Br. J. Pharmac. (1979), **66**, 553–564

THE EFFECTS OF α-ADRENOCEPTOR AGONISTS AND ANTAGONISTS ON RESPONSES OF TRANSMURALLY STIMULATED PROSTATIC AND EPIDIDYMAL PORTIONS OF THE ISOLATED VAS DEFERENS OF THE RAT

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1 The effects of α-adrenoceptor agonists and antagonists on contractile responses of transmurally stimulated prostatic and epididymal portions of the rat isolated vas deferens were examined.

2 Responses to single stimuli consisted of two phases, the first predominant in the prostatic and the second in the epididymal portion. The first phase was resistant to α-adrenoceptor antagonists but the second was reduced in a dose-related manner in the order of potency prazosin > azapetine > phentolamine > labetalol > yohimbine.

3 Both phases of the response to a single stimulus were reduced by clonidine but only the first could be reliably restored by yohimbine.

4 Trains of transmural stimuli produced biphasic responses, an early rapid component predominant in the prostatic and a slower secondary component predominant in the epididymal portion. The effects of α-adrenoceptor antagonists on these responses were complex. Prazosin produced the most straightforward inhibition of responses with relative resistance of the early rapid component. Only yohimbine and phentolamine produced increases in responses which could be pre-junctional in origin.

5 The α-adrenoceptor agonists, oxymetazoline and clonidine, reduced while phenylephrine increased responses to trains of stimuli.

6 These results are discussed in relation to the nature of the innervation of rat vas deferens and the usefulness of the preparation in pharmacological tests for activity at α-adrenoceptors.

Introduction

There is now considerable evidence to support the hypothesis that noradrenaline released from noradrenergic nerves regulates its own release by a negative feedback mechanism involving inhibitory presynaptic α-adrenoceptors. The principal evidence comes from the observation that in noradrenergically innervated tissues, the overflow of noradrenaline (NA) on nerve stimulation, taken to be an index of transmitter release, can be reduced by α-adrenoceptor agonists (Starke, 1972a, b; Langer, Enero, Adler-Graschinsky & Stefano, 1972) and increased by α-adrenoceptor antagonists (Brown & Gillespie, 1957; Kirpekar & Puig, 1971; Haggendal, Johansson, Jonason & Ljung, 1972; Dubocovich & Langer, 1974; Starke, Borowski & Endo, 1975). More recently it has been suggested that agonists (Starke, Endo & Taube, 1975; Drew, 1977) and antagonists (Starke *et al.*, 1975; Drew, 1976) display a degree of selectivity for pre- and postsynaptic α-adrenoceptors. As a consequence, antagonists which preferentially act on prejunctional α-adrenoceptors should, under certain circumstances, be capable of increasing the effector organ response to adrenergic nerve stimulation when used at concentrations where the post-junctional α-adrenoceptors are not significantly antagonized or where the post-junctional adrenoceptors are of a different type, as in the heart (Drew, 1976; Docherty & McGrath, 1977; Doxey, 1977; Langer, Adler-Graschinsky & Giorgi, 1977).

In the vas deferens it is well established that the height of the contractile response to nerve stimulation is resistant to reduction by α-adrenoceptor antagonists (Boyd, Chang & Rand, 1960; Ambache & Zar, 1971) at concentrations which increase the nerve-induced output of NA (Stjarne, 1973; Vizi, Somogyi, Hadhazy & Knoll, 1973). In order to explain this it has been suggested that the effector response is adrenergic but resistant to post-junctional blockade (Swedin, 1971; Furness, 1974) or alternatively due to a separate set of 'non-adrenergic' nerves (Ambache

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0007-1188/79/080553-12 $01.00

& Zar, 1971). Since under certain experimental conditions this response can be *increased* by α-adrenoceptor antagonists, it has been further postulated that the NA liberated by the adrenergic nerves might act pre-junctionally on the 'non-adrenergic' nerves to limit the release of the (unknown) transmitter (Ambache, Dunk Verney & Zar, 1972).

However, since nerve stimulation also produces a subsequent contractile component which *is* susceptible to post-junctional α-adrenoceptor blockade (Swedin, 1971; Anton, Duncan & McGrath, 1977), the effects of α-adrenoceptor antagonists would be expected to be complex.

In this study we have employed a range of α-adrenoceptor agonists and antagonists with varying selectivity for pre- and post-junctional receptors (Starke, 1977; Drew, 1977; Doxey, Smith & Walker, 1977; Blakeley & Summers, 1977; Cambridge, Davey & Massingham, 1977) and a bisected preparation of rat vas deferens which enables partial separation of the adrenergic and 'non-adrenergic' responses which predominate at different ends of the organ (Anton *et al.*, 1977; McGrath, 1977; 1978).

The effects of the drugs were tested against responses to single pulses, a procedure which allows further separation of the two components and avoids the possible interference from feedback mechanisms (McGrath, 1977; 1978), and against trains of pulses at frequencies of 2 and 10 Hz which, according to studies on noradrenaline overflow, should permit presynaptic α-adrenoceptor feedback to occur.

Methods

Vasa deferentia were removed from Wistar rats (200 to 250 g) which had been stunned and killed by exsanguination. Each vas deferens was divided into two portions of equal length and mounted in a jacketed 30 ml organ bath in Krebs-bicarbonate solution at 37°C. The composition of the Krebs-bicarbonate was (mM): $NaHCO_3$ 25, NaCl 119, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.0 and glucose 11.1, gassed with 95% O_2 and 5% CO_2. Threads were sewn through the wall of each end of each portion of vas deferens. One end was attached to an isometric tension transducer (Type, Grass FTO3) and the other to a hook at the base of the organ bath. The resting tension was 0.5 to 1 g. Stimulation was by a ring and hook electrode (see Anton *et al.*, 1977) with pulses of 0.3 or 0.5 ms duration and supramaximal voltage. Responses to (a) single pulses or (b) trains of pulses lasting 16 s at 2 and 10 Hz were recorded on a Devices M2 recorder, Grass Model 7 Polygraph or Tektronix D13 Storage oscilloscope. For details of experimental procedure see Anton *et al.* (1977) and McGrath (1978). Responses to single stimuli were separated by at least 5 min and no trains were delivered (McGrath, 1978). The maximum tension and that at 50, 150, 250, 450, 650 and 850 ms after each pulse was measured and the time course of the response replotted as a percentage of the maximum as shown in Figure 1. Responses to trains of stimuli were measured at two points: (1) the peak of the early rapid phase which reaches a maximum between 1 and 2 s; (2) the peak of the slower 'secondary' response which reaches its maximum between 3 and 15 s. Since many of the drugs modified the time course of the response so that these peaks moved in time or even merged (see Figure 6) the maximum responses between the times given in (1) and (2) were taken when discrete maxima were absent.

Drugs were dissolved in 0.9% w/v NaCl solution (saline) and added to the organ bath in a maximum volume of 0.3 ml and allowed at least 15 min to equilibrate before responses were recorded. Subsequently the bath was washed with fresh Krebs solution and a higher concentration of drug added. In this way dose-response curves for each preparation were recorded. The significance of differences between points along the dose-response curves were estimated by the paired *t* test.

Drugs

The following drugs were used: azapetine phosphate (Ilidar, Roche); clonidine hydrochloride (Catapres, Boehringer Ingelheim); labetalol hydrochloride (Allen & Hanburys); oxymetazoline hydrochloride (Merck); phentolamine mesylate (CIBA); phenylephrine hydrochloride (Sigma); prazosin hydrochloride (Pfizer); yohimbine hydrochloride (Baird Pharmaceuticals).

Results

Transmural stimulation of isolated segments of rat vas deferens with single pulses and trains of impulses at 2 and 10 Hz produced biphasic responses. The biphasic response to a train of stimuli is anatomically based since, on bisection of the vas deferens, the prostatic and epididymal portions produce responses in which either the initial phase or the subsequent phase predominated (Anton *et al.*, 1977).

The effects of α-adrenoceptor antagonists on responses of epididymal and prostatic portions of the vas deferens to a single transmural impulse

Single impulse stimulation of the epididymal portion of the vas deferens produces a response consisting of a small initial fast component (250 ms peak) on which is superimposed a larger but slower (650 ms peak) second component whereas in the prostatic por-

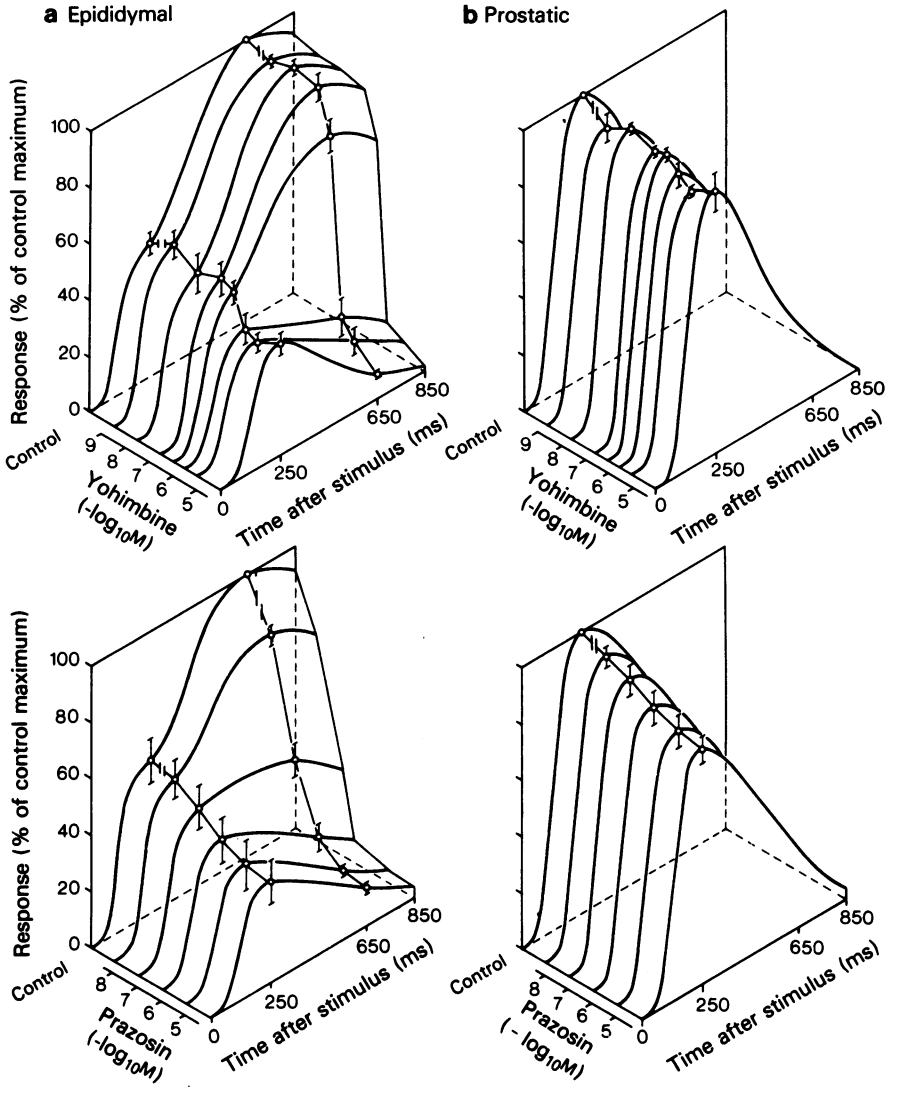

Figure 1 The effects of yohimbine and prazosin on responses of epididymal (a) and prostatic (b) portions of the vas deferens to a single transmural impulse (0.3 ms pulse width, supramaximal voltage) ($n \geq 6$). Each graph shows the relationship between response (expressed as a % of the maximum response in the control) and time after the stimulus for concentrations of yohimbine between 10^{-9} and 6×10^{-5} M (top panels) and prazosin between 6×10^{-9} and 6×10^{-5} M (bottom panels). For clarity standard error bars are shown only for points at 250 ms (a and b) and 650 ms (a) after each stimulus.

tion of the vas deferens the response consists principally of a single fast (250 ms) component (McGrath, 1977; 1978).

Since both segments of the rat vas deferens respond to a single impulse given at 5 min intervals there is in this situation little likelihood of presynaptic α-adrenoceptor feedback inhibition. Under these conditions each α-adrenoceptor antagonist should block the second part of the response in the epididymal portion of the vas deferens (McGrath, 1978). Figure 1 shows that precisely this effect was obtained with both yohimbine and prazosin; both drugs significantly reduced ($P < 0.05$) the second component in the epididymal portion at concentrations of yohimbine $\geq 3 \times 10^{-7}$ M, prazosin $\geq 6 \times 10^{-9}$ M but had no significant effect on the responses of the prostatic portions of the vas deferens or on the first part of the response in the epididymal portion.

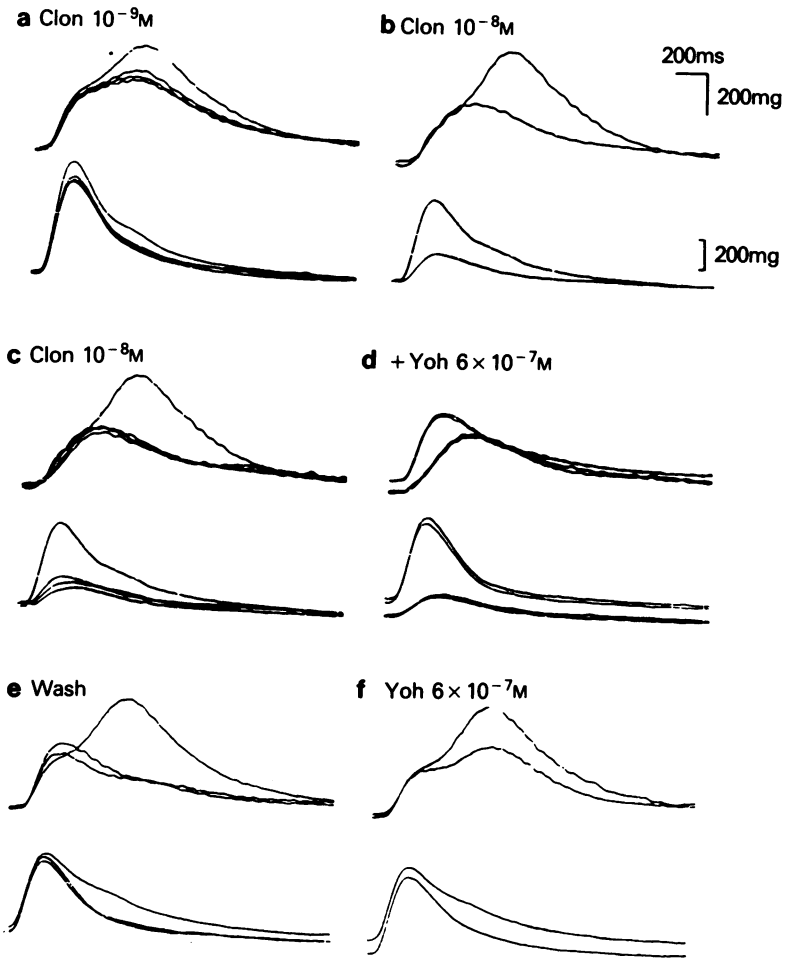

Figure 2 Effects of clonidine and yohimbine on the response of bisected portions of vas deferens to a single stimulus (0.3 ms pulse width, supramaximal voltage). Panels (a–f) are consecutive; in each panel the upper oscilloscope traces represent the responses of the epididymal portion and the lower those of the prostatic portion: (a) from top to bottom: control then inhibition by clonidine (Clon 10^{-9} M at 5, 10 and 15 min). (b) from top to bottom: control 20 min after wash; then inhibition by clonidine 10^{-8} M at 5 min. (c) as (b) plus (from top to bottom) clonidine 10^{-8} M at 10 and 15 min. (d) lower coincident taces, clonidine 10^{-8} M at 20 and 25 min; upper coincident traces 5 and 10 min after addition of yohimbine (Yoh 6×10^{-7} M) showing reversal of inhibition. (e) from bottom to top; 2 traces after 15 and 20 min in the presence of yohimbine (6×10^{-7} M) and clonidine then (upper trace) 20 min after wash. (f) upper trace control; lower trace 10 min after yohimbine 6×10^{-7} M.

An examination of the effects of a series of α-adrenoceptor antagonists was made, the threshold concentration which inhibited the second part of the response in the epididymal portion being used as an index of postsynaptic α-blockade. The threshold doses were, in order of potency, prazosin (6×10^{-9} M), azapetine (10^{-8} M), phentolamine (10^{-7} M), labetalol (3×10^{-7} M), and yohimbine (3×10^{-7} M). An additional effect which was observed with azapetine ($>10^{-6}$ M) (McGrath, 1978), labetalol ($>10^{-7}$ M) and which can be seen to a lesser extent with yohimbine ($>10^{-5}$ M) (Figure 1) was a potentiation of the nerve-induced response which could be postsynaptic in origin (McGrath, 1978). At concentrations which produced less than total block of the adrenergic component, none of these drugs prolonged the adrenergic response which would have indicated blockade of the neuronal re-uptake of NA (McGrath, 1977; 1978). Uptake blockade can therefore be eliminated as a contributory factor in potentiation of responses in the subsequent experiments using trains of pulses.

Interaction of clonidine and yohimbine in prostatic and epididymal segments of the vas deferens stimulated by single transmural nerve impulses

The preferential presynaptic α-agonist clonidine (10^{-9} to 10^{-8} M) produced an inhibition of the response of the epididymal portion of the vas deferens which was most marked on the second component (Figure 2a, b). This effect was partly reversed by the addition of yohimbine (6×10^{-7} M) (Figure 2d). After washout the secondary component returned (Figure 2e) but could be reduced by addition of yohimbine (6×10^{-7} M) (Figure 2f). Clearly there are two components involved in the response to yohimbine; a presynaptic component which is responsible for the reversal of the inhibition by clonidine and a postsynaptic component involving a degree of postsynaptic α-adrenoceptor blockade.

In the prostatic portion of the vas deferens the response was reduced about 10% by clonidine (10^{-9} M) (Figure 2a) and 65% by clonidine (10^{-8} M) (Figure 2b) but unlike the epididymal portion this response could be completely restored by yohimbine (6×10^{-7} M) (Figure 2d). After washout (Figure 2e) yohimbine (6×10^{-7} M) removed the small second component which is present on the decreasing portion of the response (McGrath, 1978) but had no effect on the first component (Figure 2f).

The effects of α-adrenoceptor agonists and antagonists on responses of prostatic and epididymal segments of the vas deferens to transmural nerve stimulation at 2 and 10 Hz

(a) *Effects of α-adrenoceptor antagonists on the initial rapid phase of the response* The effects of five adrenoceptor antagonists were studied on transmurally evoked responses to nerve stimulation. The antagonists were chosen as a series varying in properties from preferential postsynaptic antagonists such as labetalol (Blakeley & Summers, 1977) and prazosin (Cambridge *et al.*, 1977) to a preferential presynaptic antagonist, yohimbine (Starke *et al.*, 1975).

Labetalol produced a biphasic effect on the initial phase of the response to transmural stimulation at both frequencies and in both segments of the vas deferens. Initially responses were potentiated (3×10^{-8} M) but higher concentrations produced a dose-dependent inhibition of the initial phase of the response (Figure 3a). Because of the biphasic nature of the response, inhibition of the initial phase was only significant ($P < 0.05$) compared with the control in the epididymal segment stimulated at 10 Hz. Prazosin produced significant ($P < 0.05$) inhibition of all initial phases of responses with no evidence of a biphasic effect (Figure 3a). Azapetine produced a biphasic effect on the concentration-response curve, significant ($P < 0.05$) inhibition occurring up to 3×10^{-7} M followed at 10^{-6} M by a return towards control levels (Figure 3a). Yohimbine potentiated the initial phase ($P < 0.05$), the effect being most marked at a stimulation frequency of 10 Hz. At concentrations above 10^{-7} M the response tended to reach a plateau or return towards control values (Figure 4a). Phentolamine also potentiated responses particularly at lower concentrations (3×10^{-8} M) and the pattern observed was similar to that seen with yohimbine. Twitches recorded following stimulation at 2 Hz were potentiated only at the lowest concentration whereas those recorded at 10 Hz were potentiated in both segments ($P < 0.05$) over the entire concentration range (3×10^{-8} M to 10^{-6} M) (Figure 4a).

(b) *Effects of α-adrenoceptor antagonists on the secondary phase of the response* The effects of labetalol, prazosin and azapetine on the secondary response were qualitatively similar to the effects on the twitch response, labetalol producing potentiation followed by inhibition, prazosin producing only inhibition and azapetine producing inhibition followed by a return towards control responses at higher concentrations (Figure 3b). Yohimbine produced potentiation of responses which were only significant with stimulation at 2 Hz in the prostatic portion whereas phentolamine had only an inhibitory effect on secondary responses to stimulation at 10 Hz in the prostatic portion in a concentration of 10^{-6} M (Figure 4b). Higher concentrations of phentolamine were not tested in this study but under identical conditions they reduce the secondary response to 5 or 20 Hz (Anton *et al.*, 1977).

(c) *Effects of α-adrenoceptor agonists on the initial rapid phase of the response* The effects of three adrenoceptor agonists on transmurally evoked responses to nerve stimulation were studied. Oxymetazoline and clonidine are believed to stimulate preferentially presynaptic and phenylephrine postsynaptic α-adrenoceptors (Starke *et al.*, 1975; Drew, 1977). Oxymetazoline and clonidine powerfully inhibited the initial phase of the response in the prostatic segment of the vas deferens at both frequencies of stimulation, the threshold concentration for inhibition being about 10^{-9} M. The initial phase in the epididymal segment was less susceptible to inhibition by either drug. Oxymetazoline produced significant inhibition only at the highest concentration (3×10^{-8} M) at 2 Hz. Phenylephrine on the other hand significantly ($P < 0.05$) potentiated twitch responses at both frequencies of stimulation. The potentiation was most marked in the epididymal segment of the vas deferens (Figure 5a).

(d) *Effects of α-adrenoceptor agonists on the secondary phase of the response* In the prostatic segment oxy-

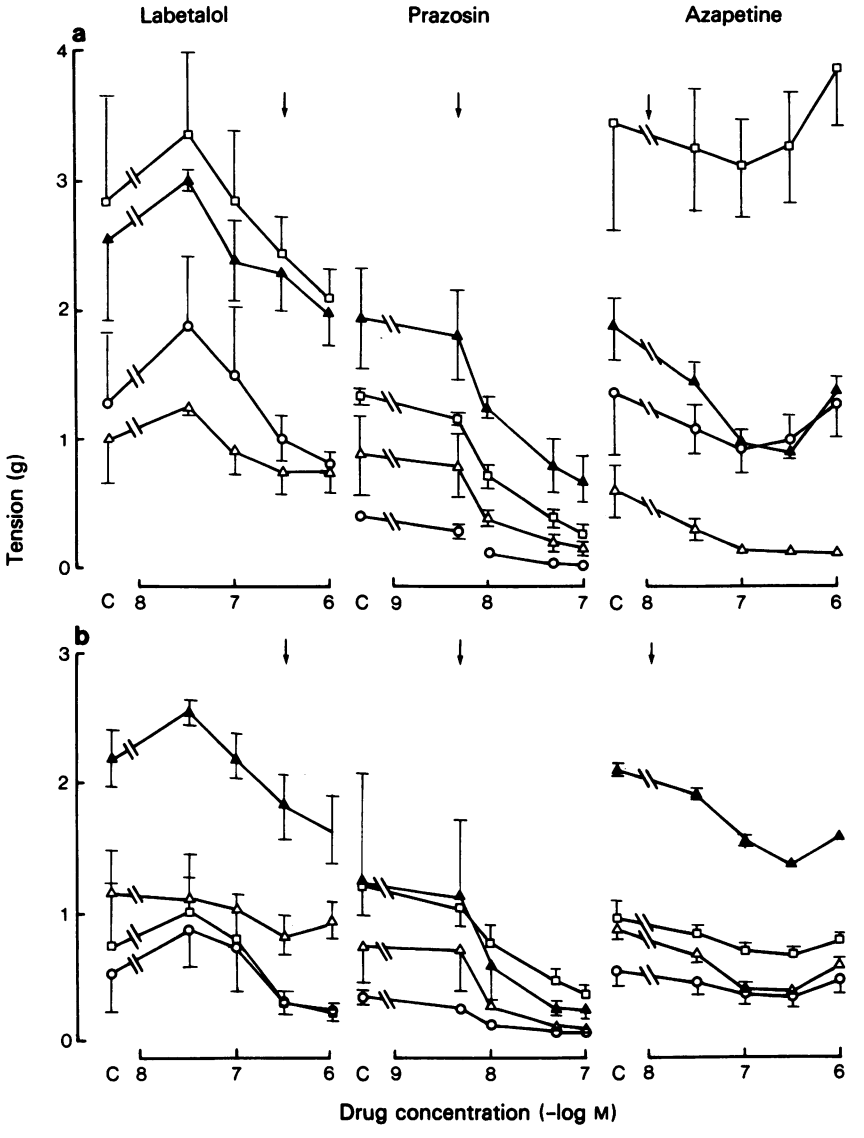

Figure 3 The effects of the α-adrenoceptor antagonists labetalol, prazosin and azapetine on responses of the epididymal and prostatic portions of the bisected vas deferens of the rat. Stimulus trains were delivered (0.3 ms pulse width, supramaximal voltage, 16 s duration) and responses measured during the initial rapid phase (a) or the subsequent secondary phase (b). Prostatic portion, 2 Hz (○), 10 Hz (□); epididymal portion 2 Hz (△), 10 Hz (▲). The arrows show the concentration of each drug at which inhibition occurred of the response to a single impulse. C = initial drug-free control. Vertical bars represent standard errors.

metazoline and clonidine produced significant inhibition of the secondary response at both frequencies of stimulation and at all but the lowest concentration. The secondary response to 2 Hz in the epididymal segment was not significantly affected by clonidine or by oxymetazoline. At the higher frequency of stimulation oxymetazoline produced a biphasic effect, enhancement at lower concentrations followed by inhibition at higher concentrations ($>3 \times 10^{-9}$ M, Figure 5b). Phenylephrine produced a dose-dependent increase in the secondary response with the effect being most marked on stimulation at 2 Hz. The potentiation reached a plateau with the highest concentration used (10^{-6} M).

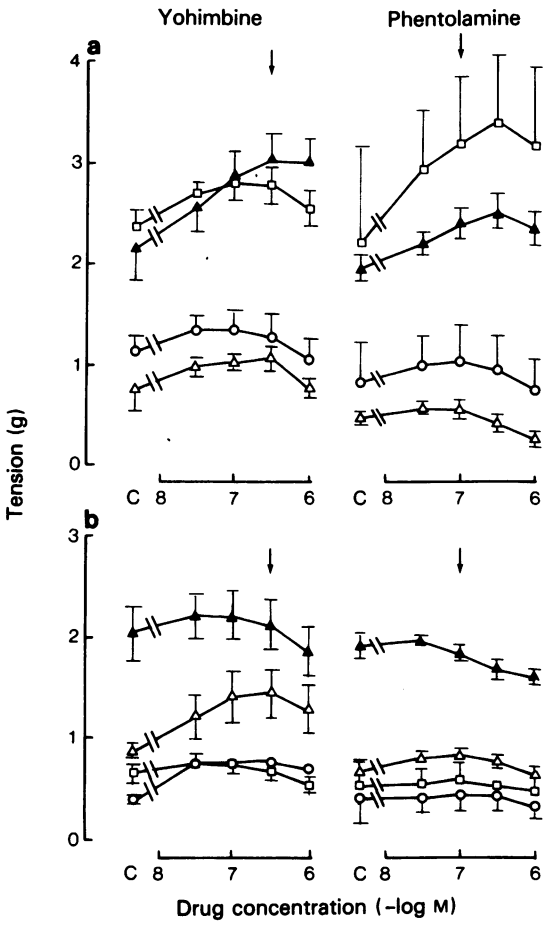

Figure 4 The effects of the α-adrenoceptor antagonists, yohimbine and phentolamine, on responses of epididymal and prostatic portions of the bisected vas deferens of the rat. Stimulus trains were delivered (0.3 ms pulse width, supramaximal voltage, 16 s duration) and responses measured during the initial rapid phase (a) or the subsequent secondary phase (b). Prostatic portion, 2 Hz (○), 10 Hz (□); epididymal portion, 2 Hz (△), 10 Hz (▲). The arrows show the concentration of each drug at which inhibition occurred of the response to a single impulse. C = initial drug-free control. Vertical bars represent standard errors.

Discussion

The five α-adrenoceptor antagonists produced relatively straightforward effects on the response to a single stimulus. They all produced a dose-dependent inhibition of the second component which has previously been identified as adrenergic (McGrath, 1977; Booth, Connell, Docherty & McGrath, 1978) but did not reduce the first component. The order of potency for this effect was similar to that previously found for antagonism of the contractile effects of exogenously administered α-adrenoceptor agonists (Drew, 1976; Blakeley & Summers, 1977; Borowski, Starke, Ehrl & Endo, 1977; Doxey *et al.*, 1977).

A further effect, that occurred with high concentrations of some agents and which had not been anticipated, was potentiation of the first phase together with the induction of spontaneous activity. Since the order of potency for this effect, azapetine > labetalol > yohimbine is the same as that for post-junctional α-adrenoceptor antagonism it may, therefore reflect some partial agonist activity. The contractile effect, like that of other α-agonists including NA (Pennefather, Vardolov & Heath, 1975), was greater in the epididymal portion. A variety of drugs can produce depolarization (Sjostrand & Swedin, 1968; Sjostrand, 1973) of vas deferens without producing contraction but will potentiate the nerve-induced contraction. The other possible explanation for the potentiating effect of these α-adrenoceptor antagonists on the response to a single pulse, namely removal of negative feedback due to spontaneous release of NA, is unlikely since there was no correlation between the order of potency with respect to antagonism of pre-junctional α-receptors. In fact the two most effective agents, azapetine and labetalol, are particularly selective for *post*-junctional receptors (Blakeley & Summers, 1977; Borowski *et al.*, 1977). This confirms the finding of Drew (1977) that the responses of rat vas deferens to low frequency (0.3 Hz) stimulation can be potentiated by high concentrations of α-blockers which are relatively selective for post-junctional receptors and is in agreement with his conclusion that this effect is of post-junctional origin.

The responses of the bisected tissue to a single stimulus provided the opportunity to compare the effects of the pre-junctional α-adrenoceptor agonist clonidine on the adrenergic (epididymal, second component) and 'non-adrenergic' (prostatic, first component) responses. Both responses were inhibited in a dose-dependent manner but the adrenergic component was susceptible to lower concentrations. This effect could be partly reversed in both halves of the tissue by yohimbine in the concentration range 10^{-7} M to 6×10^{-7} M but, since post-junctional α-antagonism occurs above 10^{-7} M, complete restoration of the adrenergic response was not possible. Thus, even yohimbine, which preferentially blocks pre- as opposed to post-junctional α-receptors (Starke *et al.*, 1975; Drew, 1976) could not be employed to block pre-junctional receptors without some interference from the post-junctional effect.

The analysis of effects of the α-adrenoceptor antagonists on responses to trains of stimuli is facilitated by consideration of the effects on a single stimulus. It is also worth stressing that when examining responses to trains of pulses, the initial and subsequent

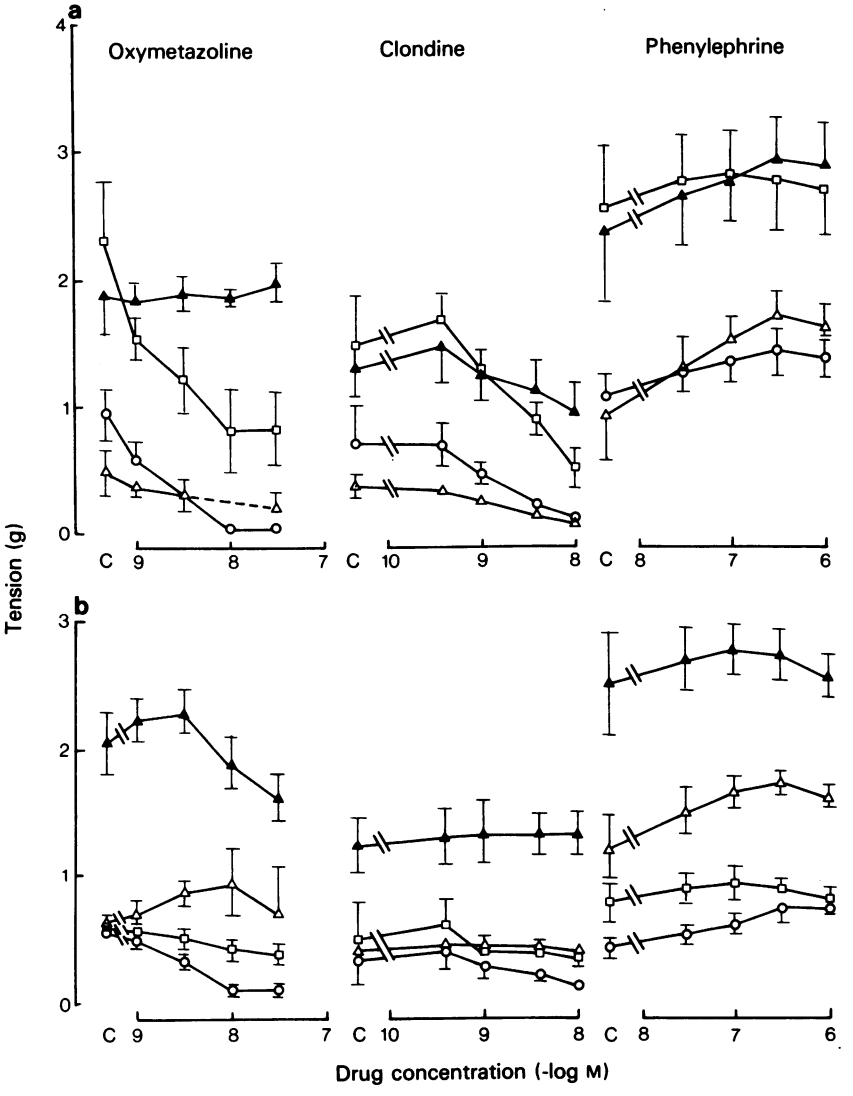

Figure 5 The effects of the α-adrenoceptor agonists, oxymetazoline, clonidine and phenylephrine, on responses of epididymal and prostatic portions of the bisected vas deferens of the rat. Stimulus trains were delivered (0.3 ms pulse width, supramaximal voltage, 16 s duration) and responses measured during the initial rapid phase (a) or the subsequent secondary phase (b). Prostatic portion, 2 Hz (○), 10 Hz (□); epididymal portion, 2 Hz (△), 10 Hz (▲). C = initial drug-free control. Vertical bars represent standard errors.

phases do not precisely correspond with the 'first' and 'second' components of the response to a single pulse. Since the initial phase reaches a peak at between 1 and 2 s, it must contain an element from the 'second' adrenergic component due to at least the first pulse. The contribution from the second and subsequent pulses will, of course, depend on facilitation and feedback mechanisms (McGrath, 1978).

Prazosin produced the most straightforward effects on the responses to trains of stimuli. Both components of the response, in both parts of the organ, were reduced in a dose-dependent manner with the 'twitch' being relatively resistant. This fits with the observation that only the post-junctional adrenergic response was removed from the response to a single pulse and the previous evidence that prazosin is selective for post-junctional α-adrenoceptors (Cambridge *et al.*, 1977; Doxey *et al.*, 1977).

Azapetine (6×10^{-9} M) produced a similar pattern to prazosin but as the concentration increased

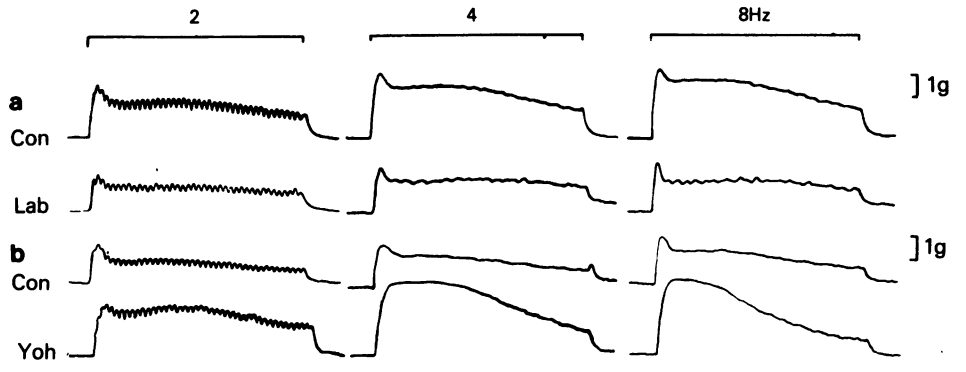

Figure 6 Effects of (a) labetalol (Lab, 3×10^{-5} M) and (b) yohimbine (Yoh, 6×10^{-7} M) on the response of the isolated vas deferens of the rat to field stimulation (at bars) for 20 s (0.3 ms pulse width, supramaximal voltage) at the frequencies indicated. Control responses (Con) are compared with responses after labetalol or yohimbine. Tissues in (a) and (b) are contralateral vasa deferentia from the same rat.

($>3 \times 10^{-7}$ M) the degree of inhibition became less. This is consistent with the potentiation of responses to single pulses and induction of spontaneous activity found with these higher concentrations.

Labetalol also produced some inhibition of both components of the response at concentrations higher than those for either prazosin or azapetine. While this is consistent with the concentrations necessary to inhibit the adrenergic part of the response to a single pulse, a potentiating effect (see above) could be detected on the response to a single pulse with a concentration of 10^{-7} M. Compared with azapetine, the threshold dose for potentiation was similar but the extent of potentiation was less, allowing a net inhibition of the responses to a train with the higher doses of labetalol but not azapetine.

Both yohimbine and phentolamine potentiated responses in each portion of the vas to stimulation with trains of pulses. In each case this was most marked on the initial phase stimulated at 10 Hz. Yohimbine but not phentolamine also potentiated the 'secondary' responses to 2 Hz stimulation. This correlates with the known relative selectivity of these drugs for pre- and post-junctional α-receptors (Drew, 1976; 1977; Doxey *et al.*, 1977). In the case of the 'secondary' component where a major part of the control response is adrenergic any potentiation due to pre-junctional α-blockade will be opposed by a post-junctional inhibition if this occurs with the same concentration, as is the case with phentolamine. With yohimbine, in contrast, lower concentrations are more selective for pre-junctional receptors so that a clearer picture of the effects of pre-junctional blockade can be seen at doses of $<10^{-7}$ M which are below the threshold for post-junctional blockade.

While these results fit into a pattern which correlates well with the known spectrum of activity of the α-adrenoceptor antagonists, the extent of endogenous activation of pre-junctional α-adrenoceptors which is uncovered, for example by the low concentrations of yohimbine, requires careful interpretation. If each component of the response to a train of pulses is the resultant of the effects of two transmission processes (Anton *et al.*, 1977; McGrath, 1978) and if each of these is susceptible to pre-junctional inhibition via α-adrenoceptors, as seems likely from the effects of clonidine on the responses to single stimuli, then increases in either component of the response could be produced by removal of inhibition from either transmission process. The resolution of this problem requires the ability to separate the two transmission processes by (1) selective blockade of the effector response and (2) detection of the transmitter output. Since neither of these manoeuvres is yet possible for the uncharacterized 'non-adrenergic' component, analysis is limited by the following factors:

(a) *Adrenergic response* Since α-adrenoceptor blockers such as yohimbine and phentolamine, can increase the nerve-induced output of noradrenaline from vas deferens at frequencies of 2 to 10 Hz (Stjarne, 1973; Vizi *et al.*, 1973), it is likely that the doses which produced increased responses at 2 and 10 Hz in the present study increased the output of noradrenaline from the adrenergic nerves. At concentrations below the threshold for post-junctional blockade it is likely that more noradrenaline is available to act on the smooth muscle *but* the contribution to the overall response of the NA cannot be determined since there is no method which selectively removes the 'non-adrenergic' component and the latter may also have been modified by the α-blocker (see below).

(b) '*Non-adrenergic*' *response* Although the initial phase of the response at 10 Hz could be clearly in-

creased by yohimbine and phentolamine, for the reasons mentioned in (a) it is not clear how much of this resultant response is or is not adrenergic. However, with concentrations of yohimbine as high as 6×10^{-6} M, which are well in excess of the concentration necessary to block the adrenergic response to a single stimulus, an initial response of greater height and duration than in controls is still found in both portions of the tissue while the 'secondary' response is virtually absent. This implies that the 'non-adrenergic' response is normally restrained during trains of field stimuli by a presynaptic α-adrenoceptor effect of noradrenaline. One possible qualification to this interpretation might be the excitatory effect of the high dose of yohimbine which could produce a similar effect. However, a similar net result can be achieved by combining a low concentration of yohimbine with a post-junctional blocking dose of prazosin which lacks the excitatory property (unpublished observations). Potentiation of the secondary response by yohimbine could also be due to an increase in the 'non-adrenergic' response. Figure 6 illustrates how the two phases merge after yohimbine to form an intermediate type of time course. Within this response the 'non-adrenergic' component may be maintained longer (as also seen after additional post-junctional α-adrenoceptor blockade) and thus contribute relatively more to the 'secondary' component.

Figure 6 also demonstrates the difficulty of separating the two components of the response to a train of pulses when the whole tissue is employed and the two components are of similar height, particularly when assessing the effects of a pre-junctional antagonist like yohimbine which alters the time course of the response. In contrast a preferential post-junctional antagonist like labetalol reduces both components with a tendency to produce a greater reduction of the slower component. The relative effects of each agent can be more clearly seen by bisecting the tissue and in particular it is possible to detect the potentiation and prologation of the early rapid component by yohimbine, a process which is completely obscured in the whole tissue by the slower component.

Stimulus frequency was critical for demonstration of an endogenous prejunctional α-adrenoceptor effect; in 20 preliminary experiments, 1 to 2 Hz was the threshold for potentiation by α-blockers, 10 Hz was optimal and 20 to 30 Hz gave maximal effector responses which could not be increased (see also Anton *et al.*, 1977).

The above interpretation is in accord with the conclusion of Ambache *et al.* (1972) that during field stimulation the NA liberated from adrenergic terminals in the vas deferens can restrain the effects of the simultaneously stimulated 'non-adrenergic' nerves by an action at α-adrenoceptors located on the 'non-adrenergic' terminals. However, the present study indicates that results fully consistent with this hypothesis can only be obtained if the dual nature of the effector response is considered.

Explanations of these results in terms of the other hypotheses advanced to explain the unusual innervation of this tissue are even more complex. On current evidence we favour the 'non-adrenergic' explanation of the α-blocker resistant' component (Ambache & Zar, 1971) since the first phase of the response to a single stimulus survives even destruction of the adrenergic terminals with 6-hydroxydopamine (Booth *et al.*, 1978). Whatever process is involved in this component, the present results suggest that it is accessible to pre-junctional modification by exogenously administered α-adrenoceptor agonists or antagonists or by endogenously released noradrenaline.

The effects of the α-adrenoceptor agonists confirmed in general their known properties. Thus the pre-junctional agonists, clonidine and oxymetazoline, inhibited both components of the responses to a train of pulses in the prostatic portion but had relatively less effect on the epididymal portion. The residual response in the epididymal portion could be expected since the first component of the response to a single pulse was not completely blocked by clonidine. In contrast, phenylephrine, a potent agonist at post-junctional α-adrenoceptors (Starke *et al.*, 1975), produced no detectable inhibition but potentiated the responses.

For any detailed analysis these effects of α-adrenoceptor agonists must be viewed against the complex effects of endogenous pre-junctional inhibition uncovered by the antagonists. In the absence of drugs, in any train of pulses, only the first is likely to occur in the absence of 'feedback' and thereafter the response will depend on the extent of pre-junctional α-adrenoceptor inhibition plus all the other facilitatory and inhibitory mechanisms which occur during continuous transmission. The effects of a drug must, therefore, vary during any train of impulses due to the changing environment in which it is acting. This will be particularly crucial for α-adrenoceptor agonists in the vas deferens since (1) the receptors on which they act will be progressively occupied to an increasing extent by endogenous noradrenaline as the train continues, (2) by decreasing the output of noradrenaline the agonists will reduce the post-junctional effect and the resultant contractile effect but will also reduce the endogenous pre-junctional effects and hence tend to increase the output and effect of the transmitter from the 'non-adrenergic' nerves, (3) if pre-junctional adrenoceptors are present on two different types of nerves these receptors or the effects of their activation may differ.

These results indicate that the nerve-induced contraction of the bisected vas deferens is suitable as a pharmacological preparation for the assessment of (1)

post-junctional blockade of α-adrenoceptors when employing single stimuli and the epididymal portion; (2) pre-junctional blockade of endogenous α-adrenoceptor activation but only (a) with trains of stimuli at 2 to 10 Hz, (b) concentrations of drugs which do not block post-junctional α-adrenoceptors, (c) provided that the basis of the effector response is of no interest, (d) by considering the time course as well as the height of the response; (3) pre-junctional α-adrenoceptor agonism or antagonism by exogenous agents (a) employing single pulses (b) considering the responses of the two portions separately.

In conclusion, the effects on the nerve-induced contractions of the rat vas deferens of drugs which act at pre- and/or post-junctional α-adrenoceptors can be explained in terms of the complex innervation of this tissue whether trains of pulses of individual stimuli are employed. However, the responses of the bisected tissue to a single stimulus provide the simplest and most reliable test for such actions.

This work was supported by grants from the M.R.C. and the Medical Research Funds of Glasgow University. The authors wish to thank Mr G. J. Connell for excellent technical assistance; Dr G. P. Levy of Allen & Hanbury's Research Ltd. for gifts of labetalol, Pfizer Ltd. for gifts of prazosin and Boehringer Ingelheim for gifts of clonidine.

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(Received October 10, 1978.)