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THE RESPONSE OF THE RABBIT RECTOCOCCYGEUS MUSCLE TO STIMULATION OF EXTRINSIC INHIBITORY NERVES AND TO SYMPATHOMIMETIC DRUGS

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1 The effects of stimulating sympathetic or non-adrenergic non-cholinergic (NANC) nerves or of the addition of noradrenaline (NA) or isoprenaline (Iso) were investigated on carbachol-induced tone and on contractions produced by acetylcholine (ACh) and by pelvic nerve stimulation, in the rabbit rectococcygeus muscle.

2 Each procedure reduced carbachol-induced tone; sympathetic and NANC nerve stimulation were equipotent but both were less effective than sympathomimetic drugs, of which Iso was the better. Both Iso and NA, but not sympathetic nerve stimulation, inhibited the contractions produced by pelvic nerve stimulation in a concentration-dependent manner. Against ACh-induced contractions, only Iso was effective. The effects of NANC nerve stimulation on the motor responses to pelvic nerve stimulation or to ACh were not investigated.

3 The inhibitory effects of sympathetic nerve stimulation, of Iso and of NA were reduced by propranolol (3×10^{-6} M) but unaffected by phentolamine (3×10^{-5} M).

4 In the presence of high (45 mM) concentrations of KCl, Iso and NA produced a concentration-dependent inhibition of tone that was antagonized by propranolol (3×10^{-6}M).

5 Methoxamine (4×10^{-7} to 4×10^{-5}M) and phenylephrine (5×10^{-7} to 5×10^{-5}M) which interact mainly with α_1-adrenoceptors, produced only small, transient reductions in carbachol-induced tone which were subject to tachyphylaxis, unlike those produced by Iso and NA. These inhibitory effects were antagonized by phentolamine (3×10^{-6}M) or azapetine (3×10^{-6}M).

6 Phenylephrine (5×10^{-4} M) and high doses (3×10^{-5}M or greater) of NA enhanced the contractile response to pelvic nerve stimulation and, on occasion, produced muscle contraction. These effects were antagonized by phentolamine (3×10^{-6}M).

7 These results suggest that inhibition of the rectococcygeus, a muscle which has no intramural nerve plexus, can be inhibited by stimulation of extrinsic NANC nerves, the transmitter for which is unknown and by sympathetic nerve stimulation via α- and β-adrenoceptors located postsynaptically on the muscle. Excitatory α-adrenoceptors may also be present.

Introduction

The principal inhibitory nerve supply to the gastrointestinal tract is provided by sympathetic and non-adrenergic, non-cholinergic (NANC) nerves. Analysis of the mechanisms underlying the inhibitory responses produced by nerve stimulation is complicated by the location of neurones within the gut wall. For example, the myenteric plexus, the major intramural nerve plexus, is the termination of many adrenergic fibres (Norberg, 1964) and the principal source of NANC nerves (Furness, 1969).

To overcome this difficulty, a preparation was used which possesses no intramural neurones and enables the inhibitory effects of extrinsic nerve stimulation on smooth muscle to be studied: namely the doubly-innervated preparation of the rabbit rectococcygeus muscle. Although not an integral part of the alimentary tract, the rectococcygeus is continuous with the longitudinal muscle of the colon and like the latter, receives both an excitatory and an inhibitory innervation (King, McKirdy & Wai, 1977; King & Muir, 1978; Blakeley, Cunnane & Muir, 1979). The inhibitory responses of the rectococcygeus to stimulation of both sympathetic and NANC nerves were examined and the mechanisms underlying these responses were investigated by use of drugs.

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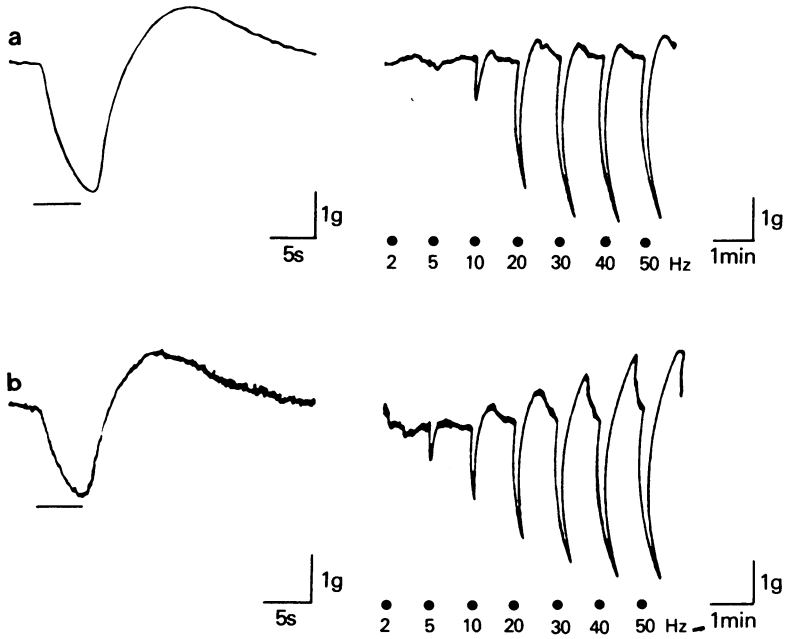

Figure 1 The inhibitory effect of nerve stimulation, at the frequencies shown, on carbachol (6×10^{-5} M)-induced tone in the rabbit rectococcygeus produced by stimulation (5 s, 0.25 ms, supramaximal voltage) of (a) the hypogastric and (b) the pelvic nerves. In each case the inhibitory response (a, b, left hand panels 30 Hz) had a similar time course and shape and was followed by an indomethacin-insensitive rebound excitation; the optimum frequency in each case was approximately 40 Hz (right hand panels). (a) and (b) were taken from the same experiment.

Methods

The rectococcygeus muscle, with attached hypogastric and pelvic nerves, was removed from Dutch rabbits (1.5 to 2 kg) as described previously (King *et al.* 1977). The preparation was mounted vertically in an organ bath (100 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 119, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11.7. The nerves were placed in separate fluid electrodes (Garry & Wishart 1951) and stimulated supramaximally at fixed frequencies for fixed periods of time as indicated in the text by the use of a Digitimer and Devices output stage. The frequencies employed ranged from 2 to 50 Hz, the duration of the stimulation period did not exceed 15 s and the pulse width was 0.5 ms. Generally, we used the minimum frequency of stimulation of the pelvic (30 Hz) or hypogastric (30 to 40 Hz) nerves required to produce the maximum response of the rectococcygeus. Tension was measured isometrically and displayed on a pen recorder.

The hypogastric nerve trunk was stimulated at a point within a distance of 1 cm from the inferior mesenteric ganglion. The pelvic nerves were stimulated peripheral to the large ganglion which is situated at the bifurcation of the pelvic nerve into its anterior and posterior branches (Langley & Anderson, 1895). Stimulation was therefore probably postganglionic in each case. Indeed, hexamethonium or tubocurarine, each in concentrations up to 3×10^{-4} M was ineffective in modifying the inhibitory response to stimulation of either the hypogastric or pelvic nerve. Although small ganglia can be observed along the intrinsic nerves to the muscle (Langley & Anderson, 1895) they are not important in transmission along the inhibitory pathways (Cocks, Crowe & Burnstock, 1979).

The following drugs were used; they were added singly or cumulatively as indicated in the text and are expressed as final bath concentrations: acetylcholine chloride, (−)-adrenaline tartrate, atropine sulphate, azapetine phosphate, carbachol chloride, cocaine hydrochloride, 6-hydroxydopamine hydrochloride, desmethylimipramine hydrochloride, guanethidine sulphate, hexamethonium bromide, indomethacin acetate, (±)-isoprenaline sulphate, methoxamine hydrochloride, (−)-noradrenaline bitartrate, 17β-oestradiol benzoate, phentolamine mesylate, (−)-phenylephrine hydrochloride, (±)-sotalol hydrochloride, tubocurarine chloride and yohimbine hydro-

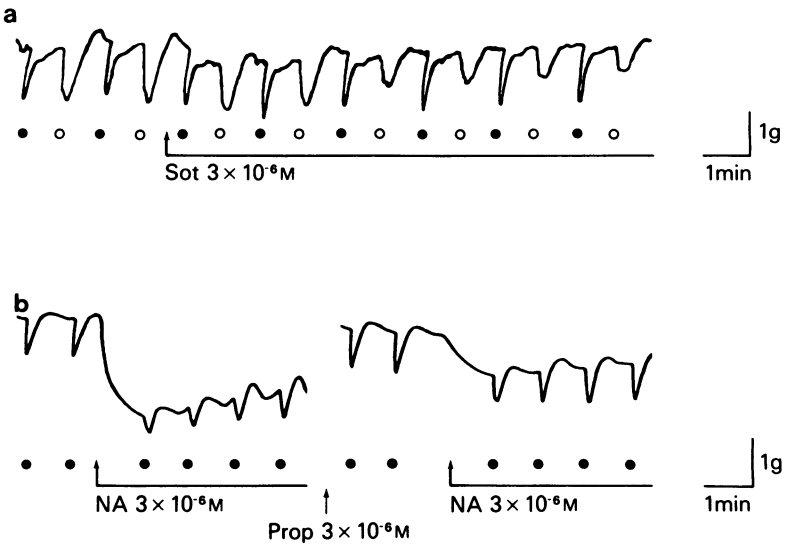

Figure 2 (a) The effect of sotalol (Sot 3 × 10^{-6} M ↑), on the ability of pelvic (●) or hypogastric (○) nerve stimulation (each for 5 s every min, 30 Hz, 0.25 ms supramaximal voltage) to inhibit carbachol (6 × 10^{-5} M)-induced tone in the rabbit rectococcygeus. Sotalol reduced the response to hypogastric (but not to pelvic) nerve stimulation indicating the involvement of β-adrenoceptors in the sympathetic response. (b) The antagonism, in another experiment, by propranolol (Prop 3 × 10^{-6} M ↑ added 5 min before start of right hand trace and present thereafter throughout the experiment) of the inhibitory action of noradrenaline (NA, 3 × 10^{-6} M) on carbachol (6 × 10^{-5} M)-induced tone in the rectococcygeus. Time between panels was 10 min. The inhibitory response produced by pelvic nerve stimulation (●, 5 times every min, 30 Hz, 0.25 ms supramaximal voltage) was temporarily reduced during exposure to NA which lowered tone.

chloride. Drugs were dissolved in 0.9% w/v NaCl solution (saline) and kept on ice during use. Solutions of adrenaline, noradrenaline and isoprenaline contained ascorbic acid (10^{-6} M) and disodium edetate (EDTA, 10^{-6} M). 6-Hydroxydopamine solutions also contained ascorbic acid and were kept at 4°C and bubbled with oxygen-free nitrogen for 1 h before use.

Indomethacin acetate was dissolved in 1% Na_2CO_3 and adjusted to pH 7 with 1 M HCl solution.

Analysis of results and statistics

Regression lines were fitted mathematically to the linear portions of log concentration-response curves from individual experiments. EC_{50} values (concentrations of agonists required to produce 50% of their own maximum effect) were calculated by analysis of the straight (20 to 80%) part of the log concentration-response curves.

Results were expressed as means ± s.e. mean with n as the number of experiments. A paired Student's t test was used to test for significance ($P < 0.05$) between means.

Results

Inhibition of carbachol-induced tone

The rectococcygeus muscle has no resting tone. In order to demonstrate the ability of the muscle to relax, the tone was raised by carbachol (6 × 10^{-5} M). This dose of carbachol produced a maximal contraction of the muscle. Although the initial high level was not maintained, sufficient carbachol-induced tone persisted for prolonged (2 h) periods to enable inhibitory responses to be demonstrated. The ability of hypogastric and pelvic nerve stimulation, NA and Iso to reduce carbachol-induced tone was investigated.

Stimulation (2 to 50 Hz) of the hypogastric and of the pelvic nerve each produced a frequency-dependent inhibition of tone; the optimum frequency in either case was approximately 30 to 40 Hz (Figure 1). Inhibition was followed by a rebound excitation which was unaffected by indomethacin (3 × 10^{-5} M for 1 h), which in this concentration was presumed to block prostaglandin synthesis. Both the inhibitory response to hypogastric nerve stimulation and the

subsequent excitation were abolished by 6-hydroxydopamine (6×10^{-4} M for 1 h *in vitro*: Wadsworth, 1973) and by guanethidine (3×10^{-7} to 3×10^{-5} M) and reduced (by about 50% in four experiments) by the β-adrenoceptor blocking drugs sotalol (3×10^{-6} M) (Figure 2a) and propranolol (3×10^{-6} M). The α-adrenoceptor antagonists phentolamine (3×10^{-7} to 3×10^{-5} M) and yohimbine (1.2×10^{-7} to 1.2×10^{-6} M) were ineffective. The inhibitory response to pelvic nerve stimulation was unaffected by any of these drugs or by atropine (10^{-6} M). At this concentration, atropine reduced tone slightly but left the inhibitory response to pelvic nerve stimulation unimpaired. These results confirmed the non-adrenergic, non-cholinergic nature of the inhibitory response.

Either NA (3×10^{-8} to 3×10^{-5} M) or Iso (2×10^{-8} to 2×10^{-5} M) inhibited carbachol-induced tone in a concentration-dependent fashion. The inhibition, in each case, was antagonized by propranolol (3×10^{-6} M, Figure 2) suggesting the involvement of β-adrenoceptors in the response.

Quantitatively, sympathetic nerve stimulation reduced carbachol-induced tone by approximately 25% and was much less effective than either NA or Iso. Sympathetic nerve stimulation was approximately equipotent with pelvic nerve stimulation. However, in only about half of the preparations did stimulation of the sympathetic nerves produce inhibition, compared with the almost invariable inhibition of carbachol-induced tone in response to pelvic nerve stimulation.

The α-adrenoceptor stimulants methoxamine (4×10^{-7} to 4×10^{-5} M) and phenylephrine (5×10^{-7} to 5×10^{-5} M) also produced a small transient inhibition of carbachol-induced tone. The inhibition was concentration-dependent but tachyphylaxis developed readily to repeated doses of each drug. The inhibition produced by each α-adrenoceptor agonist was abolished by the α-adrenoceptor blocking agents, aza-

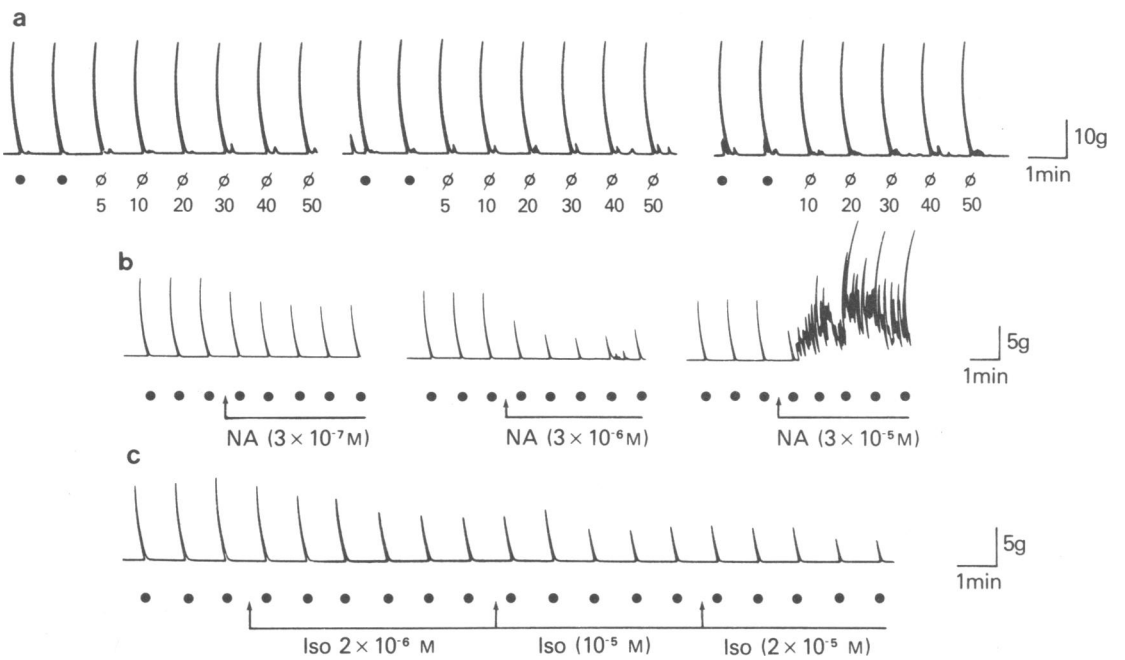

Figure 3 In the rabbit rectococcygeus (a) the ineffectiveness of hypogastric nerve stimulation (0.25 ms pulse width, supramaximal voltage at the frequencies indicated) on the motor response to pelvic nerve stimulation (●, 10 Hz for 1 s at 0.25 ms width and supramaximal voltage). Hypogastric nerve stimulation started 5, 10 or 15 s (left, middle and right hand panels respectively) immediately before and continued during the period of pelvic nerve stimulation. The combined period of hypogastric and pelvic nerve stimulation is indicated by ϕ. Time between panels was 1 min in each case. (b) The effect of increasing cumulatively, the bath concentrations of NA (↑) on the motor response to pelvic nerve stimulation (●, parameters as in (a)). NA was washed out after 5 min and the time between the panels was 2 min in each case. NA 3×10^{-7} and 3×10^{-6} M antagonized but at higher (3×10^{-5} M) concentrations became motor and enhanced the pelvic motor response. (c) In contrast to that of NA, the cumulative effect of isoprenaline (Iso ↑ 2×10^{-6}, 10^{-5} and 2×10^{-5} M) on the pelvic motor response (● parameters as in (a)) was entirely inhibitory.

petine (3×10^{-6} M) and phentolamine (3×10^{-6} M) but not by propranolol (3×10^{-6} M). This inhibition of carbachol-induced tone was quite different in pattern from that produced via β-adrenoceptors by Iso and NA. Furthermore, in the presence of methoxamine or phenylephrine the inhibitory effects of Iso and NA could still be demonstrated readily.

Inhibition of the contractile response to parasympathetic nerve stimulation and to acetylcholine

In the absence of carbachol, the tissue contracted to pelvic nerve stimulation. The ability of three inhibitory stimuli, hypogastric nerve stimulation, NA and Iso to reduce the height of the contractile response to pelvic nerve stimulation or to added ACh was next investigated. The hypogastric nerves were stimulated for 5, 10 or 15 s before and during the period of pelvic nerve stimulation (Figure 3a). Alternatively, muscles were exposed to the added catecholamines, either for 5 min or until the maximum inhibitory effect was obtained. During this period, the pelvic nerves were stimulated intermittently (10 pulses, 10 Hz every 60 s) (Figure 3b,c). When ACh replaced pelvic nerve stimulation, muscles were exposed to it (2.2×10^{-8} to 2.2×10^{-4} M) for 30 s; NA and Iso (3×10^{-8} to 3×10^{-6} M) were added 30 s before ACh.

Against the pelvic motor response, Iso and NA, produced a concentration-related inhibition of the evoked contractions with maximal inhibition of 50% at 5×10^{-6} M (NA) and of 80% at 2×10^{-5} M (Iso). Somewhat surprisingly, sympathetic nerve stimulation (2 to 50 Hz) did not affect the responses. It still failed to inhibit (or enhance) even in the presence of phentolamine (3×10^{-6} M) or drugs which reduce neuronal (cocaine at 3×10^{-6} M or desmethylimipramine at 3×10^{-6} M) and extraneuronal (oestradiol 3×10^{-6} M) uptake of NA. The effects of both NA and Iso were reduced significantly by propranolol (3×10^{-6} M), probably confirming the involvement of β-adrenoceptors in the inhibitory response.

When examined against the effects of ACh, only Iso inhibited the contractile response (Figure 4), doing so in a concentration-dependent manner. Its effect was to shift the ACh concentration-response curve to the right without affecting the maximum height. Regression analysis of the straight part of the ACh concentration-response curve showed a significant ($P < 0.01$, $n = 4$) change in the EC_{50} value in the absence ($5.8 \pm 0.2 \times 10^{-6}$ M) and presence ($1.1 \pm 0.1 \times 10^{-4}$ M) of Iso. The action of Iso was antagonized by propranolol confirming the role of β-adrenoceptors in the inhibitory response. Sympathetic nerve stimulation failed to inhibit the contractile response to ACh. The presence of neuronal (cocaine or desmethylimipramine) and extraneuronal (oestradiol) blockers of NA uptake failed to influence the ineffectiveness of sympathetic nerve stimulation. NA (3×10^{-5} M) added exogenously likewise failed to inhibit the contraction produced by ACh.

In contrast to the inhibitory effects produced by lower doses, NA in concentrations of 3×10^{-5} M and higher enhanced the contractile response to pelvic nerve stimulation and, on occasion, produced contraction of the muscle. NA (3×10^{-5} M) significantly enhanced the maximum contraction which could be obtained to pelvic nerve stimulation (Figure 5). When the frequency-response curves to nerve stimulation alone and in the presence of NA were expressed as a % of the maximum response obtained in each case to pelvic nerve stimulation, they were almost superimposable (Figure 5). This observation

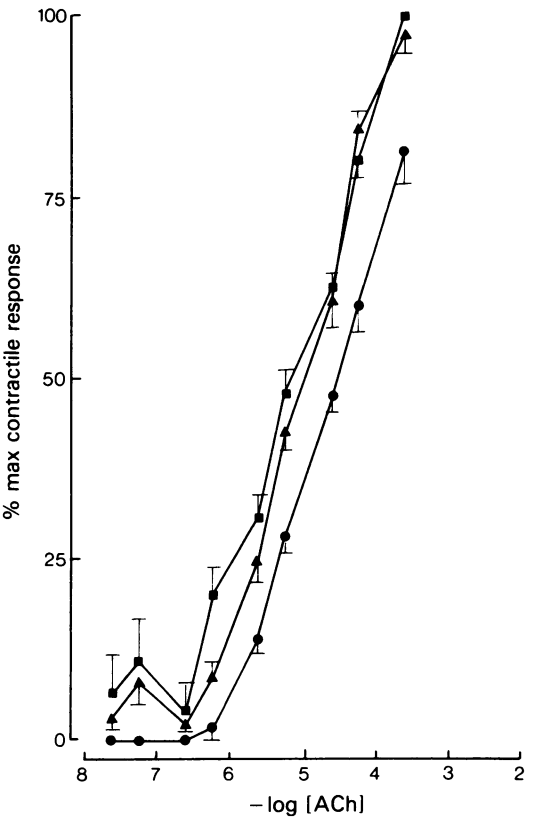

Figure 4 The inhibitory effect of isoprenaline (Iso, 2×10^{-5} M) on the motor response of the rabbit rectococcygeus to acetylcholine (ACh). In the presence of Iso, the control ACh dose-response curve (■) was displaced to the right (●). The mean EC_{50} values for ACh in the absence ($5.8 \pm 1.1 \times 10^{-6}$ M) and presence ($1.1 \pm 0.5 \times 10^{-4}$ M) of Iso differed significantly ($P < 0.01$, $n = 4$). The inhibitory effect of Iso was blocked by propranolol (3×10^{-6} M); in the presence of both these drugs, the dose-response curve and the EC_{50} values for ACh (▲) were not significantly different from those of controls.

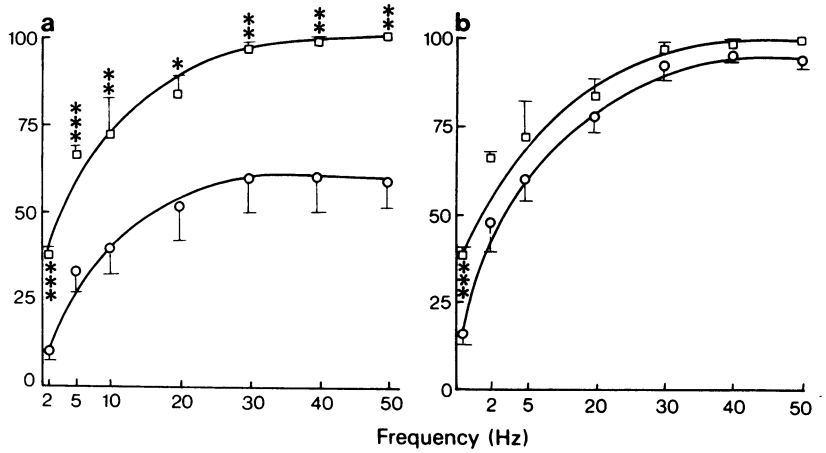

Figure 5 The effect of noradrenaline, (NA, 3×10^{-5} M) on the motor response to pelvic nerve stimulation (10 pulses at 0.25 ms width and supramaximal voltage at the stipulated frequencies, abscissa scale) in the rabbit rectococcygeus. (a) When expressed as a % of the maximum response obtained to pelvic nerve stimulation alone (○), the frequency-response curve in the presence of NA (□) was enhanced significantly. (b) When expressed as a % of its own maximum, the frequency-response curves were superimposable at all points except the first which differed significantly ($P < 0.001$). Each point represents the mean of nine observations (vertical lines show s.e. mean) and asterisks refer to significance of differences: $***P < 0.001$; $**P < 0.01$; $*P < 0.02$. These results suggest that the enhancement of the pelvic nerve response produced by NA was not due to any change in the receptor characteristics and could have arisen from the synergism of two separate excitatory stimuli.

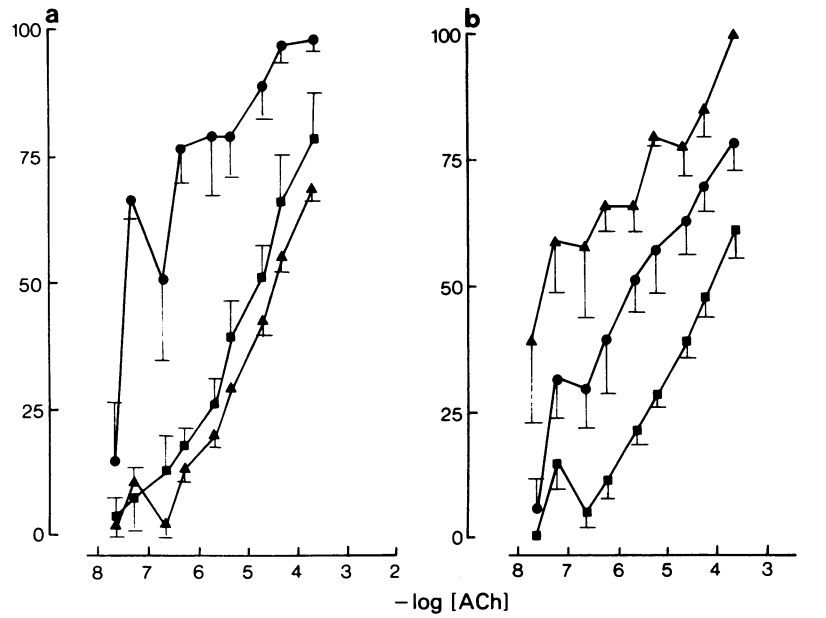

Figure 6 (a) The effect of noradrenaline (NA 3×10^{-5} M) on the motor response of the rabbit rectococcygeus to acetylcholine (ACh). The motor response (ordinate scale) is expressed as a % of the maximum control, ACh (■) response. In the presence of NA (●) the motor response to ACh was enhanced; the mean EC_{50} values for ACh in the absence (control, $5.0 \pm 1.0 \times 10^{-6}$ M) and in the presence of NA ($1.7 \pm 0.16 \times 10^{-7}$ M) differed significantly ($P < 0.02$, $n = 3$). The enhancement was antagonized by phentolamine (3×10^{-6} M, ▲) in the presence of which the mean EC_{50} value for ACh was not significantly different from that of control (■). (b) The ability of NA to enhance (●) the motor response to ACh was further increased by propranolol (3×10^{-6} M). In the presence of NA (3×10^{-5} M) and propranolol, the ACh dose-response curve (▲), expressed as a % of the maximum control ACh response (■), was displaced to the left. The mean EC_{50} values for ACh alone ($5.2 \pm 2.2 \times 10^{-6}$ M) and in the presence of NA and propranolol ($3.3 \pm 0.5 \times 10^{-7}$ M) differed significantly ($P < 0.001$, $n = 4$).

suggested that the amine was not altering directly the transmitter-receptor interaction but that the enhancement probably arose from a synergistic action of the amine with ACh. That this effect involved postsynaptic α-adrenoceptors was suggested by its being mimicked by phenylephrine (5×10^{-6} M), which is relatively specific for α_1-adrenoceptors and which was some ten times more potent than NA. The effect of phenylephrine was antagonized by the α-adrenoceptor antagonist phentolamine (3×10^{-6} M). On the other hand, propranolol (3×10^{-6} M) further increased the enhancement produced by the sympathomimetic amines.

Sympathetic nerve stimulation (2 to 50 Hz for 15 s before and during the period of pelvic nerve stimulation), either alone or with propranolol or with drugs which reduce the neuronal (cocaine, desmethylimipramine) and extraneuronal (oestradiol) uptake of NA, failed to enhance the pelvic motor response. Thus, while added catecholamines could, depending upon the dose employed, inhibit or enhance the response to pelvic nerve stimulation, sympathetic nerve stimulation was ineffective in either respect (Figure 3).

Phenylephrine, NA and adrenaline (Adr, 3×10^{-5} M) also enhanced the contractile responses produced by ACh (Figure 6). Based on the changes in the EC_{50} values the order of potency was phenylephrine > Adr > NA. Again the enhancement produced by each of these drugs was antagonized by phentolamine (3×10^{-6} M) and that of NA and Adr (but not of phenylephrine) was increased by propranolol (3×10^{-6} M). However, sympathetic nerve stimulation alone, with propranolol, or with cocaine or desmethylimipramine and oestradiol, failed to enhance the ACh response. These results suggested the presence of α-excitatory as well as β-inhibitory adrenoceptors in the preparation.

The postsynaptic location of the α-excitatory adrenoceptors was confirmed in preparations depolarized by adding sufficient KCl to the Krebs solution to give a final concentration of approximately 45 mM KCl. After 20 min of equilibration, NA (1.5×10^{-7} to 3×10^{-5} M) or Iso (10^{-7} to 2×10^{-5} M) was added. Each drug produced a dose-dependent, propranolol-sensitive inhibition of KCl-induced tone; Iso was the more effective and produced up to 50% inhibition (Figure 7). In doses greater than 3×10^{-6} M, NA produced small transient contractions of the muscle unaccompanied by a further reduction in tone. In the presence of phentolamine (3×10^{-6} M) these contractions were abolished and the inhibitory effect of NA enhanced. In the presence of both phentolamine and propranolol (3×10^{-6} M), only the inhibitory effect of NA was observed. The inhibition of KCl tone induced by Iso was unaffected by phentolamine (3×10^{-6} M).

Discussion

Stimulation of the extrinsic nerves (sympathetic or

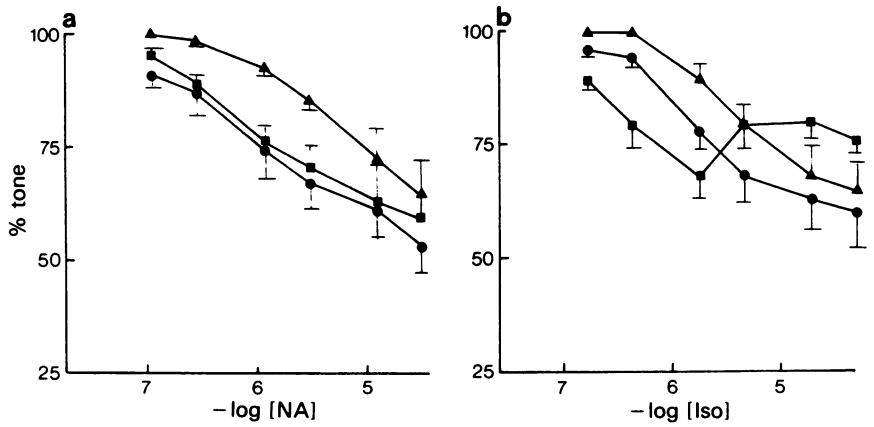

Figure 7 The effect of (a) isoprenaline (Iso) (■) and (b) noradrenaline (NA) (■) on KCl (45 mM)-induced tone in the rabbit rectococcygeus. Drugs were added cumulatively; 3 min were allowed to elapse between additions of each dose. Both drugs inhibited tone; NA (but not Iso) produced contractions at higher concentrations (b). The decrease in tone was inhibited significantly in the presence of propranolol and phentolamine (each 3×10^{-6} M, ▲) but unaffected by phentolamine alone (3×10^{-6} M, ●). The mean EC_{50} value for NA in the presence of phentolamine and propranolol ($2.70 \pm 0.36 \times 10^{-5}$ M) was significantly different ($P < 0.01$, $n = 3$) from that for NA in the presence of phentolamine (●) only ($0.98 \pm 0.33 \times 10^{-5}$ M, $n = 3$). The mean EC_{50} value for Iso in the presence of phentolamine and propranolol ($3.9 \pm 0.33 \times 10^{-5}$ M) was significantly different ($P < 0.01$, $n = 3$) from that for Iso and phentolamine (●) only ($8.5 \pm 0.42 \times 10^{-6}$ M). These results suggest a postsynaptic location of α-excitatory and β-inhibitory adrenoceptors.

NANC) or addition of NA or Iso each inhibited carbachol-induced tone in the rabbit rectococcygeus. Iso was the most effective and both drugs were better than either form of nerve stimulation. The size of the inhibitory response to stimulation of sympathetic and of NANC nerves was comparable. The inhibitory effects produced by sympathetic nerve stimulation, Iso and NA were antagonized by sotalol and by propranolol indicating the involvement of β-adrenoceptors, but phentolamine, which blocks α-adrenoceptors, was ineffective. There was no specific antagonist for NANC nerve-mediated responses.

Against pelvic nerve or ACh-induced contractions, only Iso inhibited the response to either stimulus; NA was effective only in the presence of phentolamine while sympathetic nerve stimulation, even in the presence of drugs which prevented NA uptake and blocked α-adrenoceptors, was totally ineffective. The effects of both Iso and NA were reduced significantly by propranolol.

The ability of NA to reduce carbachol-induced but not ACh-induced tone may be related to the particular spasmogen employed. The contraction produced by cholinoceptor stimulants in the muscle comprises both a phasic and a tonic component (see King *et al*, 1977). Carbachol was used to raise tone for prolonged periods during which time the muscle was presumably depolarized making it incapable of further contraction. Under these circumstances, stimulation of α-excitatory adrenoceptors was ineffective and so both NA and Iso inhibited tonic muscle contractions via β-adrenoceptors. When ACh was added instead, it was present for brief (30 s) periods; the depolarization produced was presumably much less than with carbachol and the muscle remained capable of further excitation. It is suggested that the α-adrenoceptor excitatory effect of NA would then offset the β-adrenoceptor-mediated inhibition of tone; indeed, the catecholamine failed apparently to relax the muscle unless in the presence of phentolamine which blocked α-adrenoceptors. Isoprenaline, on the other hand, which interacts only with β-adrenoceptors, inhibited ACh-induced contractions.

The effects of NANC nerve stimulation could not be investigated. These nerves comprise part of the extrinsic pelvic nerve supply and the mechanical response of the muscle to their stimulation can be demonstrated only in the presence of tone.

These results confirmed the importance of β-adrenoceptors in the mediation of catecholamine-induced inhibition in the rectococcygeus. The location of the β-adrenoceptors is probably the smooth muscle itself, for the following reasons. Isoprenaline which acts mainly on β-adrenoceptors on smooth muscle was, by far, the most effective inhibitor. In preparations exposed to high potassium, so as to depolarize both muscle and nerves, both Iso and NA produced a propranolol-sensitive reduction in tone. It is therefore unlikely that the site of the receptors was on nerve fibres.

In addition, the α-adrenoceptor agonists phenylephrine and methoxamine inhibited carbachol-induced tone by an effect less pronounced than, though quite distinct from, that produced via β-adrenoceptors by either Iso or NA. The brief inhibition suggests that the inhibitory mechanism activated by phenylephrine and methoxamine is probably minor compared with that mediated via β-adrenoceptors. The location of the α-adrenoceptors is uncertain. Failure to detect the response in KCl-depolarized preparations where the inhibition produced by NA was entirely blocked by propranolol, favours a presynaptic site. Inhibitory α-adrenoceptors are present on parasympathetic nerve terminals in the rabbit colon (Gillespie & Khoyi, 1977). On the other hand, the effectiveness of the α_1-adrenoceptor agonists phenylephrine and methoxamine, and of the α_1-adrenoceptor inhibitors azapetine and phentolamine, supports a postsynaptic location. Catecholamines hyperpolarize smooth muscle membranes by a postsynaptic α-adrenoceptor action, antagonized, in the taenia, by phentolamine (Bülbring & Tomita, 1969). In the rectococcygeus, NA also hyperpolarizes the muscle membrane. The extent of the hyperpolarization in some preparations exceeded that evoked by pelvic nerve stimulation (Blakeley, *et al.*, 1979). Consistent with the present findings, the α-adrenoceptor inhibitory effect rapidly declined. The cause of this decline in the α-mediated inhibitory response is uncertain. It could have arisen from a desensitization of the inhibitory α-receptor or could represent the resultant of two adrenergic components, the initial inhibitory effect being opposed by the subsequent excitatory action of NA at higher doses. In the colon, exogenously added NA inhibited smooth muscle directly. The receptors involved were resistant to block by propranolol (Gillespie & Khoyi, 1977). It is tempting to suggest that the inhibitory effect produced by phenylephrine and methoxamine may be mediated via similar receptors. If this were the case, failure to detect these receptors in depolarized preparations may be due to their sparse occurrence.

In addition to inhibitory α- and β-adrenoceptors, the present results indicate that excitatory α-adrenoceptors may also be present. NA in high doses and phenylephrine enhanced the contractile response to pelvic nerve stimulation and to ACh; moreover NA but not Iso, produced contractile responses in preparations depolarized by high concentrations of KCl. These excitatory responses were blocked by phentolamine. Neither the location of these receptors nor their function is certain.

The rectococcygeus can also be inhibited by stimulaton of NANC nerves. The response obtained was readily distinguishable from that produced by sympa-

thetic nerve stimulation or by sympathomimetic drugs. The location of NANC receptors cannot be inferred from the present results. The transmitter in these nerves has not been identified precisely though ATP or a closely related purine has been proposed (Burnstock, 1972). In this 'purinergic' hypothesis, although both presynaptic and postsynaptic receptors for purine compounds are envisaged, the ability of added ATP, which interacts mainly with postsynaptic receptors in the presence of indomethacin, to inhibit tone in the rectococcygeus (Cocks *et al.*, 1979) suggests a postsynaptic site is mainly involved.

The question also remains as to whether NANC or sympathetic nerves provide the more important physiological inhibitory mechanisms. Both the frequency (hypogastric 30 to 40 Hz, pelvic 30 Hz) and the number of pulses (100 to 150 in each case) required to produce a maximal inhibition were comparable. The present results give some indication that NANC nerves may be the more important. Sympathetic nerve stimulation itself was effective only against carbachol-induced tone and did not inhibit the contractions produced by pelvic nerve stimulation or ACh. Moreover sympathetic nerve stimulation, unlike stimulation of NANC nerves, failed, on occasion, to produce an inhibitory response. Together with the sparse adrenergic innervation of the muscle (Burnstock *et al.*, 1978) these results suggest that NANC nerves provide the major inhibitory mechanism in the rectococcygeus. Interestingly, during the present investigation, spontaneous contractions occurred in a few preparations; these contractions were reduced significantly by stimulation of the pelvic nerves in the presence of atropine. In contrast, even prolonged (30 s) stimulation of the hypogastric nerves was ineffective.

It is interesting to compare the present findings with those in the rabbit colon (Garry & Gillespie, 1955) with which the rectococcygeus is continuous. Both tissues receive a NANC and a sympathetic (hypogastric) innervation; both nerves are inhibitory. In the colon, α-adrenoceptors on preganglionic parasympathetic fibres play an important inhibitory role in the control of synaptic transmission in Auerbach's plexus (Gillespie & Khoyi, 1977). On the other hand, β-adrenoceptors located postsynaptically in the colon may inhibit both spontaneous tone and drug-induced contractions but have little significant effect on the motor nerve pathway. Such differences between the rectococcygeus and the colon reflect their anatomical and functional differences. In the colon, extrinsic sympathetic nerve stimulation is more effective in inhibiting synaptic transmission at Auerbach's plexus (Gillespie & Khoyi, 1977); NANC cell bodies in the plexus may also modify neural transmission as well as inhibiting smooth muscle directly (Daniel, 1978). In the absence of intrinsic neurones, in the rectococcygeus, neural inhibition by extrinsic sympathetic and NANC nerve stimulation is mediated more directly on the smooth muscle itself.

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