leu^2-enkephalin (○) on the response of the prostatic portion measured 250 ms after stimulation (a) or of the epididymal portion measured 650 ms after stimulation (b): each expressed as a % of pre-drug control values. The effect of the addition of naloxone to the final concentration of each agonist; FK 33-824, + naloxone 10^{-7} M (▽), + naloxone 10^{-6} M (▼); D-Ala^2, D-Leu^2-enkephalin, + naloxone 10^{-7} M (□), naloxone 10^{-6} M (■); ($n \geq 6$). (c) and (d) show the reversal by naloxone of the effects of D-Ala^2, D-Leu^5-enkephalin on the time course of the response (g tension) in the prostatic (c) or epididymal (d) portions. Control (○), D-Ala^2, D-Leu^5-enkephalin 10^{-5} M (●), + naloxone 10^{-7} M (□), + naloxone 10^{-6} M (△). (n = 6). Vertical bars representing s.e. are shown only at 450 ms (c) or 650 ms (d).

effect of FK 33-824 (10^{-6} M) was significantly ($n = 8$, paired t test test, $P < 0.05$) but incompletely reversed by naloxone (10^{-6} M) in each portion (Figure 9a and b) but, in contrast to D-Ala^2, D-Leu^5-enkephalin, complete recovery of the epididymal response could not be attained even by increasing the concentration of naloxone to 10^{-5} M.

ATP ATP produced concentration-dependent, reversible, inhibition of responses in either end of the vas (Figure 5) without producing any detectable potentiation. A short lived (< 10 s), small (< 50 mg) contraction was sometimes found at ATP 10^{-4} M (epididymal end, 3 out of 6; prostatic end, 1 out of 6). Inhibition of the prostatic (250 ms) response was statistically significant ($n = 6$, paired t test, $P < 0.05$) with a concentration of ATP of 10^{-5} M and above but inhibition of the epididymal response was not significant until 3×10^{-4} M. On a percentage basis, the inhibition was significantly greater on the prostatic than on the epididymal portion at each concentration of ATP tested. The inhibitory effect of ATP was not affected by yohimbine (6×10^{-6} M), sotalol (3×10^{-6} M) or naloxone (10^{-7} M) and could be obtained after the adrenergic component had been removed by prazosin (6×10^{-6} M) or WB 4101 (10^{-7} M). This effect, therefore, appears to involve a different mechanism from

the α-adrenoceptor agonists and is exerted more effectively against the 'non-adrenergic' response.

Discussion

The effects of each of a wide variety of drugs differed when tested on the two halves of the bisected vas deferens of the rat. This should be considered whenever this organ is used to study the physiology of sympathetic nerves or the pharmacology of drugs which act on sympathetic nerves or smooth muscle.

By the use of single stimulus pulses, endogenous feedback mechanisms were avoided and, consequently, the effects of agonist drugs against neurotransmission could be interpreted more simply than when trains of pulses were employed (cf. Brown *et al.*, 1979).

α-Adrenoceptor agonists exhibited particularly distinct differences between the two portions. Reserpine-treatment indicated that excitatory and inhibitory actions were independent of functioning adrenergic nerves. Excitatory effects could be detected, as increases in height and duration of nerve-induced responses, at concentrations of agonists lower than those resulting in contraction of the tissue. This has been shown previously with a variety of smooth muscle stimulants in guinea-pig vas deferens (Sjostrand & Swedin, 1968; Sjostrand, 1973) and illustrates that, even if the more sensitive epididymal portion is taken, the contractile effects will underestimate the post-junctional excitatory effects of agonists in this tissue. The potentiation of the nerve-induced response in the epididymal portion did, however, provide a reproducible index of the excitatory effects of agonists at equilibrium, which would appear to be more satisfactory than measurement of the accompanying complex contractile effects (Waddell, 1916; Macht, 1917; MacDonald & McGrath, 1980). The inhibitory action of α-adrenoceptor agonists was exerted against each of the components of the nerve-induced response. This raises the possibility that both 'adrenergic' and 'non-adrenergic' transmission sites in this tissue may possess inhibitory α-adrenoceptors. From the point of view of assaying agonist potency at such receptors, however, the 'non-adrenergic' response of the prostatic portion gave a more reliable comparison between agents since the excitatory effects in the epididymal portion produced 'physiological antagonism' of the inhibitory action. The excitatory and inhibitory effects could thus be more clearly seen in the epididymal and prostatic portions, respectively.

The orders of potency for pre- and post-junctional effects of α-adrenoceptor agonists corresponded to those found in other tissues or in other preparations of vas deferens (Starke, Endo & Taube, 1975; Drew, 1977; Brown *et al.*, 1979). The excitatory, post-junctional effects and their sensitivity to prazosin or WB 4101 (Cambridge, Davey & Massingham, 1977; Kapur & Mottram, 1978; Butler & Jenkinson, 1978) indicate an α_1-adrenoceptor as defined by Langer (1974); this contrasts with the rat blood pressure and anococcygeus in which a prazosin-resistant but yohimbine-sensitive response can be found (Docherty *et al.*, 1979; Drew & Whiting, 1979; Docherty & McGrath, 1980). Similarly, the pre-junctional, inhibitory effects and their sensitivity to yohimbine indicate an α_2-adrenoceptor (see note on terminology of adrenoceptors, Starke & Langer, 1979). This preparation may, therefore, be more suitable than some others, e.g. anococcygeus, rat blood pressure, for the initial screening for effects on such receptors. When examining α-adrenoceptor antagonist properties it would seem that, of the drugs tested, phenylephrine was the most suitable α_1-adrenoceptor agonist and xylazine or guanabenz the most selective α_2-agonists since clonidine or oxymetazoline could affect each receptor over a narrow range of concentrations. No qualitative differences between the post-junctional effects of prazosin and WB 4101 were noted.

The effects of the β-adrenoceptor agonists, isoprenaline and salbutamol, and their reversal by sotalol in the prostatic portion show the presence of inhibitory β-adrenoceptors in the rat vas deferens. However, in the case of isoprenaline this effect was complicated by an α-adrenoceptor agonist action. This confirms the previous reports of inhibitory β-adrenoceptors in guinea-pig, rat and mouse vasa (Large, 1965; Ganguly & Bhattacharya, 1970; Hedqvist & Euler, 1976; Jenkins, Marshall & Nasymth, 1977; MacDonald & McGrath, 1980). The failure of sotalol to antagonize satisfactorily the inhibitory effects of isoprenaline or salbutamol on the nerve-mediated response of the epididymal portion raises the possibility of an additional inhibitory effect of these drugs. However, the inhibitory effect of salbutamol on the phenylephrine-induced contractions (and its removal by sotalol) confirms the presence of post-junctional inhibitory β_2-adrenoceptors in both the prostatic and epididymal ends of the vas.

Noradreanline could display both the α_1 and α_2-agonism found with the synthetic drugs and clearly the concentration range for the two actions overlapped. Two differences from the other agonists stood out which deserve further investigation; (1) noradrenaline showed potentiation of nerve-induced responses at the same concentration as the threshold for contraction and (2) excitatory effects appeared in the prostatic and epididymal ends at the same concentration, although the effects in the epididymal end were more pronounced. Extrapolation from the effects of synthetic agonists to the physiological effects of noradrenaline should, thus, proceed with care.

The results with 'opioid' agonists indicate that each type of nerve-induced contraction in rat and mouse

vas deferens can be reduced by drugs with affinity for δ- or μ-opioid receptors (Kosterlitz, 1978). However, the precise effects of opioids at the two types of neurotransmission site in this organ were different. The ineffectiveness of morphine on rat vas deferens reflects its relatively low effectiveness at the receptors on which FK 33-824 acts, e.g. 10^3 times less potent (Roemer *et al.*, 1977). Part of the depressant effect of FK 33-824 is likely to be non-specific due to the high concentration necessary for its effect compared with that in other tissues (Roemer *et al.*, 1977; Kosterlitz, Lord, Paterson & Waterfield, 1980). The clearest and least equivocal effect of opioids on rat vas was the naloxone-sensitive inhibition by D-Ala^2, D-Leu^5-enkephalin of the adrenergic component of the response to nerve stimulation. This confirms that 'enkephalin-receptors' are present at adrenergic nerve terminals in this organ.

The absence of an effect of D-Ala^2, D-Leu^5-enkephalin on the adrenergic nerve-mediated response of rat anococcygeus indicates a tissue as well as species variation in the distribution of opioid receptors at the adrenergic junction. This is further reinforced by the observation that adrenergic nerve-induced contractions of cat anococcygeus (Gillespie & McGrath, 1974a) are inhibited by morphine or by Met- or Leu-enkephalin to approximately the same extent as those in mouse vas deferens, whereas rat anococcygeus is resistant to each (Tilmisany, 1976; M.G.C. Gillan & J.C. McGrath, unpublished observations).

Of the four types of agonist tested which produced inhibition of nerve-induced responses without the complicating factor of excitation, i.e. ATP, guanabenz/xylazine, salbutamol and enkephalin analogues, ATP was the only one which produced a significantly greater percentage inhibition on one portion of the tissue compared with the other. Since the inhibitory effect of ATP was greatest against the 'non-adrenergic' response this is further evidence for the independence of this component from adrenergic transmission. This may help to explain the observation of Clanachan *et al.* (1977) that when the isolated central portion of rat vas deferens was field stimulated at 5 Hz for 30 s, the initial 'twitch' phase (partly 'non-adrenergic'*) of the contraction was slightly but significantly reduced by ATP (3×10^{-6} M) whereas the sustained contraction (mainly adrenergic*) and the overflow of [^{3}H]-noradrenaline was only partly reduced by ATP (10^{-4} M) (*according to the interpretation of Brown *et al.*, 1979). The contractile effects of high concentrations of ATP which were noted by Clanachan *et al.* (1977) were hardly detectable in this study but this may be partly due to tachphylaxis which is commonly found with the cumulative addition of ATP.

While the bisected vas can provide a sensitive assay for prejunctional inhibitory effects, the physiological systems being affected are not clearly delineated. In particular, the results from the prostatic portion cannot be directly compared with the effects of the same drugs on the overflow of transmitter noradrenaline induced by trains of stimuli, since there is no evidence to connect the main part of the prostatic response with adrenergic transmission. This has not so far produced any major confusion since the effects of several drugs on the two detection systems have been similar. (Indeed this might support the concept that only one type of transmission is present). However, the present results with ATP and with opioid receptor agonists indicate differences in the effects exerted against the two types of transmission.

It is concluded that bisection of the rat vas deferens can improve its usefulness in pharmacological investigation since a wide variety of agonist drugs produce different effects, both qualitatively and quantitatively, in the two portions.

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