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The effects of bromocriptine on pre-synaptic and post-synaptic α-adrenoceptors in the mouse vas deferens

ALAN GIBSON* AND MORTEZA SAMINI

Department of Pharmacology, Chelsea College, University of London, London, SW3 6LX, U.K.

Noradrenaline (NA) and dopamine (DA) contracted the mouse vas deferens and reduced the responses to low frequency nerve stimulation (0·1 Hz). The relative potencies of antagonists suggested that these effects were due to stimulation of post-synaptic and pre-synaptic α-adrenoceptors respectively. Bromocriptine produced a non-competitive antagonism of contractile responses to NA ($pD_2' = 7·6$) and DA ($pD_2' = 8·0$) but had no effect on responses to carbachol. Bromocriptine also reduced single twitch responses of the vas to low frequency field stimulation (0·1 Hz), but did not affect stimulation at higher frequencies (1–20Hz). Yohimbine selectively and rapidly reversed the inhibiting effects of bromocriptine on single twitches, although they could not easily be reversed by washing. Bromocriptine produced a yohimbine-reversible reduction in the stimulated overflow of tritium from vasa previously loaded with ^{3}H–NA. Thus the mouse vas deferens does not appear to contain specific DA receptors and the results suggest that bromocriptine acts as a pre-synaptic α-adrenoceptor agonist and post-synaptic α-adrenoceptor antagonist in this tissue.

Bromocriptine (2-bromo-α-ergocryptine) is an ergot alkaloid derivative which is used clinically in the treatment of acromegaly, parkinsonism, and certain cases of infertility (Hokfelt & Nillius 1978), and it is believed that the therapeutic effects of the drug are related to its ability to stimulate specific dopamine (DA) receptors in the central nervous system and pituitary gland. Although it was originally thought to have only weak actions on other amine receptor systems (Thorner 1975), recent evidence has suggested that bromocriptine may be a potent post-synaptic α-adrenoceptor antagonist, having activity equal to that of established α-adrenoceptor antagonists such as phentolamine and phenoxybenzamine (Lew et al 1977; Gibson & Samini 1978). In addition, it is possible that bromocriptine might also interact with pre-synaptic α-adrenoceptors (Langer 1977) on which other ergot alkaloid derivatives have been shown to act (Hughes 1973; Marshall et al 1977). Knowledge of the effects of bromocriptine on α-adrenoceptors is of importance since the drug is increasingly being used as a highly selective DA receptor agonist in experiments which might elucidate the physiological function of DA in the central and peripheral nervous systems (Thorner 1975; Greenacre et al 1976; Stumpe et al 1977). In the periphery, it has been suggested that specific DA receptors exist in the vas deferens of certain species and that DA may be a neurotransmitter in this tissue (Simon & Van Maanen 1976; Tayo 1977). In the present study therefore we have investigated the effects of bromocriptine on pre- and post-synaptic receptors in the mouse vas deferens which possesses post-synaptic α-adrenoceptors (Jones & Spriggs 1975) and which is a useful model for the study of drugs acting on pre-synaptic receptors (Henderson et al 1972; Marshall et al 1978).

* Correspondence.

METHODS

Post-synaptic studies

Male mice (LACA; 20–30 g) were killed by stunning and exsanguination. Single vas deferens preparations were dissected and set up in 5 ml organ baths containing Mg^{2+}-free Krebs bicarbonate solution (mM: Na Cl, 118·1; KCl, 4·7; KH_2PO_4, 1·2; $CaCl_2$, 2·5; $NaHCO_3$, 25·0; glucose, 11·1) which was maintained at 37 °C and was gassed continuously with 5% CO_2 in oxygen. A resting tension of 500 mg was placed on the tissue and changes in tension were recorded by a Grass FTO3 force-displacement transducer attached to a Devices M2 pen-recorder. Field stimulation was applied by 2 parallel longitudinal platinum electrodes running along each side of the tissue. These were attached to a SRI square wave pulse generator (1 ms; 150 V). Nerve-mediated responses obtained using these stimulation parameters were blocked by tetrodotoxin (5 $\mu g\ ml^{-1}$).

Agonists were added to the organ bath in volumes not exceeding 25 μl and were left in contact with the tissue for 1 min. The response was measured as the

peak rise in tension produced by each dose. The time interval between successive doses was 10 min and only one dose-response curve was obtained from each tissue. The pD_2 values of agonists were calculated from the curves (Ariëns & van Rossum 1957).

Antagonists were left in contact with the tissue for 30 min before addition of the first agonist dose. pA_2 and pD'_2 values were obtained by repeating agonist dose-response curves in medium containing various concentrations of antagonist (Van den Brink & Lien 1977).

Pre-synaptic studies

Tissues were dissected and set up in organ baths as described above.

Dose-response curves for agonists acting pre-synaptically were obtained by measuring the reduction of twitch height during low frequency (0·1 Hz) nerve stimulation and by calculating the ID50 (dose of agonist producing 50% reduction in twitch height). Agonists were left in contact with the tissue for 2 min and the response measured as the percentage reduction in the height of the tenth twitch after addition of the drug. The interval between successive doses was 10 min and only one dose-response curve was obtained from each tissue.

Antagonists were left in contact with the tissue for 30 min before measuring agonist activity.

Tritium overflow studies

For these experiments both vasa were set up in series and were incubated in Mg^{2+}-free Krebs solution (37 °C, 45 min) containing 2·5 μM[^{3}H]-noradrenaline (^{3}H-NA, 14 Ci mmol^{-1}, Amersham). Following incubation the tissue was transferred to a radioactivity-free medium in a 1 ml organ bath. Cocaine (10 μM), corticosterone (40 μM) and yohimbine (51·2 nM) were added to the Mg^{2+}-free Krebs solution in order to increase nerve-mediated tritium overflow. 0·1 ml samples of medium were taken every 30 min for a 90 min post-incubation rest period, the organ bath being washed out after each sample. The tissues were then stimulated (0·2 Hz) for a 15 min period and 15 min later a further 0·1 ml sample was taken. Radioactivity in the samples was then counted in an Intertechnique SL40 liquid scintillation counter using a Toluene-Triton X-100 scintillant. Results were expressed as the percentage change in the 30 min stimulation sample compared with the sample taken immediately before stimulation. Bromocriptine was added to the medium immediately before nerve stimulation.

Drugs used

Bromocriptine methane sulphonate (Sandoz); carbachol (Koch Light); cocaine hydrochloride (May and Baker); corticosterone (Sigma); dopamine hydrochloride (Sigma); haloperidol (Searle); naloxone hydrochloride (Endo Laboratories); noradrenaline bitartrate (Sigma); normorphine hydrochloride (Chelsea College Pharmacy Department); phentolamine mesylate (Ciba); pimozide (Janssen); Yohimbine (Aldrich).

RESULTS

Post-synaptic studies

The mouse vas deferens contracted to both NA ($pD_2 = 5·14 \pm 0·03$; maximum response = 1·22 ± 0·08 g) and to DA ($pD_2 = 3·83 \pm 0·11$; maximum response = 0·59 ± 0·05 g). The responses to both agonists were competitively antagonized by phentolamine (pA_2 against NA = 7·76 ± 0·05; against DA = 7·94 ± 0·13) and by haloperidol (pA_2 against NA = 6·90 ± 0·12; against DA = 6·80 ± 0·10).

Unlike NA and DA, bromocriptine did not by itself contract the vas. On the contrary, it produced a non-competitive antagonism of responses to both NA ($pD'_2 = 7·62 \pm 0·05$; Fig. 1) and DA ($pD'_2 = 7·99 \pm 0·12$). After washing bromocriptine out of the organ bath the block of responses to NA and DA persisted and was undiminished 1 h later. However the antagonism was selective against the catecholamines since the responses to carbachol were unaffected by bromocriptine (Fig. 1).

Presynaptic studies

Single twitch responses of the vas to low frequency (0·1 Hz) nerve stimulation were inhibited by NA (ID50 = 0·65 μM) and by DA (ID50 = 2·7 μM). The effects of yohimbine and of haloperidol on these inhibitions are shown in Fig. 2. Yohimbine (512 nM) antagonized the responses to both NA and DA, whereas haloperidol caused a slight potentiation of the effects of both agonists. This potentiation of the pre-synaptic effects of NA and DA by haloperidol has been observed previously in guinea-pig vas and may be due to inhibition of neuronal uptake (Bell & Matalanis 1977; Janssen 1967).

Bromocriptine also inhibited single twitch responses of the vas at 0·1 Hz but had no effect on the responses to higher frequencies of nerve stimulation (1–20 Hz, Fig. 3). The characteristics of the response to bromocriptine were unlike those of NA and DA. Firstly, there was a much slower onset of action and secondly the response did not disappear on washing bromocriptine out of the organ bath, the effect per-

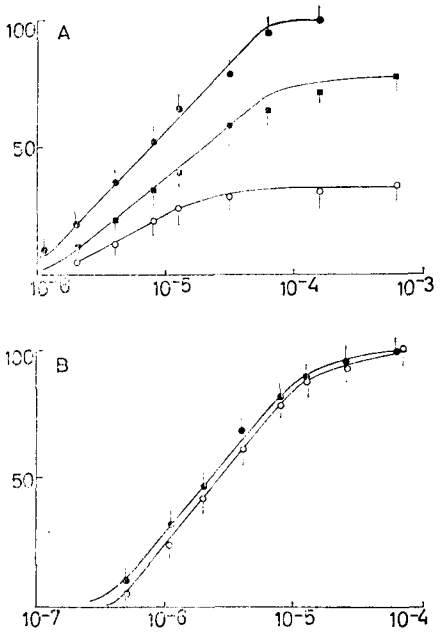

FIG. 1. Dose-response curves of contractile responses of the mouse vas deferens to noradrenaline (A) and carbachol (B) acting alone (●—●) or in the presence of 10 nM bromocriptine (■—■) or 40 nM bromocriptine (○——○). Each point is the mean ± s.e. of at least 6 vas deferens preparations. Abscissa: concentration (M). Ordinate: response (% of control maximum response).

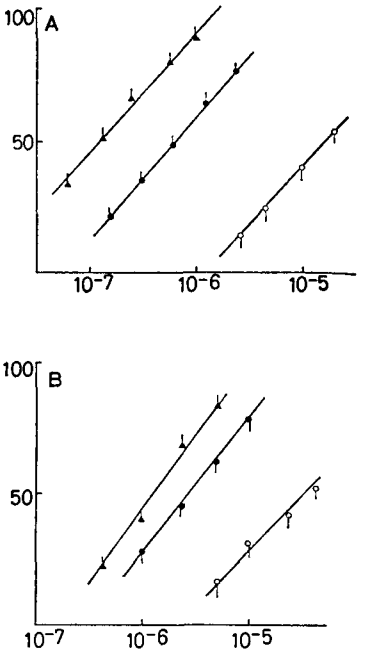

FIG. 2. Dose-response curves of inhibitory effects of noradrenaline (A) and dopamine (B) on single twitch responses of the mouse vas deferens (0·1 Hz; 1 ms; 150 V), acting alone (●——●) or in the presence of 2 μM haloperidol (▲——▲) or 512 nM yohimbine (○——○). Each point is the mean ± s.e. of at least 6 vas deferens preparations. Abscissa: concentration (M). Ordinate: % inhibition of twitch.

sisting for at least one hour after washout. However, the effects of bromocriptine were rapidly and completely reversed by yohimbine (Figs 3 and 4), and to a lesser extent by phentolamine (Fig. 4). The effects of bromocriptine were not reversed by either haloperidol or pimozide (both 10 μM).

The selectivity of the yohimbine antagonism was determined by observing its effect on the inhibitory effects of normorphine, which reduces single twitch, responses of the mouse vas deferens by acting on pre-synaptic opioid receptors (Henderson et al 1972). The results are shown in Fig. 5. Yohimbine had no effect on the dose-response curve to normorphine, while the opioid antagonist naloxone had no effect on the dose-response curve to bromocriptine.

Tritium overflow studies

Following incubation of the vas in ^{3}H-NA, nerve stimulation (0·2 Hz, 15 min) increased the overflow of tritium from the tissue by 40% (Fig. 6). In the presence of 13·5 μM bromocriptine the stimulated overflow was reduced by only 8%. This effect of bromocriptine was completely reversed by increasing the yohimbine concentration in the bathing medium to 10μM. Bromocriptine did not influence the resting output of tritium from the tissue.

DISCUSSION

Simon & Van Maanen (1976) reported that the rat vas deferens contained specific post-synaptic DA receptors and postulated that DA may be an excitatory transmitter in this tissue. In addition, Tayo (1977) has suggested that DA may exert a dual influence on the rat vas, producing both pre-synaptic inhibition and post-synaptic excitation. Since the nature of the excitatory transmitter in the mouse vas deferens is also uncertain (Jones & Spriggs 1975; Jenkins et al 1977) it seemed possible that there might be DA receptors present in this tissue. However in the present study there was no evidence for the existence of specific DA receptors in the mouse vas either pre-synaptically or post-synaptically. The relative effects of antagonists on the excitatory and inhibitory effects of DA would suggest that they are mediated by post-synaptic and pre-synaptic α-adrenoceptors respectively. It is of course possible that the receptor populations in the rat and mouse

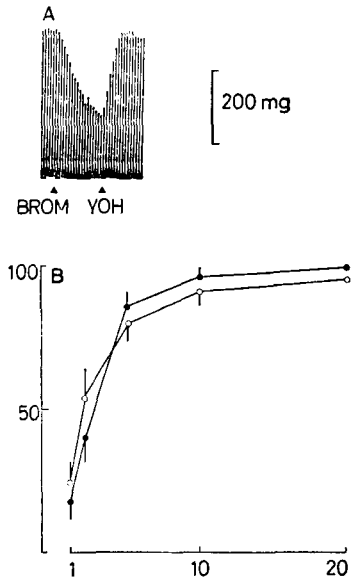

FIG. 3. A. Single twitch responses of the mouse vas deferens in response to field stimulation (0·1 Hz; 1 ms; 150 V). Brom—10 μM bromocriptine; YOH—10 μM yohimbine.
B. Frequency-response curves of the mouse vas deferens in response to field stimulation (1 ms; 150 V), under control conditions (●——●) or in the presence of 13·2 μM bromocriptine (○——○). Each point is the mean ± s.e. of at least 6 vas deferens preparations. Abscissa: frequency (Hz). Ordinate: response (% of control maximum response).

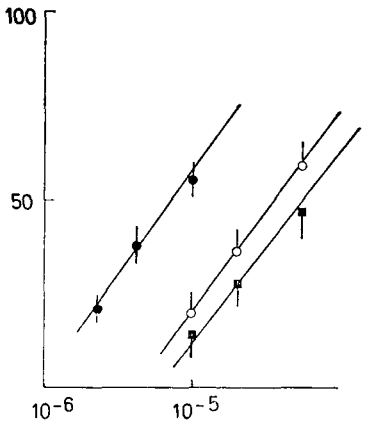

FIG. 4. Dose-response curve of the inhibitory effects of bromocriptine on single twitch responses of the mouse vas deferens to field stimulation (0·1 Hz; 1 ms; 150 V), acting alone (●——●) or in the presence of 500 nM yohimbine (○——○) or 2·5 μM phentolamine (■—■). Each point is the mean ± s.e. of at least 6 vas deferens preparations. Abscissa: concentration (M). Ordinate: % inhibition of twitch.

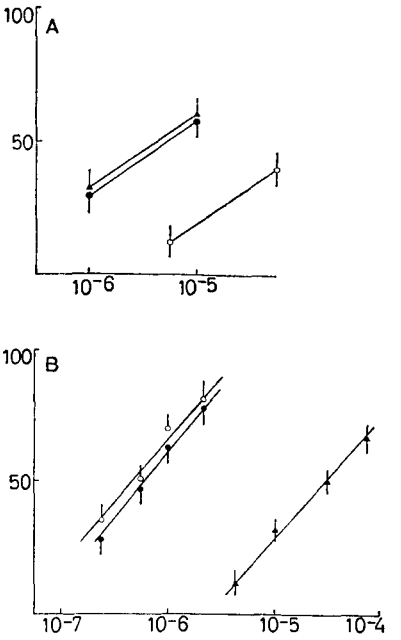

FIG. 5. Dose-response curves of the inhibitory effects of bromocriptine (A) and normorphine (B) on single twitch responses of the mouse vas deferens to field stimulation (0·1 Hz; 1 ms; 150 V), acting alone (●——●) or in the presence of 512 nM yohimbine (○——○) or 100 nM naloxone (▲——▲). Each point is the mean ± s.e. of at least 6 vas deferens preparations. Abscissa: concentration (M). Ordinate: % inhibition of twitch.

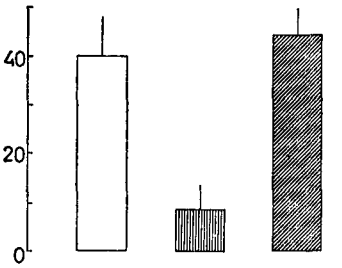

FIG. 6. Histogram of the % increase in tritium overflow from ^{3}H-NA loaded mouse vas deferens preparations in response to nerve stimulation (0·2 Hz; 1 ms; 150 V). Each column represents the mean ± s.e. of 6 vas deferens preparations. Cocaine (10 μM) and corticosterone (40 μM) were present in all cases. Clear column: +51·2 nM yohimbine. Stippled column: +51·2 nM yohimbine and 13·6 μM bromocriptine. Hatched column: +10 μM yohimbine and 13·6 μM bromocriptine.

vas deferens are different. Certainly in the mouse the maximum contraction produced by DA was only 50% of that produced by NA, whereas in the rat both agonists produce a similar maximum response (Simon & Van Maanen 1976). However, even in the rat vas deferens it has been suggested that the post-synaptic responses to DA are due to activation of

α-adrenoceptors (van Rossum 1965; Patil et al 1973). The present results are in agreement with those of Hurst et al (1979) who found no evidence of pre-synaptic DA receptors in the mouse vas deferens.

Perhaps the most interesting results of this study concern the effects of bromocriptine on single twitch responses of the vas to nerve stimulation, and the evidence would suggest that bromocriptine acts as a pre-synaptic α-adrenoceptor agonist in this tissue. Firstly, the frequency-dependent reductions in response to nerve stimulation were reversed by yohimbine, a selective pre-synaptic α-adrenoceptor antagonist (Starke et al 1975). This effect was not merely due to a non-specific increase in transmitter output produced by yohimbine since it had no effect on the inhibitory actions of normorphine. Secondly, the effects of bromocriptine were not reversed by either haloperidol or pimozide, thus ruling out DA receptor activation. Thirdly, bromocriptine produced a yohimbine-reversible reduction in stimulated overflow of tritium from tissues previously loaded with ^{3}H-NA. Separation of the labelled overflow into NA and its metabolites was not attempted in this study. Thus bromocriptine appears to act like LSD and ergometrine in the mouse vas and stimulates pre-synaptic α-adrenoceptors (Hughes 1973; Marshall et al 1977). However, if bromocriptine is a presynaptic agonist then the nature of the agonism is puzzling. Although the effects of bromocriptine were rapidly reversed by yohimbine they could not be reversed by washing, at least up to 1 h later. The mechanisms of this prolonged agonism may be due to some intracellular site of action or perhaps to the existence of different binding sites on the membrane receptor (Dolphin et al 1977; Ruffolo et al 1977). It is unlikely that bromocriptine is acting by release of NA from pre-synaptic nerves since it did not by itself increase tritium overflow, although DA may act in this way (Hurst et al 1979).

The results confirmed that bromocriptine is a potent post-synaptic α-adrenoceptor antagonist (Lew et al 1977; Gibson & Samini 1978), yet it did not block the responses of the vas to higher frequencies of nerve stimulation (1–20 Hz). The responses of the vas deferens of several species may be divided into two components, a twitch followed by a slower, more prolonged contraction (Swedin 1971; Birmingham & Freeman 1976). The initial twitch is resistant to α-adrenoceptor antagonists which completely abolish the second slower contraction. In the present study we did not attempt to differentiate the components of the response to nerve stimulation, but the resistance of the total response to bromocriptine is further evidence that excitatory transmission in the mouse vas deferens is complex (Jenkins et al 1977).

The results presented suggest that bromocriptine has complex effects on α-adrenoceptor systems and that interaction with α-adrenoceptors may explain some of the effects of the drug (Hökfelt & Nillius 1978).

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