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α-ADRENOCEPTORS IN THE MOUSE VAS DEFERENS AND THEIR EFFECTS ON ITS RESPONSE TO ELECTRICAL STIMULATION

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1 Noradrenaline (ID_{50}, 0.75 μM) and clonidine (ID_{50}, 2.8 nM) produced a dose-related inhibition of the twitch response of the isolated vas deferens of the mouse to electrical stimulation, their effectiveness decreasing as frequency of stimulation increased from 0.2 to 16 hertz.

2 Phenylephrine (1.0–3.0 μM) produced a dose-related contraction of the mouse isolated vas deferens and potentiated the responses to field stimulation.

3 Yohimbine (10 nM) antagonized the inhibitory effects of noradrenaline and clonidine, but had no effect on the motor activity of phenylephrine. At a concentration of 128 nM yohimbine potentiated the twitch response by 110% at 1 Hz, but its effectiveness decreased with increasing frequency of stimulation up to 16 hertz.

4 Thymoxamine (0.3 μM) antagonized the effects of phenylephrine, but not those of clonidine.

5 From a consideration of the known characteristics of pre- and postsynaptic α-adrenoceptors, it is concluded that the inhibitory effect of noradrenaline is produced by stimulation of the former and the effects of phenylephrine by stimulation of the latter.

Introduction

Ambache & Zar (1971) were the first to report an inhibitory effect of noradrenaline on twitch responses to electrical stimulation in the isolated vas deferens of the guinea-pig. This was confirmed by von Euler & Hedqvist (1975) and shown to be true for the mouse vas deferens by Jenkins, Marshall & Nasmyth (1976). On the other hand, the more specific α-adrenoceptor agonist phenylephrine potentiated the twitches and contracted the tissue (Jenkins *et al.* 1976). It was also noted by Ambache & Zar (1971), by Jenkins *et al.* (1976) and by Jenkins, Marshall & Nasmyth (1977) that the concentration of noradrenaline required to contract the vas deferens was higher than that required to inhibit the responses to electrical stimulation. By contrast, the contraction to both phenylephrine and noradrenaline was more susceptible to blockade with phentolamine than was the inhibitory effect. Nevertheless, the inhibitory effect was antagonized by phentolamine and as it was not blocked by propranolol Jenkins *et al.* (1977) concluded that it was mediated by α-adrenoceptors.

Kirpekar & Puig (1971), Farnebo & Hamberger (1971) and Enero, Langer, Rothlin & Stefano (1972) have suggested that α-adrenoceptors on the sympathetic neurone inhibit the output of noradrenaline when they are stimulated. The inhibitory effect of exogenous noradrenaline on the twitch response of the electrically stimulated isolated vas deferens of the mouse could therefore be due to preferential stimulation of a presynaptic α-adrenoceptor reducing the output of the transmitter. In contrast, the contractor effects of phenylephrine and noradrenaline are probably caused by an action on the postsynaptic α-adrenoceptor. In this case, the potentiation of the twitch response by phenylephrine could be the result of summation of its effect on the postsynaptic receptor with the effects of released transmitter. The results of the experiments described here support this concept. A preliminary report of the work has been presented to the British Pharmacological Society (Marshall, Nasmyth, Nicholl & Shepperson, 1977).

Methods

T.O. strain mice weighing 25–40 g were used. A single vas deferens was removed and dissected free of blood vessels and mesentery and suspended in a 2 ml bath

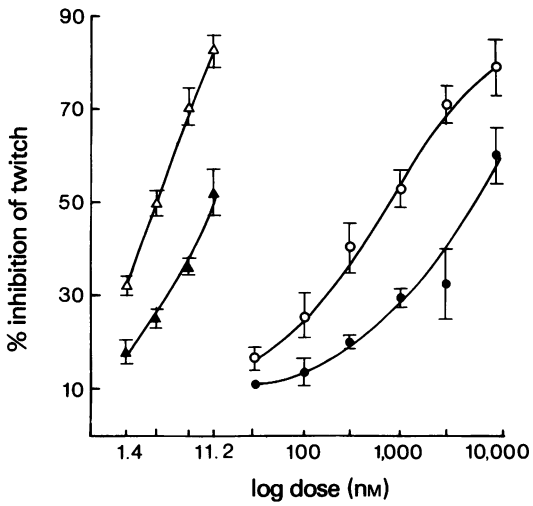

Figure 1 Inhibition of twitch responses of the isolated vas deferens of the mouse to field stimulation (64 V, 2.0 ms, 0.2 Hz) by clonidine and noradrenaline. Clonidine control: (△); clonidine 2 min after the addition of yohimbine (10 nM): (▲); noradrenaline control: (○); noradrenaline 2 min after the addition of yohimbine (10 nM): (●).

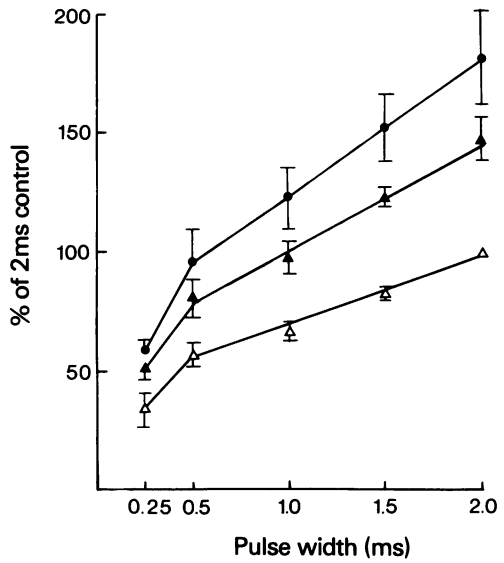

Figure 2 Response of the mouse vas deferens to field stimulation (64 V, 0.2 Hz) at various pulse widths and its potentiation by yohimbine. In the absence of yohimbine: (△); yohimbine 3.2 nM: (▲); yohimbine 12.8 nM: (●). Because the tissues varied considerably from one to another in the tension developed in response to stimulation, the response at 2.0 ms in the absence of yohimbine was taken as 100% in each tissue. The responses at other pulse widths, in the presence or absence of yohimbine, in the same tissue were then expressed as a percentage of this value.

between two parallel platinum electrodes. The bath contained Krebs solution of the following composition (mM): NaCl 119.0, KCl 4.7, $CaCl_2$ 2.5, $NaH_2PO_4.2H_2O$ 1.2, $NaHCO_3$ 25.0 and glucose 11.0. Magnesium was omitted because, in agreement with Hughes, Kosterlitz & Leslie (1975), it was easier to obtain good responses in its absence. The solution was maintained at 37°C and bubbled with 95% O_2 and 5% CO_2. A resting tension of 500 mg was applied to the tissue. Field stimulation was provided by a Grass S48 stimulator at 64 V with frequencies of 0.2 to 16 Hz and pulse widths of 0.25–2.0 milliseconds. Tetrodotoxin (0.63 μM) abolished the twitches elicited by these stimulation parameters, indicating that only nerve fibres were stimulated.

Contractions of the vas deferens were recorded isometrically via an FT.03 transducer attached to a Grass polygraph. Agonists were added to the bath with a micrometer syringe after 6 control stimuli in each case, unless otherwise stated. The effect of the drug was determined by measuring the height of the eighth response after adding the drug. Unless otherwise stated, the results are the mean ± s.e. mean of three observations at each parameter in each of at least four different tissues. The concentrations given refer to the final concentration in the bath.

The drugs used were: clonidine hydrochloride (Catapres, Boehringer Ingelheim); noradrenaline bitartrate (Levophed, Winthrop Laboratories); (−)-phenylephrine hydrochloride; (Sigma) thymoxamine hydrochloride (Opilon, Warner); and yohimbine hydrochloride (Sigma).

Results

Effects of presynaptic α-adrenoceptor agonists and antagonists

Both noradrenaline and clonidine produced a dose-related inhibition of the twitch response to electrical stimulation (0.2 Hz, 2.0 ms) as shown in Figure 1. The ID_{50} for noradrenaline was 0.75 μM and that for clonidine 2.8 nM. The addition of yohimbine (10 nM) to the bath 2 min before starting stimulation of the tissue, shifted the dose-response curves for both drugs to the right by more than half a log unit (Figure 1). The twitches elicited at 0.2 Hz with pulse widths varying from 0.25 to 2.0 ms were potentiated by yohimbine in a dose-related manner by concentrations of 3.2 and 12.8 nM (Figure 2). A concentration

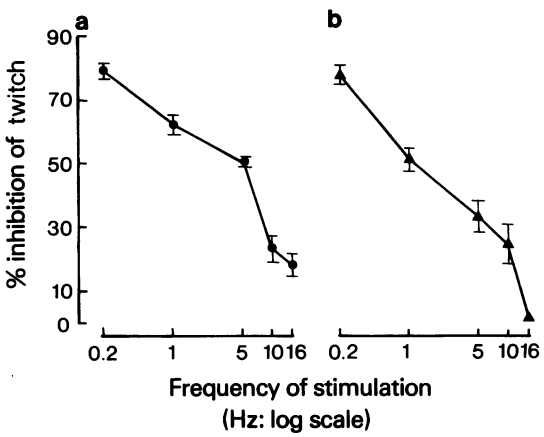

Figure 3 The inhibitory effect of (a) noradrenaline (3.0 μM) and (b) clonidine (5.6 nM) on twitch responses of the isolated vas deferens of the mouse at various frequencies of stimulation.

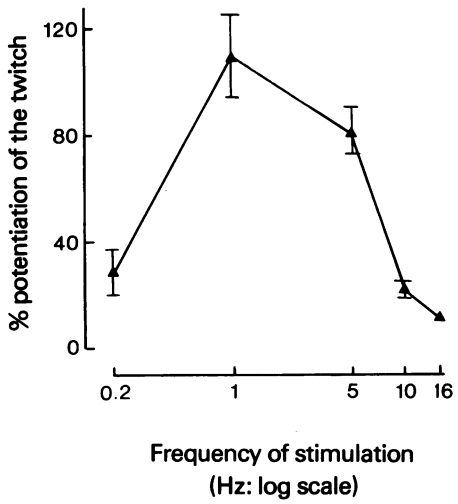

Figure 4 Potentiation by yohimbine (128 nM) of the twitch response of the isolated vas deferens of the mouse at different rates of stimulation.

of 128.0 nM yohimbine produced no greater potentiation of the twitch than did 12.8 nM.

The effect of varying the frequency of stimulation from 0.2 to 16 Hz on the inhibition of twitches produced with 2 ms pulses by noradrenaline (3.0 μM) and by clonidine (5.6 nM) added 30s before stimulation began is shown in Figure 3. At frequencies higher than 1 Hz the responses were no longer discrete, so they were allowed to reach a maximum before stopping the stimulation. This was achieved with a train of 50 pulses at 5 Hz and 120 pulses at 10 and 16 hertz. The inhibition was greatest (78%) at 0.2 Hz and fell as the frequency of stimulation increased to less than 20% for noradrenaline and less than 10% for clonidine at 16 hertz. Yohimbine (198 nM) potentiated the twitch by about 30% at 0.2 Hz and by 110% at 1.0 Hz, but by only 10% at 16 Hz (Figure 4).

Effects of postsynaptic α-adrenoceptor agonists and antagonists

In three experiments, phenylephrine (1.0 and 3.0 μM) produced a dose-related contraction of the tissue and a potentiation of the twitch response to electrical stimulation (0.2 Hz, 2.0 milliseconds). These effects were not affected by yohimbine (10 nM) which was sufficient to antagonize the inhibition produced by noradrenaline or by clonidine.

In another three experiments, thymoxamine (0.3 μM) blocked the contraction of the tissue and reduced the potentiation of the twitch responses caused by phenylephrine (0.3 μM). This dose of thymoxamine was without effect on the inhibition of the twitch produced by clonidine (Figure 5).

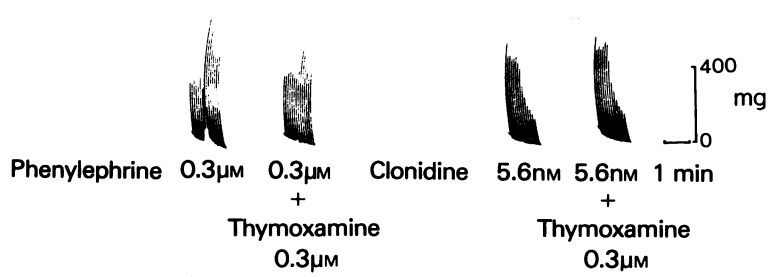

Figure 5 Contracture and potentiation of the twitch responses of the isolated vas deferens of the mouse to field stimulation (64 V, 2.0 ms, 0.2 Hz) by phenylephrine (0.3 μM) and inhibition of both effects by thymoxamine (0.3 μM). Inhibition of the responses by clonidine (5.6 nM) and the failure of thymoxamine (0.3 μM) to affect it.

Discussion

It is remarkable that three known α-adrenoceptor agonists, clonidine, noradrenaline and phenylephrine should produce such different effects on the twitch response of the mouse vas deferens. Clonidine produced only inhibition of the response, as did noradrenaline at the lowest concentrations used. However, as the concentration of noradrenaline was increased, the inhibition of the twitch response also increased, but the tone of the muscle was increased too and a contracture was superimposed upon the twitches. By contrast, phenylephrine superimposed a contracture like noradrenaline, but unlike noradrenaline, it potentiated the twitches. Since noradrenaline also stimulates β-adrenoceptors, it might be supposed that they mediated the inhibition of the twitch response and that the contracture was caused by the stimulation of α-adrenoceptors. However, Jenkins *et al.* (1977) reported that the inhibitory effect of noradrenaline on the twitch responses is not blocked by the β-adrenoceptor blocking agent, propranolol. They also showed that both the inhibition and the contracture were inhibited by the α-adrenoceptor blocking agent, phentolamine. Since phenylephrine is a more specific α-adrenoceptor agonist than noradrenaline, it must be supposed that all three α-adrenoceptor agonists produce their effects via this receptor, but because their effects are dissimilar, we must also suppose that more than one kind of α-adrenoceptor is involved.

We know that not only are there post-junctional α-adrenoceptors but also pre-junctional receptors situated on the sympathetic neurone (Kirpekar & Puig, 1971; Farnebo & Hamberger, 1971; Enero, *et al.*, 1972), which, when stimulated, inhibit the output of noradrenaline. Using the field-stimulated pulmonary artery, Starke, Endo & Taube (1975) showed that phenylephrine preferentially stimulated postsynaptic α-adrenoceptors, whereas the presynaptic receptors were preferentially stimulated by clonidine (Starke, Montel, Gayk & Merker, 1974). This could certainly account for the results reported here and would explain why propranolol did not block the inhibition of the twitch response produced by noradrenaline.

Yohimbine has been shown to block presynaptic α-adrenoceptors in lower concentrations than are required to block postsynaptic α-adrenoceptors (Starke, Borowski & Endo, 1975). The ability of yohimbine to antagonize the inhibitory effects of noradrenaline and clonidine on the twitch response is thus consistent with the suggestion that their effects are mediated by a presynaptic receptor. That yohimbine potentiated the twitch response suggests that endogenous noradrenaline constantly stimulates the presynaptic receptor at low rates of neuronal stimulation particularly at 1 hertz. Its inability to affect the potentiation produced by phenylephrine is also consistent with the suggestion that the effects of the latter drug were produced by preferential stimulation of postsynaptic α-adrenoceptors. In contrast, thymoxamine was found to be 300 times less potent than yohimbine in blocking presynaptic α-adrenoceptors in the cardiovascular system (Drew, 1976). Predictably, it blocked the contracture and the potentiation of the twitch produced by phenylephrine but in the same concentration, it was without effect on the inhibition produced by clonidine.

It is characteristic of presynaptic α-adrenoceptors that their ability to modify the response to sympathetic nerve stimulation becomes less as the frequency of stimulation increases (Starke, 1972; Vizi, Somogyi, Hadhazy & Knoll, 1973). As would be expected, therefore, the inhibitory effects of clonidine and noradrenaline and the potentiation of the twitch responses by yohimbine were less at a stimulation frequency of 16 Hz than they were at 0.2 to 1.0 hertz.

It is concluded that the potentiation of the twitch response produced by phenylephrine and the contracture produced by phenylephrine and noradrenaline are mediated by postsynaptic α-adrenoceptors. The inhibition of the twitch response produced by clonidine and by noradrenaline is mediated by stimulation of presynaptic α-adrenoceptors with a consequent reduction in the secretion of the motor transmitter.

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