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The effect of clonidine on the response to stimulation of non-adrenergic non-cholinergic nerves in the guinea-pig urinary bladder in-vitro

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The effects of clonidine on the response of the guinea-pig urinary bladder detrusor muscle to stimulation of non-adrenergic non-cholinergic (NANC) nerves were investigated in-vitro. In tissues from both treated and chemically sympathectomized animals, in the presence of atropine (10^{-5} M) to inhibit cholinergic responses, clonidine (10^{-10}–10^{-6} M) invariably enhanced the contractile response to NANC nerve stimulation at 2, 5, 10 and 20 Hz. The enhancement was not inhibited by yohimbine (10^{-5} M), phentolamine (10^{-5} M), or the histamine H_1- or H_2-receptor antagonists mepyramine (10^{-6} M) or cimetidine (10^{-5} M) respectively. Phentolamine (10^{-5} M), like clonidine, enhanced the response to stimulation of NANC nerves at 2, 5, 10 and 20 Hz. Xylazine, another α_2-agonist, which, unlike both clonidine and phentolamine, is not a substituted 2-imidazoline compound, failed to enhance the response in the bladder to NANC nerve stimulation. The results suggest that clonidine enhances the response to NANC nerve stimulation, independently of its effects on α-adrenoceptors, by increasing the amount of NANC transmitter available.

In several smooth muscles, for example the rat anococcygeus (Gillespie 1972) and the rabbit recto-coccygeus (King & Muir 1981), the response to stimulation of the autonomic nerves is the resultant of more than one transmitter. In the detrusor muscle of the guinea-pig urinary bladder, field stimulation releases two excitatory transmitters, acetylcholine (ACh) which contributes some 20–30% to the observed contractile response (Ursillo 1961) and, the as yet unknown, transmitter from non-adrenergic non-cholinergic (NANC) nerves. Noradrenaline (NA) which is released from sympathetic nerves interacts mainly with β-adrenoceptors in the bladder (Raezer et al 1973; Krell et al 1981) to produce inhibition.

In such muscles, due to the presence of sympathetic nerves, the response to field stimulation will be affected clearly by drugs which interact with adrenoceptors. Clonidine, for example, modifies the excitatory, adrenergically-mediated, response of the rat anococcygeus to field stimulation both presynaptically via α_2-adrenoceptors (Idowu & Zar 1976) and also postsynaptically via α_1-adrenoceptors (Docherty & McGrath 1980). Clonidine, it is reported, also enhances the response to field stimulation in atropine-treated guinea-pig detrusor (Krell et al 1981) and antagonizes the effects of adenine nucleotides—proposed transmitters in NANC nerves (Burnstock 1972)—both centrally (Stone & Taylor 1978a,b) and peripherally (Ambache et al 1977). These results suggested the involvement of the NANC transmitter in the response to the drug, and suggested the possibility that clonidine, in addition to its effects on adrenoceptors, could also modify the response to the transmitter liberated from NANC nerves. The guinea-pig urinary bladder was chosen; in this tissue the NANC transmitter is excitatory. This obviates the need, in other tissues which contain NANC inhibitory nerves, to raise tone in order to examine the NANC response.

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METHODS

The detrusor was dissected from the urinary bladder of guinea-pigs of either sex (250–600 g), without removal of the mucosa, by the method of Ambache & Zar (1970). Detrusor strips (1 cm long and 4–5 mm wide) were each passed through a Ag–AgCl ring electrode for field stimulation and one end attached by thread to the base of an organ bath (10 ml) which contained Krebs solution at 37 °C gassed with 95% O_2 and 5% CO_2. The Krebs solution had the following composition (mM): NaCl 111·8, KCl 4·7, $CaCl_2$ 2·5, NaH_2PO_4 1·13, $MgCl_2$ 1·3, $NaHCO_3$ 25·0 and glucose 5·5. Since clonidine adheres to glass, the organ bath was the barrel of a (10 ml) polythene disposable syringe and the drug was added cumulatively without washing. The other end of each detrusor strip was attached to a strain gauge by thread and changes in tension produced by

drugs or by field stimulation (supramaximal voltage 0·1–0·5 ms at the frequencies indicated in the text) recorded isometrically and displayed on a pen recorder. A weight (1–3 g) was applied when each bladder preparation was set up; this minimized spontaneous activity and 20–30 min were allowed to elapse before commencement of each experiment when the weight was removed.

The regimen for pretreatment with 6-hydroxydopamine (6-OHDA) was that of Gibson & Gillespie (1973). 6-OHDA was given i.p. in doses of 2×50 mg kg^{-1} on day 1 and 2×100 mg kg^{-1} on day 4 and experiments were carried out on day 5 or 6. A small piece of bladder from each pretreated animal was subjected to a modification of the Falck histochemical procedure (Gillespie & Muir 1970) and examined microscopically for the presence of adrenergic nerves.

Drugs used were adenosine triphosphate, atropine sulphate, cimetidine, clonidine hydrochloride, 6-hydroxydopamine hydrochloride, mepyramine maleate, (−)-noradrenaline bitartrate, phentolamine mesylate, xylazine hydrochloride, yohimbine hydrochloride. They are expressed as final bath concentrations. All were dissolved in distilled water except 6-OHDA which was dissolved in 0·001 M HCl saturated with N_2 and NA which was dissolved in a solution of NaCl (0·9%) ascorbic acid (10^{-7} M) and disodium edetate (EDTA) (5×10^{-8} M).

Analysis of results

Log frequency response curves were constructed from individual experiments in the presence and absence of drugs. The mean (± s.e. of mean of a number, n, of experiments) effect of each dose of drug at different frequencies was then calculated and a paired Student's *t*-test used to test for significance ($P < 0·05$) between means.

RESULTS

Effect of clonidine on the contractile response to field stimulation

Field stimulation (0·1 ms supramaximal voltage using a maximum of 20 pulses every 60 s) of the isolated detrusor muscle strip produced a twitch response which reached a peak within 1 s; relaxation took 5–10 s. Under these conditions, the responses were entirely reproducible for periods up to 45 min, the longest period investigated. To block the motor effect of cholinergic transmission, atropine (3×10^{-5} M) was used routinely in all these experiments. In its presence, clonidine (10^{-10} to 10^{-6} M) enhanced the response to field stimulation at each frequency in both untreated and chemically sympathectomized tissues. The maximum enhancement (at 20 Hz, 10^{-6} M clonidine) measured some 200% of the control response (Fig. 2a). In untreated animals, as Fig. 1 shows, differences at each dose level were significant at all frequencies except 2 Hz. Only two experiments were conducted at this frequency, since it was not always possible to obtain a measurable response. In both cases clonidine, at each dose level increased the response to field stimulation.

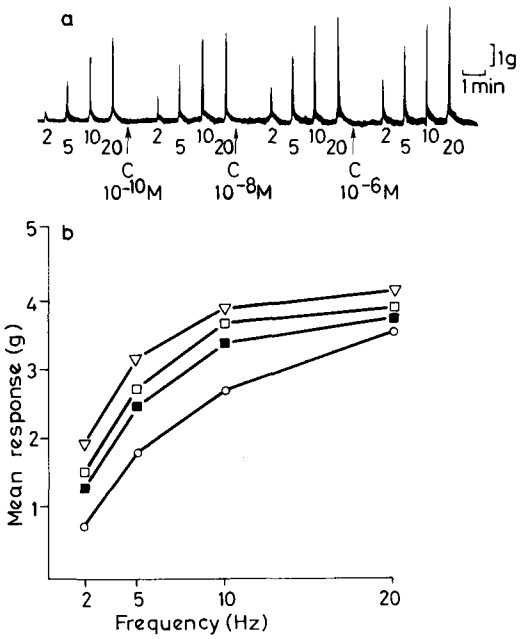

FIG. 1. (a) The enhancement by clonidine (C) (10^{-10}, 10^{-8} and 10^{-6} M) in the presence of atropine (10^{-5} M) of the excitatory response of the detrusor muscle of the guinea-pig bladder to field stimulation (10 pulses 2, 5, 10, 20 HZ, at 0·5 ms width supramaximal voltage). Each point in the graph (b) is the mean response (g, n = 8) at 5, 10 and 20 Hz; at 2 Hz, n = 2. Mean response at 5, 10 and 20 Hz in the presence and absence (controls) of clonidine at each dose level, differed significantly ($P < 0·05$ or better). At 2 Hz, clonidine enhanced the response to field stimulation at each dose level which measured (g) 0·49, 0·55; 1·21, 1·35; 1·47, 1·57; 2·2, 1·6 in control and clonidine-treated (10^{-10}, 10^{-8} and 10^{-6} M) muscles respectively. C 10^{-6}, ▽; C 10^{-8} □; C 10^{-10}, ■; control, ○.

Effect of other drugs interacting with α-adrenoceptors

The possibility that the enhancement produced by clonidine involved postsynaptic α_1-adrenoceptors was examined in both untreated and chemically sympathectomized animals. Phentolamine (10^{-5} M) which inhibits both α_1- and α_2-adrenoceptors (Docherty & McGrath 1980) not only failed to

inhibit the enhancement produced by clonidine but itself enhanced the response to field stimulation. The absence of α-adrenoceptor involvement in the enhancement produced by clonidine was evident also in chemically sympathectomized tissues. Both clonidine (10^{-10}–10^{-6} M) and phentolamine (10^{-5} M) enhanced significantly the response (Fig. 2A) to field stimulation, the effects of each drug being synergistic.

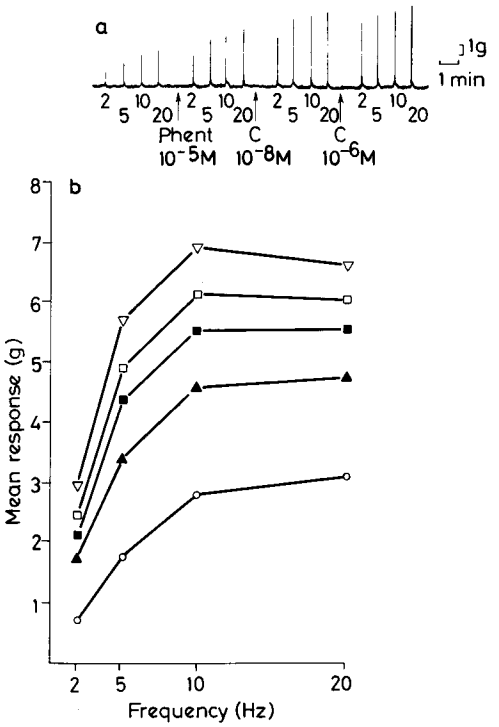

FIG. 2. (a) The enhancement by phetolamine (Phent) (10^{-5} M) alone and in the presence of clonidine (C) (10^{-8}, 10^{-6} M) on the excitatory responses of the detrusor muscle of the guinea-pig bladder to field stimulation (20 pulses every min, 2, 5, 10, 20 Hz, 0·5 ms supramaximal voltage). Atropine (10^{-5}) was present throughout. (b) Each point in the graph is the mean response (g) at each frequency in 5 experiments. Mean differences at each frequency between controls and either phentolamine alone or phentolamine and clonidine were significant ($P < 0.05$ or better) at each dose level. These experiments, carried out in chemically sympathectomized animals, suggest that the enhancement produced by clonidine and phentolamine is synergistic and independent of α-adrenoceptors. Phentolamine 10^{-5} M, ▲; all other symbols as for Fig. 1.

Further evidence that the action of clonidine was independent of its α-agonist properties came from two sets of experiments employing drugs with a more selective affinity for α_2-receptors. In the first, yohimbine (10^{-10} and 10^{-8} M) which is a relatively selective α_2-antagonist (Starke et al 1975) and which itself alone did not enhance the response to field stimulation also failed to inhibit the enhancement of the response to field stimulation produced by clonidine (10^{-10} M). In the second series, xylazine, like clonidine a selective α_2-antagonist (Docherty & McGrath 1980) in the dose range (10^{-10}–10^{-6} M) at which the latter was effective, failed to modify the response of the detrusor to field stimulation at 2, 5, 10 or 20 Hz.

Effect of histamine receptor antagonists

Histamine contracts the guinea-pig bladder via H_1-receptors, an effect antagonized by mepyramine (Ambache & Zar 1970). Though clonidine interacts with H_2-receptors in cardiac (Csongrady & Kobinger 1974) and smooth (Karppanen & Westerman 1973) muscle we have seen no reports of the presence of these receptors in the bladder. Clonidine and the H_1-antagonist antazoline are both 2-substituted imidazolines. The possibility that histamine receptors were involved in the enhancement by clonidine was examined therefore. No support for the involvement of either histamine receptor was obtained. Neither mepyramine (10^{-6} M) at a dose known to inhibit H_1-receptors in the bladder nor cimetidine (10^{-6} M) which inhibits H_2-receptors (Brogden et al 1978) modified the enhancement of the response to field stimulation by clonidine.

Effect of adenosine triphosphate

A dose (3×10^{-4} M) of adenosine triphosphate (ATP) was selected which produced an immediate rapid submaximal response similar to that produced by field stimulation (Ambache & Zar 1970). A single dose rather than several doses of ATP was used, to minimize the development of tachyphylaxis. The response to NANC nerve stimulation was unaltered by ATP, nor did the nucleotide affect the enhancement of the response by clonidine. From these results it seems unlikely that the enhancement produced by clonidine is mediated via postsynaptic adenine nucleotide receptors.

DISCUSSION

Clonidine enhanced significantly the overall contractile response to field stimulation, in a dose dependent fashion. In the detrusor, the contractile response to field stimulation is likely to be that to the NANC transmitter; the effect of acetylcholine being removed by atropine while the inhibitory response to simultaneously-released NA is apparently small since the magnitude of the contractile response, in

untreated and sympathectomized tissues, was very similar.

An identical finding in the detrusor (Krell et al 1981) was attributed to an interaction between clonidine and presynaptic α_2-adrenoceptors with an accompanying reduction in the amount of NA released for interaction with postsynaptic β-inhibitory adrenoceptors. The finding that the antagonist phentolamine also enhanced the NANC response led to the suggestion that phentolamine inhibited α_2-adrenoceptors and so enhanced the release of the NANC transmitter; the exact location of the receptors was unspecified.

The present results suggest that the site of enhancement by clonidine is unlikely to be on presynaptic α_2-adrenoceptors however. This is evident for the following reasons; the enhancement (a) occurred at both high (10, 20 Hz) and low (2, 5 Hz) frequencies although the effect of the drug on presynaptic α_2-adrenoceptors is more evident at low frequencies (b) was not mimicked by the selective α_2-adrenoceptor agonist xylazine nor antagonized by the α_2-antagonist yohimbine (c) was not abolished by chemical sympathectomy. The involvement of postsynaptic α_1-adrenoceptors is less likely for two reasons; (a) in the bladder the adrenoceptors are largely of the β-type and mediate relaxation; (b) phentolamine failed to block the enhancement. Interaction with α_2-adrenoceptors on cholinergic nerve terminals (Starke 1972) is also an unlikely site of enhancement since cholinergic receptors were blocked in the present experiments by atropine. Nor is there evidence, to support the participation of either H_1- or H_2-histamine receptors.

The response of the detrusor to NANC transmission was enhanced by clonidine while that to added ATP was not. Both clonidine and phentolamine inhibit the negative feedback (auto inhibition) of purines released from adrenergic nerves in vascular smooth muscle by KCl without causing an appreciable purine release (Katsuragi & Su 1981). It is tempting to suggest that, in the present experiments, the 2-imidazoline compounds clonidine and phentolamine increased the release of transmitter from NANC nerve terminals. Support for this view is the ability of phentolamine to increase the nerve-mediated release of noradrenaline in the rabbit heart presumably by interacting with α_2-adrenoceptors (Starke 1972) and of clonidine to enhance the frequency but not the amplitude of spontaneously occurring miniature end plate potentials in the rat phrenic nerve diaphragm (Lim & Muir 1982 unpublished) by interacting with catecholamine receptors in cholinergic nerve endings. Whether the effect observed in the present investigation on the bladder operates via receptors on NANC nerve terminals which control transmitter release in a way analogous to α_2-adrenoceptors on adrenergic nerve terminals or non-selectively cannot be at present ascertained.

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