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The actions of cirazoline on the rat vas deferens

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1 The pre- and postsynaptic effects of the α_1-agonist cirazoline were assessed in epididymal and prostatic portions of the rat isolated vas deferens.

2 Cirazoline produced a postsynaptic α_1-adrenoceptor mediated potentiation of the isometric contraction to single pulse field stimulation in both prostatic and epididymal portions.

3 In epididymal portions, nifedipine (10 μM) greatly attenuated the postsynaptic α_1-receptor mediated potentiation of nerve mediated contractions, uncovering a presynaptic inhibitory action of cirazoline.

4 No evidence was found for α_2-antagonism by cirazoline. It is concluded that the previously reported antagonism of the presynaptic inhibitory effects of clonidine was due to postsynaptic potentiation of nerve-mediated responses by cirazoline.

Introduction

The imidazoline derivative cirazoline is a potent α_1-adrenoceptor agonist (Roach *et al.*, 1978; Ruffolo & Waddell, 1982), which has been reported to be an α_2-adrenoceptor antagonist in pithed rat heart and rat vas deferens (Cavero *et al.*, 1982), in rabbit blood vessels (Hannah *et al.*, 1982) and guinea-pig ileum (Ruffolo & Waddell, 1982). However, cirazoline has also been shown to be an agonist at presynaptic α-adrenoceptors in cat spleen (Dubocovich *et al.*, 1980) and in the pithed rat heart (Docherty 1983a,b); in the latter study no evidence was found for α_2-receptor antagonism by cirazoline.

The purpose of the present study is to re-examine the actions of cirazoline at pre- and postsynaptic α-adrenoceptors in the rat vas deferens using procedures which allow separation of the pre- from the postsynaptic influences on neurotransmission. Some of these results have been published previously in abstract form (Docherty & McGrath, 1982).

Methods

Vasa deferentia were obtained from male Wistar rats (225–275 g) and were bisected into prostatic and epididymal portions (McGrath, 1978). Tissues were placed between platinum electrodes in organ baths (50 ml), and bathed at 37°C in Krebs-Henseleit solution of the following composition (mM): NaCl 119, $NaHCO_3$ 25, D-glucose 11.1, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.0. The tissues were attached to myograph transducers for recording of isometric tension. Responses to single pulse field stimulation (supra-maximal voltage, 0.5 ms) were obtained at intervals of 5 min. When consistent control responses had been obtained, the effects of drugs were assessed on nerve-stimulation evoked responses.

Two types of experimental protocol were employed. In protocol 1, employing both prostatic and epididymal portions, the effects of cumulative concentrations of cirazoline or the α_2-antagonist yohimbine were assessed on nerve-stimulation evoked contractions, either in the absence of any prior drug or in the presence of the α_2-selective agonist clonidine or the α_1-antagonist prazosin. In protocol 2, employing only the epididymal portions, experiments were carried out in the presence of the calcium entry blocker nifedipine (10^{-5} M), and the effects of cirazoline, alone or after prior clonidine, were assessed on nerve-stimulation evoked contractions.

In all experiments (protocols 1 and 2) consistent control responses (in absence or presence of prior drug) were obtained before beginning concentration-response curves, and two nerve-evoked responses were obtained in the presence of a given concentration of drug before the next concentration was added.

Drugs used were: cirazoline hydrochloride (gift: Synthelabo, Paris); clonidine hydrochloride (Catapres injection, Boehringer, Ingelheim); nifedipine (gift Bayer Leverkusen); prazosin hydrochloride (gift: Pfizer, Sandwich); yohimbine hydrochloride (Sigma, Poole).

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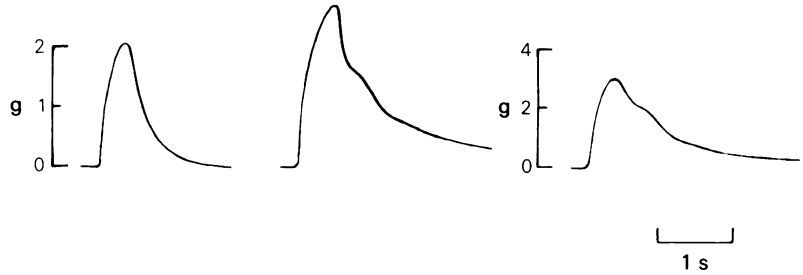

Figure 1 Effects of cirazoline on the isometric contraction to single pulse field stimulation of a prostatic portion of rat vas deferens (original recordings, curvilinear trace). Responses from left to right: control, and in the presence of two concentrations of cirazoline, 100 nM and 1 μM. Note change of calibration scale.

Results

In prostatic portions of the rat vas deferens, cirazoline (10 nM–10 μM) produced a concentration-dependent potentiation of the isometric contraction to a single stimulus pulse (protocol 1). The recordings of an original experiment are shown in Figure 1, and mean data in Figure 3. In epididymal portions, the effects of cirazoline were difficult to quantify since low concentrations (1–10 nM) potentiated nerve-evoked responses, while higher concentrations produced concentration-dependent spontaneous activity which masked any effects on nerve-evoked contractions; prazosin could not be employed to abolish these effects of cirazoline since prazosin also abolishes the nerve-evoked contraction in this portion of the vas deferens. However, the effects of cirazoline on epididymal portions was potentiation rather than inhibition (data not shown). All further experiments using protocol 1 were carried out on prostatic portions only.

In prostatic portions, clonidine (10 nM) inhibited the isometric contraction to a single stimulus pulse, and this inhibition was not prevented by pre-exposure to prazosin 30 nM (Figures 2 and 3). In the absence of prazosin, yohimbine (10 nM) caused a small reversal of the inhibitory effects of clonidine, and yohimbine (100 nM) restored the isometric contraction to pre-clonidine levels (Figures 2a and 3). Yohimbine (1 nM) had no greater effect than yohimbine (100 μM). In the absence of prazosin, cirazoline (100 nM) restored the response in the presence of

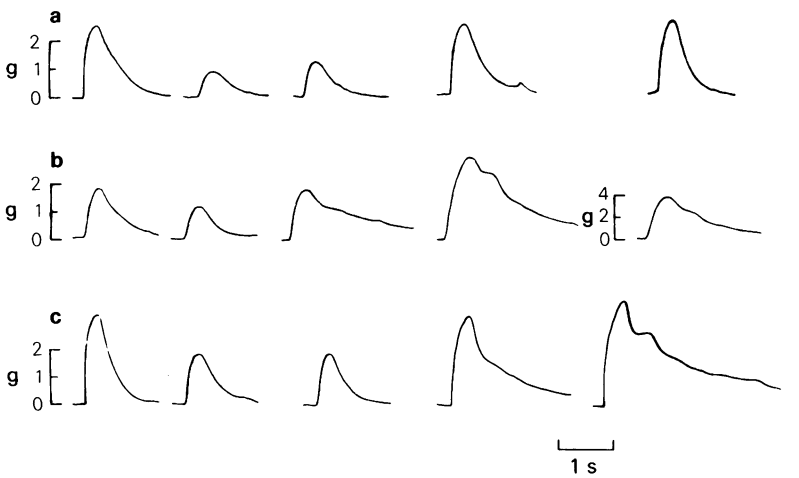

Figure 2 Effects of drugs on the isometric contraction to single pulse field stimulation of prostatic portions of rat vas deferens (original recordings, curvilinear trace). Responses from left to right: (a) control, in the presence of clonidine (10 nM), and with subsequent addition of yohimbine in 3 cumulative concentrations, 10 nM, 100 nM and 1 μM; (b) control, in the presence of clonidine (10 nM), and with subsequent addition of cirazoline in 3 cumulative concentrations, 100 nM, 1 μM and 10 μM (note change of calibration scale); (c) control in tissues pretreated with prazosin (30 nM), in the presence of clonidine (10 nM), and with subsequent addition of cirazoline in 3 cumulative concentrations, 100 nM, 1 μM and 10 μM.

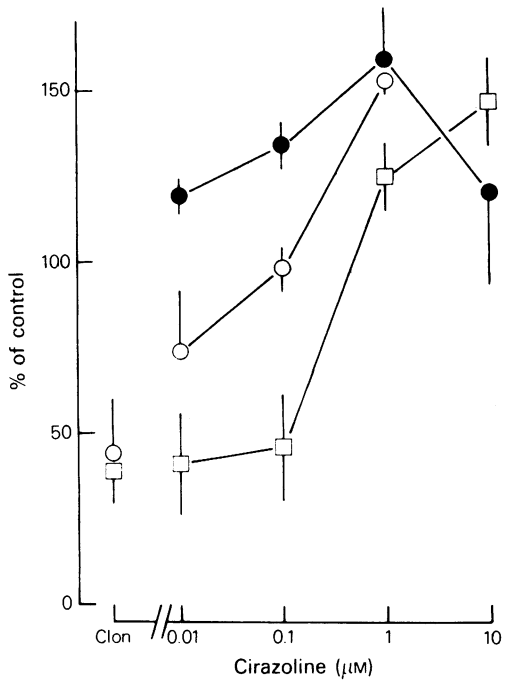

Figure 3 Concentration-response curves for the effects of cirazoline on the isometric contraction to single pulse field stimulation of prostatic portions of rat vas deferens. Symbols: in the absence of prior drug (●); following clonidine (10 nM) (○); following clonidine (10 nM) in prazosin (30 nM) pretreated tissues (□). Responses are expressed as a percentage of the preclonidine control response, and the response in the presence of clonidine is also shown (where applicable). Vertical bars represent s.e.mean. Results are the mean of at least 4 experiments.

clonidine to the pre-clonidine level, and higher concentrations of cirazoline potentiated the response above control levels (Figures 2b and 3). In the presence of prazosin (30 nM), cirazoline (100 nM) had no effect on the clonidine-induced inhibition, but cirazoline 1 μM restored the response to the control level and cirazoline 10 μM potentiated the response above control levels (Figure 2c). Prazosin produced an approximately ten fold reduction in the potency of cirazoline at reversing the inhibitory effects of clonidine.

In experiments using protocol 2, epididymal portions of the vas deferens were employed. Tissues were given nifedipine (10 μM) at the beginning of the experiment. This treatment abolishes the early non-adrenergic component of the contractile response to a single pulse, leaving only the adrenergic component so that the response becomes monophasic (Figure 4). Under these conditions, cirazoline not only failed to potentiate nerve stimulation-evoked contractions but produced a concentration-dependent inhibition of the contractile response to a single stimulus pulse (Figure 5). Following clonidine (10 nM), which caused a marked inhibition of the contractile response to a single stimulus, cirazoline (100 nM) caused a small recovery of the contractile response but higher concentrations again reduced the response (Figure 5).

Discussion

The main purpose of the present investigation was to examine the pre- and postsynaptic actions of cirazoline in influencing nerve-mediated isometric contractions of the rat isolated vas deferens. Cirazoline has been found to be a presynaptic agonist in the perfused cat spleen (Dubocovich *et al.*, 1980), but an antagonist of the presynaptic inhibitory effects of clonidine in rat vas deferens (Cavero *et al.*, 1982). Since these apparently contradictory results could be explained if cirazoline were a partial agonist at α_2-adrenoceptors (revealing its antagonist properties only in the presence of an agonist such as clonidine), it was of interest to examine whether cirazoline behaved as an α_2-agonist in the rat vas deferens in the absence of clonidine.

Whereas Dubocovich *et al.*, (1980) assessed the presynaptic effects of cirazoline against transmitter

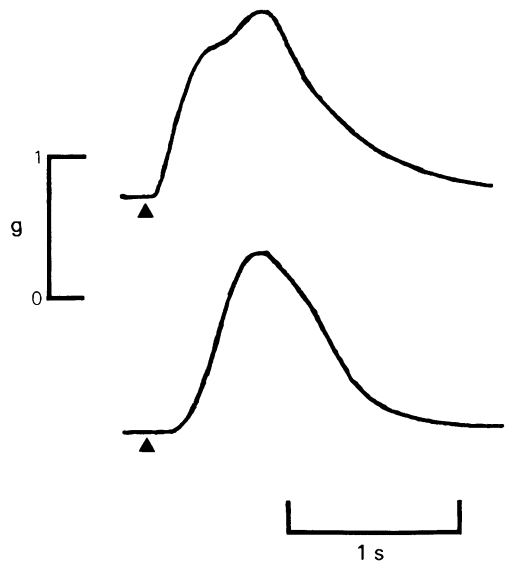

Figure 4 Effects of nifedipine on the isometric contraction to single pulse field stimulation of an epididymal portion of rat vas deferens (original recordings, rectilinear trace). Top trace: control; bottom trace: in the presence of nifedipine (10 μM). Arrows indicate when pulse was given.

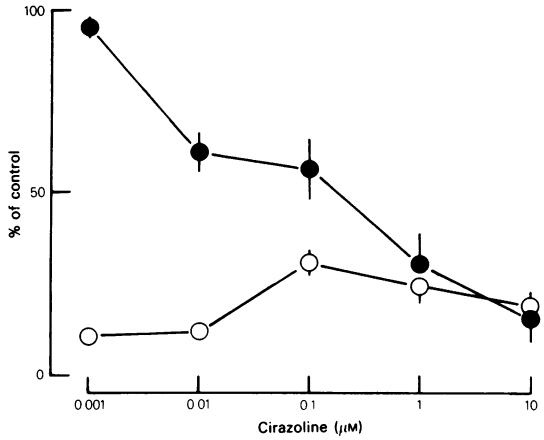

Figure 5 Concentration-response curves for the effects of cirazoline on the isometric contraction to single pulse field stimulation of epididymal portions of rat deferens in the presence of nifedipine (10 μM). Symbols: in the absence of clonidine (●): in the presence of clonidine (10 nM) (○). Responses are expressed as a percentage of control response or of the pre-clonidine control response. Vertical bars represent s.e.mean. Results are the mean of at least 4 experiments.

release by measuring directly the tritium overflow in tissues pre-incubated with [^{3}H]-noradrenaline, Cavero *et al.*, (1982) assessed presynaptic effects of cirazoline in terms of end organ response, namely isometric contraction. Caution is necessary when assessing presynaptic effects of drugs in terms of end organ response, since the net effects seen may be the result of both pre- and postsynaptic actions (see Dubocovich *et al.*, 1980). We therefore decided to investigate how the rat vas deferens could be used to elucidate pre- and postsynaptic effects of drugs when measuring only end organ responses.

The rat vas deferens gives reproducible isometric tension responses to single pulse field stimulation every 5 min, and the responses obtained are biphasic: the first (presumed non-adrenergic) component predominates at the prostatic end of the vas, and the second (adrenergic) component predominates at the epididymal end (see McGrath, 1978). To examine the effects of agonists on each component of the response in comparative isolation, tissues were bisected into prostatic and epididymal portions (McGrath, 1978; MacDonald & McGrath, 1980).

In prostatic portions, the α_2-antagonist yohimbine (Weitzell *et al.*, 1979), in the concentration range 10–100 nM, produced a concentration-dependent reversal of the inhibition by clonidine of nerve-mediated contractions, confirming that this inhibitory action of clonidine was at presynaptic α_2-adrenoceptors. Presynaptic α_2-receptors mediate a feedback inhibition whereby nerve-released noradrenaline inhibits its own further release (see Starke, 1977; 1981; Langer, 1981). Like yohimbine, cirazoline, in the concentration range 10–100 nM, reversed the inhibition by clonidine of nerve-mediated contractile responses. However, cirazoline had two actions not shared by yohimbine: firstly, cirazoline in higher concentrations did not merely reverse the inhibitory effects of clonidine, but actually potentiated responses beyond pre-clonidine control levels; secondly, in the absence of clonidine, cirazoline itself produced a concentration-dependent potentiation of nerve-evoked responses. These data confirm that cirazoline, in common with other α_1-agonists, can potentiate nerve-mediated contractions of the vas deferens by an action at postsynaptic α_1-receptors (see MacDonald & McGrath, 1980). As a corollary, the α_1-antagonist prazosin (30 nM) (Cambridge *et al.*, 1977) caused an approximate 10 fold shift in the cirazoline concentration-response curve for the reversal of the inhibitory effects of clonidine. Since this concentration of prazosin did not interfere with the inhibitory effects of clonidine, it is clear that the predominant effects of clonidine and cirazoline are by actions at different sites: namely presynaptic α_2- and postsynaptic α_1-receptors, respectively. It follows that the apparent antagonism by cirazoline of the inhibitory effects of clonidine was largely due to postsynaptic α_1-receptor agonism. No evidence was found for α_2-antagonism by cirazoline, although it cannot be ruled out completely as a contributory factor, in prostatic portions at least.

In epididymal portions, low concentrations of cirazoline potentiated nerve-mediated contractions as in the prostatic portion, but higher concentrations produced spontaneous activity, making results difficult to quantify (see also MacDonald & McGrath, 1980). The calcium entry blocker nifedipine abolishes the non-adrenergic component of the nerve-mediated response, but leaves the adrenergic component intact (Blakely *et al.*, 1981; Brown *et al.*, 1983). In addition, the postsynaptically-mediated potentiation of adrenergic nerve-induced contractions by noradrenaline (Brown *et al.*, 1983) and by the α_1-agonists cirazoline (present results) and amidephrine (Butler & Jenkinson, 1978) is greatly attenuated by nifedipine, implying that this potentiation involves calcium influx. The nifedipine treated epididymal portion is thus suitable for the investigation of the presynaptic effects of α_1-agonists in the absence of complicating postsynaptic effects. In the presence of nifedipine (10 μM), not only did cirazoline fail to potentiate the nerve-mediated contractions, but produced a concentration-dependent inhibition of responses. This presynaptic inhibitory action of cirazoline may be, at least in part, by an action at presynaptic α_1-receptors, since α_1-receptors

have been shown to occur presynaptically in the pithed rat heart (Kobinger & Pichler, 1980; 1982; Docherty 1983a,b). Even in the presence of nifedipine, cirazoline (100 nM) did produce a small reversal of the inhibitory effects of prior clonidine. However, this effect of cirazoline is presumably due to a residual post-synaptic potentiation of nerve-mediated contractions, since a change of slope occurs at this same concentration of 100 nM in the inhibitory concentration-response curve for cirazoline in the absence of clonidine. While it could be argued that the partial reversal of the inhibitory effects of clonidine demonstrated the partial agonist nature of cirazoline at α_2-receptors, this possibility is unlikely since cirazoline cannot also 'partially antagonize' its own inhibitory effects except by action at another site, presumably postsynaptic receptors. These data rule out the possibility of α_2-receptor mediated antagonism by cirazoline in the epididymal portion of the vas, and it would seem likely that this is true also for the prostatic portion.

In conclusion, cirazoline had two demonstrable effects on nerve-mediated responses in the rat vas deferens: firstly, a postsynaptic α_1-adrenoceptor mediated potentiation in both prostatic and epididymal portions, secondly, a presynaptic inhibitory action was observed in epididymal portions. No evidence was found for α_2-antagonism by cirazoline, and it is concluded that the previously reported antagonism of the presynaptic inhibitory effects of clonidine (Cavero *et al.*, 1982) was a physiological antagonism due to postsynaptic potentiation of responses by cirazoline, and demonstrates the dangers of misinterpretation inherent in assessing presynaptic effects of drugs when measuring only end organ response.

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