Br. J. Pharmac. (1985), **86**, 427–437

Mechanisms underlying the electrical and mechanical responses of the guinea-pig internal anal sphincter to field stimulation and to drugs

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1 The electrical membrane characteristics and the response of the circular muscle of the guinea-pig internal anal sphincter (i.a.s.) to field stimulation were studied *in vitro* using intracellular microelectrodes and conventional mechanical recording techniques.

2 The i.a.s. developed its own tone (3–4 g), following initial stretch (1 g) and spontaneous spike potentials were evident. In the absence of spike potentials, tone declined and disappeared. Tone was not significantly reduced by phentolamine (1×10^{-6}M). The resting membrane potential, measured between spontaneous spike potentials, was -45 ± 3.0 mV ($n = 224$); the space constant (λ) was 1.13 ± 0.1 mm ($n = 13$). Spikes usually overshot by approximately 15 mV.

3 The frequency of spike potential discharge (1–3 Hz) varied with the degree of membrane depolarization, being increased in K^+-rich and decreased in K^+-deficient solutions or by the presence of Mn^{2+}. It was not significantly affected by Cl^--withdrawal but was increased in Na^+-deficient solutions with or without tetrodotoxin (TTX; 1×10^{-6}M).

4 Field stimulation (1–20 Hz, 0.5 ms, supramaximal voltage) produced inhibitory junction potentials (i.j.ps) and relaxed tone; at high frequencies (50 Hz or greater), contractions were observed but excitatory junction potentials (e.j.ps) were not. I.j.ps and relaxations were inhibited by apamin (1×10^{-6}M), TTX (1×10^{-6}M) but not by atropine (1×10^{-6}M), phentolamine (1×10^{-6}M) or hexamethonium (1×10^{-6}M).

5 I.j.ps were reduced by hyperpolarization and enhanced by depolarization of the membrane by current pulses (15 s). The mean equilibrium potential for the i.j.p. was -94 mV (correlation coefficient, $\gamma = 0.71$, $n = 5$, $p < 0.001$). I.j.ps were enhanced in K^+-deficient solutions and reduced in K^+-rich solutions. Together these results suggest that the i.j.p. is mediated by an increased G_K. The absence of $[Ca^{2+}]_o$ or the presence of Mn^{2+} (2 mM) abolished the i.j.p.; in contrast Na^+-deficient or Cl^--free solutions were ineffective in this respect.

6 Tetraethylammonium (5–50 mM) abolished the i.j.p.; the accompanying relaxation was reduced by about 80%. The major aspect of the relaxation to nerve stimulation is mediated by membrane hyperpolarization.

Introduction

Field stimulation of inhibitory non-adrenergic non-cholinergic (NANC) nerves results in relaxation of many mammalian smooth muscles in both the gastrointestinal tract and elsewhere (see review by Gillespie, 1982). The accompanying electrical changes may differ, however, in different muscles. In the spontaneously-active taenia coli, relaxation is accompanied by a significant membrane hyperpolarization (Bennett *et al.*, 1966) and a fall in membrane resistance produced by an increased G_K. In the non-spontaneously active bovine retractor penis (BRP; Byrne & Muir, 1984; 1985), rat anococcygeus (Creed *et al.*, 1975) and canine fundus (Morgan *et al.*, 1981) a much smaller membrane hyperpolarization is observed in response to field stimulation of NANC nerves. In the BRP, this hyperpolarization is accompanied by an increase in membrane resistance (Byrne & Muir, 1984). Hitherto, detailed investigations of the electrical aspects of NANC mechanisms have largely employed propulsive smooth muscle. The purpose of the present study was to investigate the electrical basis of the inhibitory response in the circular non-propulsive muscle of the

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guinea-pig internal anal sphincter (i.a.s.), a tissue with a powerful inhibitory NANC innervation (Costa & Furness, 1973). Some of these results have been communicated previously (Lim & Muir, 1983a,b; 1984).

Methods

Anatomy

The anatomy of the anal region in the guinea-pig has been described by Furness & Costa (1973). The location of the i.a.s. coincides with a thickening (3 mm long × 5 mm broad) of the rectal wall. The sphincter consists of a band of circular fibres more densely arranged than elsewhere in the region (Figure 1).

Of the two muscle layers of the rectum, the outer, longitudinal muscle extends over the inner, circular, sphincteric area. Each muscle layer terminates 5–10 mm short of the anal margin and is separated from the skin by a band of largely connective tissue with a few circular smooth muscle fibres. The term 'internal anal sphincter' (i.a.s.) in this paper denotes the circular muscle free from the longitudinal muscle layer.

Adult guinea-pigs (300–400 g) were killed by cervical dislocation and bled. The peritoneal cavity was opened, the viscera removed and the pubic symphysis split. A segment of the rectum, including the anal region, was removed and transferred to a Sylgard-coated petri dish containing Krebs solution which was bubbled with 95% O_2 and 5% CO_2 (pH = 7.4). The Krebs solution had the following composition (mM): NaCl 118.4, $NaHCO_3$ 25.0, NaH_2PO_4 1.13, KCl 4.7, $CaCl_2$ 2.7, $MgCl_2$ 1.3, glucose 11.0. The rectum was pinned out and the skeletal muscle and connective tissue forming the external anal sphincter removed. A longitudinal incision was then made in the ventral wall of the rectum, caudally, from the region of the swelling. The mucosa and submucosa were removed and the i.a.s. identified with a dissection microscope. A horizontal strip (2 mm long × 10 mm broad, unstretched) of sphincter with attached longitudinal layer was dissected out. The longitudinal muscle layer was carefully removed. One end of the sphincter was attached, by thread, to a force displacement transducer (Grass FTO3C) and the other end passed through Ag/AgCl ring electrodes for field stimulation and pinned to the Sylgard base of a horizontal organ bath (4 ml). The sphincter was perfused with Krebs solution (6 ml min^{-1}) at 36 ± 0.5°C.

Intracellular electrical recordings were made with capillary glass micro-electrodes (resistance 20–40 MΩ) filled with 3 M KCl. Signals were passed to a unity gain impedance amplifier (W.P. Instruments Model M4A) displayed on a storage oscilloscope and u.v. recorder (EMI 8E6150 MKII) and stored on an instrumentation tape recorder (Racal Store 4DS).

To confirm that the i.a.s. was free of longitudinal muscle, noradrenaline (NA; 1×10^{-7}–1×10^{-5}M) and acetylcholine (ACh; 1×10^{-7}–1×10^{-5}M) were used routinely. NA depolarized the membrane, increased spike frequency and contracted the sphincter; separated longitudinal muscle strips were relaxed and the membrane hyperpolarized. On the i.a.s., ACh was ineffective; on the longitudinal muscle ACh depolarized the membrane, increased spike frequency and caused contractions.

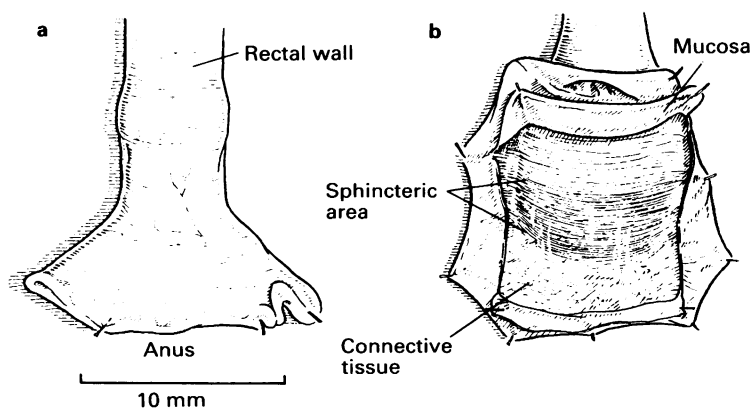

Figure 1 Diagrammatic indication of the position of the guinea-pig internal anal sphincter (× 5). In this stretched preparation in (a), the approximate location of the sphincter is indicated by a slight swelling of the rectum wall. In (b), a longitudinal incision in the rectum has been made and the mucosa and submucosa removed. The sphincter can be identified as a band of circular smooth muscle in which the fibres are more densely arranged than elsewhere in the rectum wall. The sphincter is thus easily differentiated from the connective tissue, which is adjacent to the anus.

Displacement of the membrane potential was carried out by the Abe & Tomita (1968) partition method. Values of the displaced membrane potential were obtained by subtraction of the voltage transient recorded outside the cell during the current pulse (Bywater & Taylor, 1980).

The concentration of potassium in the bathing medium $[K^+]_o$ was modified by replacing KCl with an equivalent amount of NaCl in the Krebs solution and Na^+-deficient solutions prepared by substituting NaCl with choline chloride, the other ions remaining unchanged. In Cl-free solutions, NaCl was replaced by an equivalent amount of sodium benzenesulphonate, and KCl, $MgCl_2$ and $CaCl_2$ each by an equivalent amount of K_2SO_4, $MgSO_4$ and $CaSO_4$ respectively. Ca^{2+}-free solutions were prepared by removing $CaCl_2$ from the Krebs solution without compensation.

6-Hydroxydopamine (6-OHDA) was dissolved by sonication in 0.9% saline, containing ascorbic acid (1 mg ml^{-1}) kept at 4°C on ice and bubbled with O_2-free N_2 for at least 30 min before use. The drug was given intraperitoneally in doses of 150 mg kg^- on day 1 and 250 mg kg^{-1} on day 2; experiments were carried out on day 3. A small piece of the sphincter 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.

In view of the presence of noradrenergic nerves in the sphincter and of cholinergic nerves in the adjacent longitudinal muscle (Costa & Furness, 1973), atropine (1×10^{-6}M) and phentolamine (1×10^{-6}M) were routinely present in all experiments employing field stimulation except where otherwise stated.

Drugs

The following drugs were used; concentrations in the bath refer to the salts. Except where otherwise stated, drugs were dissolved in 0.9% saline and perfused in Krebs solution. ACh (Sigma), apamin (Serva), atropine sulphate (Sigma), hexamethonium bromide (Light), 6-hydroxydopamine hydrochloride (Sigma), manganese chloride (BDH), (−)-noradrenaline bitartrate (Koch-Light), phentolamine mesylate (Ciba), tetraethylammonium hydrochloride (TEA) (Sigma) and tetrodotoxin (TTX) (Boehringer).

Analysis of results

Results were expressed as means ± s.d. of a number, n of experiments. Student's t tests were used to test for significance ($P < 0.05$) between means.

Results

Membrane properties of the i.a.s.

When set up, the i.a.s. had no existing tone. The preparation was then gently stretched by subjecting it to a tension of 1 g. During the next 15–30 min, 3–4 g tone was developed spontaneously by the sphincter and this was maintained throughout each experiment. The development of tone depended on the maintenance of a spontaneous spike potential discharge. In the absence of tone, spikes were not observed. Tone was not significantly affected by either cholinoceptor (atropine, 1×10^{-6}M) or adrenoceptor (phen-

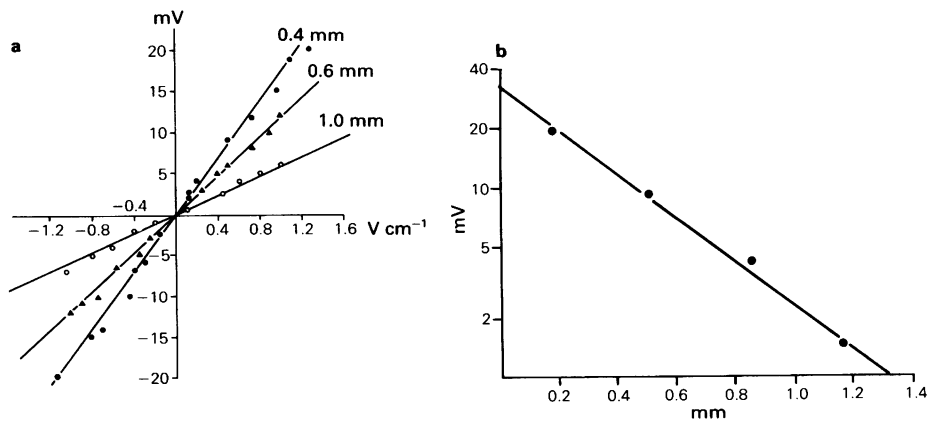

Figure 2 (a) Current (V cm^{-1})/voltage (mV) relationships at 0.4 mm (●), 0.6 mm (▲) and 1.0 mm (○) from the stimulating plate in the guinea-pig internal and sphincter in the presence of atropine (1×10^{-6}M) and phentolamine (1×10^{-6}M). No rectification was observed with either hyperpolarizing or depolarizing currents over the range employed. (b) The relationship between the amplitude of the electrotonic potential (3 s) plotted semi-logarithmically (ordinate scale) and the distance from the stimulating plate. The slope of this line was used to calculate the space constant ($\lambda = 1.13 \pm 0.12$, $n = 13$).

tolamine, 1×10^{-6}M) antagonists, suggesting its myogenic origin.

The mean resting membrane potential, measured between spontaneous action potentials, was -45.0 ± 3.0 mV ($n = 224$), significantly ($P < 0.001$) less than that of cells of the isolated longitudinal muscle layer (-51.5 ± 3.6 mV, $n = 24$). Two different patterns of spontaneous spike potentials were evident. The first, observed in 40% of sphincteric cells (and in all longitudinal muscle cells) consisted of large spikes discharging at 1–2 Hz, most of which reached 60 mV in amplitude. Each spike was preceded by a slow depolarization characteristic of pacemaker activity and followed by a hyperpolarization of about 5–7 mV. Oscillations in tone were often seen. In the remainder of cells, smaller spike potentials (30–40 mV in amplitude at 2–3 Hz), either intermittent or continuous, were observed, often superimposed on a membrane depolarization and accompanied by oscillations in tone. Spontaneous inhibitory junction potentials (i.j.ps) or excitatory junction potentials (e.j.ps) were not observed.

To study the spread of current within the tissue, rectangular pulses of constant inward or outward current were applied (for 10 s) through extracellular plate electrodes and recorded at various distances from the stimulating partition in an Abe & Tomita (1968) bath. Electrotonic potentials could be recorded up to 2 mm from the stimulating plate; they decayed exponentially with distance. Membrane potential displacement (by up to 25 mV) provided a linear current-voltage relationship, indicating the presence of cable properties. No rectification of the membrane potential was seen over the range of currents employed (Figure 2a). The mean value of the space constant (λ) calculated from the decay of the electrotonic potential was 1.13 ± 0.07 mm, $n = 13$ (Figure 2b).

Effects of alterations of external ionic environment

Reduction in $[K^+]_o$ from 4.7 mM (control) to 2.3 mM, hyperpolarized the membrane from -44 ± 1.6 mV ($n = 25$) to -60 ± 2.6 mV ($n = 11$), abolished spike discharge and reduced muscle tone (Figure 3a). Increase in $[K^+]_o$ to 10 mM then to 20 mM (Figure 3b and a, respectively) depolarized the membrane progressively to -36.1 ± 3.8 mV ($n = 11$) then to -30.3 ± 4.4 mV ($n = 12$). The frequency of spike discharge was increased at 10 mM $[K^+]_o$. As the frequency of spikes increased and the membrane potential decreased, tension rose. The relationship between membrane potential and $[K^+]_o$ for K^+-enriched solutions was not linear over the range 2.3–20 mM (Figure 3e).

Removal of $[Ca^{2+}]_o$, hyperpolarized the membrane, reduced, then abolished, spike frequency (Figure 4) and reduced tone. Similar results were obtained foll-

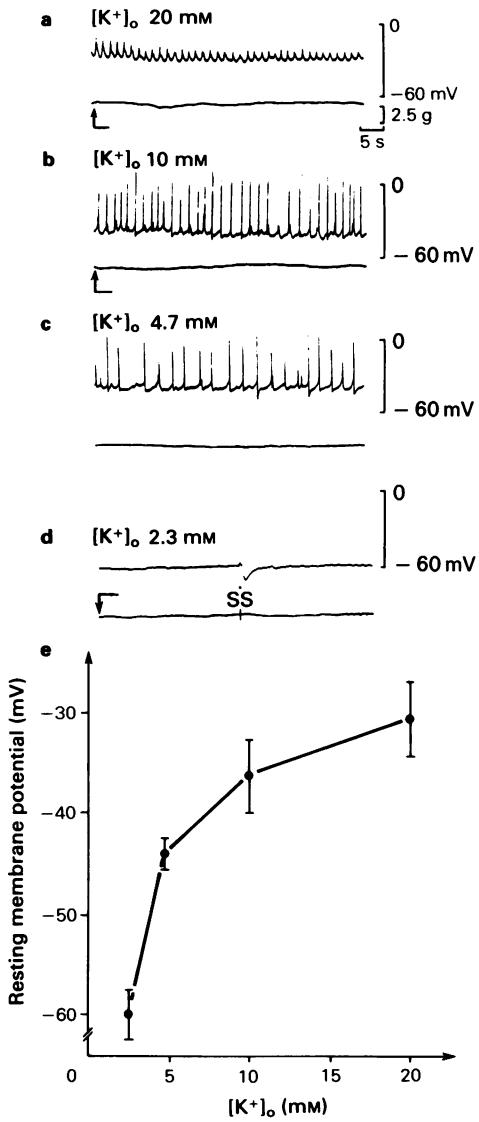

Figure 3 (a–d) The effect of altering $[K^+]_o$ on the spontaneous electrical and mechanical activity in the guinea-pig internal anal sphincter. Upper traces, show electrical and lower traces, mechanical events and the level of tone. The level of resting tone is that in (c): increases (a and b) and decreases (d) in tone are indicated by ↑ and ↓, respectively. Increase in $[K^+]_o$ from normal, 4.7 mM, (c) to 10 mM (b), increased the frequency of spike discharge and raised tone. Further increase in $[K^+]_o$ to 20 mM (a) reduced spike amplitude but did not further significantly affect spike frequency or tone. Reducing $[K^+]_o$ to 2.3 mM (d), abolished spike discharge and lowered tone. The hyperpolarization to field stimulation of inhibitory nerves (single pulse, SS, 0.5 ms, supramaximal voltage) remained, although the accompanying relaxation was not evident, presumably because of the reduced tone. Phentolamine (1×10^{-6}M) and atropine (1×10^{-6}M) were present throughout. (e) Shows that the relationship between $[K^+]_o$ and membrane potential was non-linear over the range 4.7–20.0 mM ($n = 11$).

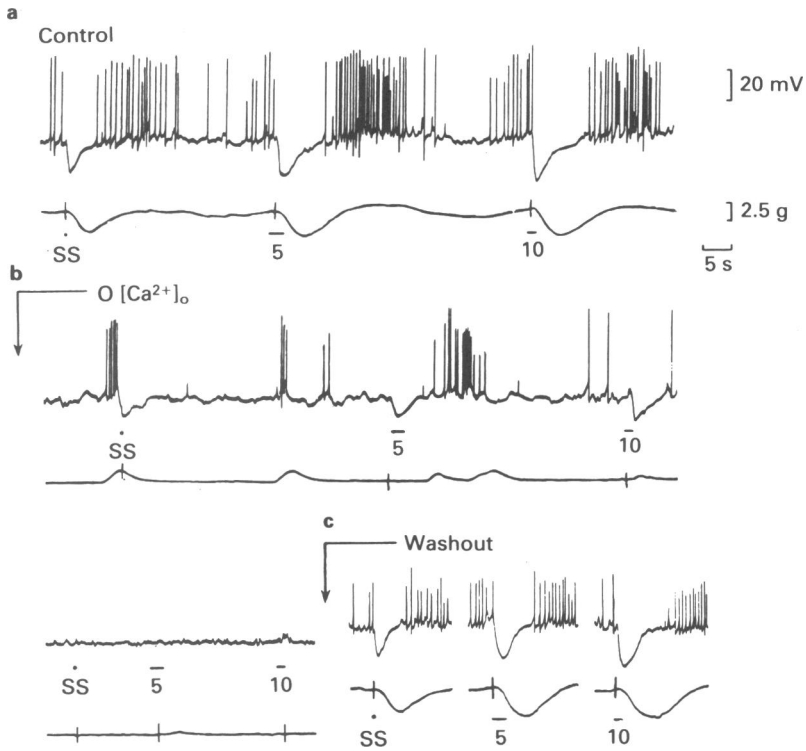

Figure 4 The reversible effect of $[Ca^{2+}]_o$-removal on spontaneous activity and on the response to field stimulation (single pulse, SS, and trains of 5 pulses at 5 and 10 Hz, 0.5 ms, supramaximal voltage) in the guinea-pig internal anal sphincter. Upper trace represents electrical and lower trace, mechanical events. (b) Removal of $[Ca^{2+}]_o$ reduced, then abolished the spontaneous membrane discharge and the hyperpolarization produced by field stimulation at each frequency. Tone was reduced and spontaneous mechanical fluctuations were evident during residual spike discharge. (c) These effects were reversible following washout. The period between the traces (c) was 12 min. Phentolamine (1×10^{-6} M) and atropine (1×10^{-6} M) were present throughout.

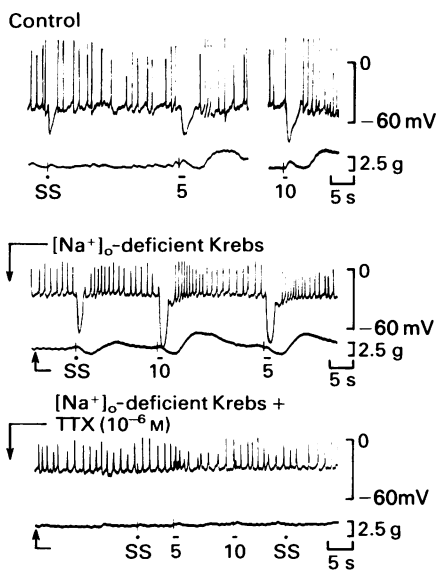

Figure 5 The effects of $[Na^+]_o$-deficient Krebs solution on the spontaneous activity and on the response to field stimulation (0.5 ms, single pulse, SS, and trains of 5 pulses at 5 and 10 Hz, supramaximal voltage) in the guinea-pig internal anal sphincter in the presence of atropine (1×10^{-6} M) and phentolamine (1×10^{-6} M). Replacement of NaCl by choline chloride depolarized the membrane and, as a result, the frequency of spike discharge and tone were increased. The amplitude of spike discharge fell progressively during membrane depolarization but was not further affected by tetrodotoxin (TTX; 1×10^{-6} M). The i.j.p. and mechanical relaxation were not reduced in $[Na^+]_o$-deficient Krebs solution; the i.j.p. was initially enhanced due to membrane depolarization but both were abolished by TTX (1×10^{-6} M).

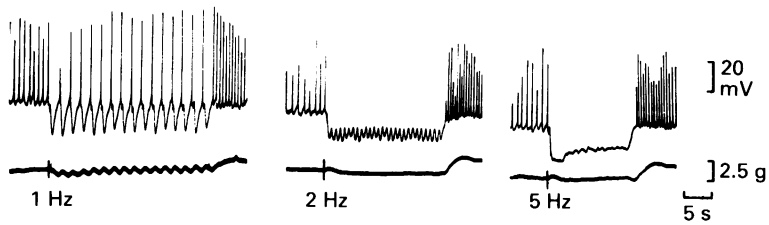

Figure 6 The electrical (upper) and mechanical response of the guinea-pig internal anal sphincter to prolonged (20 s or more) periods of field stimulation at 1, 2 and 5 Hz (0.5 ms, supramaximal voltage). At 1 Hz, field stimulation produced i.j.ps, inhibited spontaneous spike discharge and relaxed the muscle; there was no facilitation. Above 1 Hz, summation was evident; field stimulation produced a maintained hyperpolarization on which, at lower frequencies (2 Hz), discrete, small i.j.ps were superimposed. The i.j.ps gradually disappeared at frequencies above 5 Hz. Phentolamine (1×10^{-6}M) and atropine (1×10^{-6}M) were present throughout.

owing addition of Mn^{2+} (2 mM) which blocks Ca^{2+} channels (Edwards, 1982).

Na^+-deficient solutions depolarized the membrane, increased the frequency of spike discharge and raised tone. As a result of the depolarization, spike amplitude was reduced but unaffected further by TTX (1×10^{-6}M) (Figure 5). In $[Cl^-]_o$-free Krebs, neither the membrane potential nor the pattern of spike discharge was affected significantly. These results suggest that neither Na^+ nor Cl^- mediate the spontaneous discharge of spike potentials.

Evoked membrane activity

Field stimulation (1–20 Hz, 0.5 ms and supramaximal voltage) inhibited spike discharge, hyperpolarized the membrane and relaxed tone. I.j.ps in response to single pulses (maximum 20 mV) or to trains of stimuli (5–20 Hz) could reach 40 mV (20–40 mV), were frequency-dependent (optimum 5–10 Hz), but failed to facilitate. When field stimulation was continued for prolonged periods (20–30 s), discrete i.j.ps could be obtained below 1 Hz with no summation. At 2 Hz, summation was evident and a maintained membrane hyperpolarization with small discrete i.j.ps was seen. Above 2 Hz, summation of the responses produced a maintained hyperpolarization with little evidence of individual i.j.ps (Figure 6). A post-stimulus increase in spike frequency and a rebound contraction, a common characteristic of the electrical response to NANC nerve stimulation, were observed regularly throughout these experiments (see also Figures 4 and 5).

I.j.ps were not significantly affected by atropine (1×10^{-6}M), phentolamine (1×10^{-6}M) or hexamethonium (1×10^{-6}M) alone or in combination but were abolished by TTX (1×10^{-6}M), confirming their post-

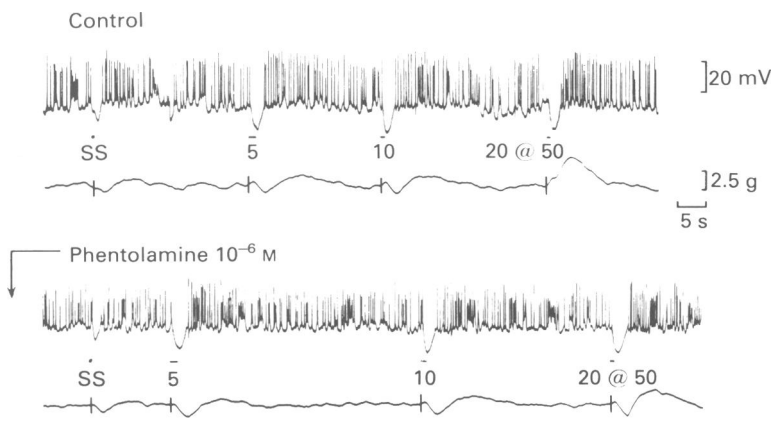

Figure 7 The effect of phentolamine (1×10^{-6}M) on the electrical (upper trace) and mechanical responses of the guinea-pig internal anal sphincter to field stimulation (0.5 ms, single pulse, SS, and trains of 5 pulses at 5 and 10 Hz and 20 pulses at 50 Hz, supramaximal voltage). Atropine (1×10^{-6}M) was present throughout. Phentolamine failed to affect the responses to field stimulation except those at 50 Hz, when the mechanical excitation was changed to a relaxation. The amplitude of the post stimulus excitatory response remained unaltered in the presence of phentolamine. There was no evidence of e.j.ps accompanying the contraction in the absence of phentolamine.

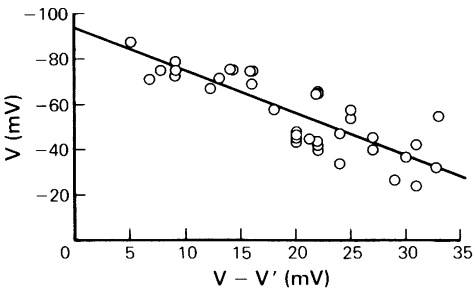

Figure 8 The linear relationship between the amplitude of the inhibitory response to nerve stimulation (V − V′) and the level of the displaced membrane potential (V) in the guinea-pig internal anal sphincter as computed by regression analysis. No hyperpolarization occurred when the membrane potential was −94 mV, suggesting that this is the equilibrium potential. Membrane potential was displaced by 15 s rectangular pulses of constant current between two stimulating plates by the method of Abe & Tomita (1968). Phentolamine (1×10^{-6}M) and atropine (1×10^{-6}M) were present throughout.

ganglionic, neural origin. Moreover, the nerves responsible for the i.j.p. were not sympathetic; i.j.ps and relaxations persisted in tissues pretreated with 6-OHDA, in which significant fluorescence in the Falk procedure was absent. This suggests that the inhibitory NANC transmitter and NA are present in different nerve fibres. E.j.ps were never observed though mechanical contractions sensitive to phentolamine (1×10^{-6}M) were sometimes seen at high frequencies (50 Hz) (Figure 7). Phentolamine reduced the amplitude of the contraction that occurred immediately following the stimulus period revealing the inhibitory response. The amplitude of the post-stimulus excitatory response remained unaltered in the presence of phentolamine. In an attempt to unmask any electrical response accompanying the contraction, the spike potential discharge, which might have obscured the e.j.ps, was abolished by reducing the bath temperature to 30–32°C. Under these circumstances, field stimulation at 50 Hz produced i.j.ps (but no e.j.ps) and phentolamine-sensitive contractions.

The effect of field stimulation on membrane resistance

The inhibitory transmitter, by opening channels in the membrane sets the membrane potential towards an equilibrium potential (E). The membrane potential at the peak of the response, V′, is given by

$$V' = V' - \frac{g}{g + G}(V - E)$$

where V and G are, respectively, the membrane potential and conductance in the absence of transmitter and g is the increase in conductance (Ginsborg, 1967). The hyperpolarization (V − V′) should therefore vary linearly with the displacement of the membrane potential (V) from the equilibrium potential (V − E) when the ionic channels are fully opened and no hyperpolarization should occur when V = E.

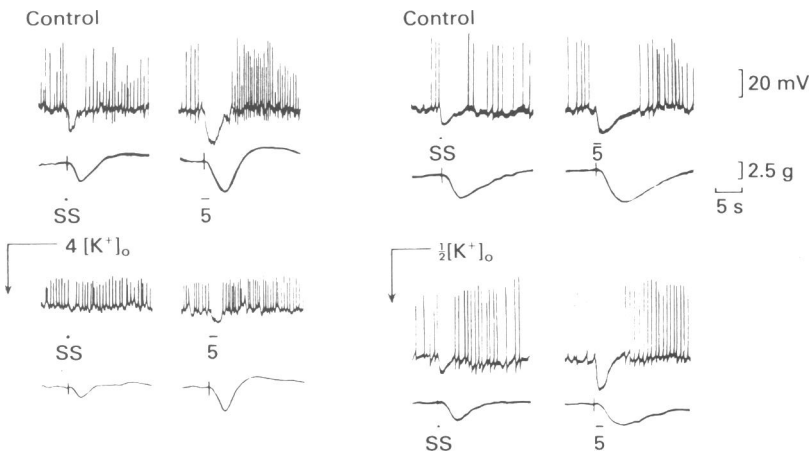

Figure 9 The effect of alteration in the $[K^+]_o$ on the i.j.ps induced by field stimulation of the inhibitory nerves (0.5 ms, single pulse, SS, and trains of 5 pulses at 5 and 10 Hz, supramaximal voltage) in the guinea-pig internal anal sphincter. In each set of records, upper trace, shows electrical and lower trace, mechanical events. Quadrupling the $[K^+]_o$, to 18.8 mM, depolarized the membrane, increased tone and reduced the i.j.ps. The anticipated accompanying reduction in the mechanical response was masked by the increase in tone. Halving the $[K^+]_o$ to 2.35 mM, hyperpolarized the membrane, decreased the tone and increased the i.j.ps. Due to the decrease in tone, no accompanying increase in the mechanical response to nerve stimulation was observed. Phentolamine (1×10^{-6}M) and atropine (1×10^{-6}M) were present throughout.

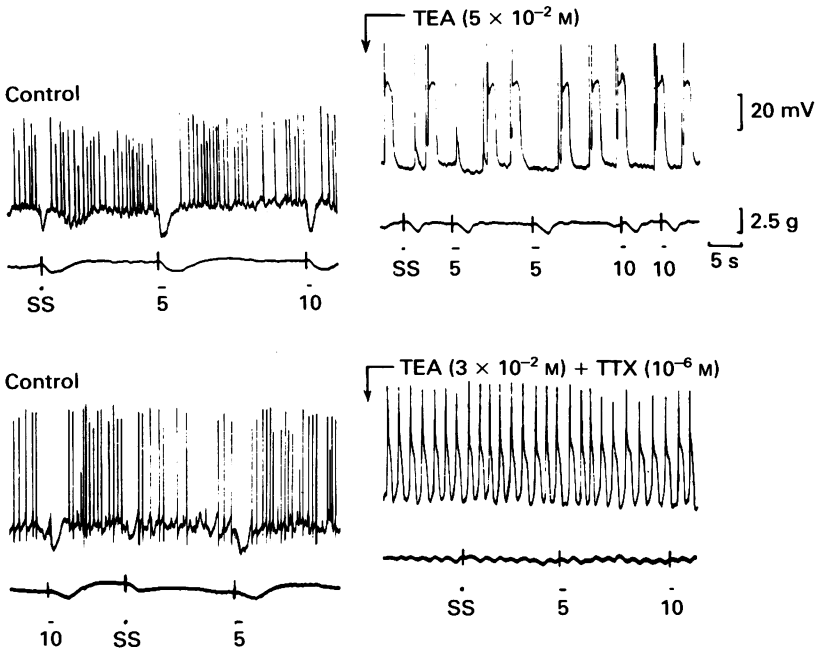

Figure 10 The effect of tetraethylammonium (TEA; 3 and 5×10^{-2}M) alone and in the presence of tetrodotoxin (TTX; 1×10^{-6}M) on the intracellularly measured inhibitory responses to NANC nerve stimulation (0.5 ms, single pulse, SS and 5 pulses at 5 and 10 Hz, supramaximal voltage) in two cells of 2 different preparations of the guinea-pig internal anal sphincter. In each record, the upper trace shows the electrical and the bottom trace, the mechanical events. TEA depolarized the membrane potential, prolonged the duration (but reduced the frequency) of spike potentials. Tone was increased, the i.j.p. abolished, and the accompanying mechanical relaxation reduced. The mechanical relaxation would have exceeded controls but for the presence of TEA since tone is necessary to display the inhibition. On this basis the reduction in the mechanical response was 80%. The residual relaxation was abolished by TTX (1×10^{-6}M). Phentolamine (1×10^{-6}M) and atropine (1×10^{-6}M) were present throughout.

The membrane potential was displaced by passage of extracellular current between two plates for 15 s in the Abe & Tomita bath and field stimulation (0.5 ms, supramaximal voltage) was applied after the beginning of the current pulse. A linear relationship between the amplitude of membrane hyperpolarization produced in response to field stimulation ($V - V'$) and membrane potential was obtained and the intercept of the line on the ordinate scale indicated that when $(V - V') = 0$, the value of V, and therefore of the equilibrium potential (E), was between -89 and -106 mV, in different cells (-94 mV, correlation coefficient, $r = 0.71$, $P < 0.001$, $n = 5$) (Figure 8). This figure is approximately that for E_K (Jones, 1981), suggesting that this ion is involved in the inhibitory NANC nerve response.

Effects of change of external ionic environment on i.j.ps

Reducing the $[K^+]_o$ by half hyperpolarized the membrane and enhanced the i.j.p. (Figure 9). Tone was reduced as a result; the relaxation produced by field stimulation was demonstrably smaller than in controls. Conversely, doubling (to 9.4 mM) then quadrupling the $[K^+]_o$ to 18.8 mM reduced the i.j.p. at each frequency. There was no apparent reduction in the relaxation to field stimulation. The anticipated reduction was offset, presumably by the increase in tone. Tone is required to display the relaxation and any increase in tone would enhance the amplitude of the mechanical inhibition. When the $[K^+]_o$ was raised to 50 mM (not shown) membrane potential was decreased, tone increased, the amplitude of spontaneous activity reduced and the i.j.ps abolished. TEA (5–50 mM) which blocks certain K channels (Imaizumi & Watanabe, 1981) abolished the i.j.p. (single pulse, SS, and trains of 5 pulses at 5 and 10 Hz, 0.5 ms, supramaximal voltage) and inhibited the relaxation by approximately 80% (Figure 10). The residual relaxation persisted throughout prolonged periods (30 min or more) of TEA perfusion, was unaffected by apamin (1×10^{-6}M) but was abolished by TTX (1×10^{-6}M). The relaxation appears, therefore, to be comprised of two components; the major one is closely dependent

upon membrane hyperpolarization, whereas the minor one may be independent of membrane potential change.

In the absence of $[Ca^{2+}]_o$, (Figure 4) or the presence of Mn^{2+} (2 mM), both the electrical and mechanical responses to field stimulation were reduced and eventually (after 15–20 min) abolished, presumably due to the inhibition of transmitter release.

In contrast, the i.j.p. and mechanical relaxation were not reduced in either Na^+-deficient (Figure 5) or Cl^--free Krebs solution. This suggests that neither of these ions play an important role in the generation of the i.j.p.

Discussion

The electrical membrane characteristics of the i.a.s. resemble those of other circular muscles of the same species e.g. caecum (Ito & Kuriyama, 1973) and stomach (Kuriyama *et al.*, 1970). The value for the space constant, λ, (1.1 mm), lower than that found for either taenia (1.7 mm) or caecum (1.1–1.8 mm) (Holman & Hirst, 1979) may have arisen from the method used. Here the voltage transient at the onset of the electrotonic potential was compensated for by subtracting the average transient voltage recorded outside the cell from that recorded intracellularly at that point (Bywater & Taylor, 1980). Membrane potential was readily affected by alterations in $[K^+]_o$; but the relationship between these two parameters was not linear. This is a similar result to that found previously (Holman, 1958; Kuriyama, 1963) for guinea-pig taenia coli for $[K^+]_o < 30$ mM. The non-linearity may be due to an increase in intracellular potassium concentration with increasing $[K^+]_o$ or to an increase in the contribution of G_{Cl} as a determinant of membrane potential (Holman, 1958; Kuriyama, 1963).

Changes in the frequency of spike potentials accompanied changes in membrane potential. Membrane depolarization by, for example, current pulses or high K^+-containing solutions increased spike frequency; hyperpolarization had the reverse effect. The spikes were Ca^{2+}-dependent, being suppressed by Ca^{2+}-withdrawal or the presence of Mn^{2+}. Tone and the ability to maintain spike potential discharge were related. Any reduction in the rate of spike discharge lowered tone. Tone was maximal when spike potential discharge was well maintained, for example in K^+-rich solutions, but fell when spike frequency was reduced or abolished. The ability of the sphincter to maintain tone physiologically may, therefore, arise from the presence of pacemaker activity which depolarizes the membrane and initiates spike potentials via Ca^{2+}-operated voltage-dependent channels.

There is no evident role for a tonic sympathetic discharge in the maintenance of tone as proposed already for the human anal sphincter (Frenckner & Ihre, 1976). Adrenergic nerves apparently play little part in maintaining sphincteric tone in the guinea-pig; phentolamine (1×10^{-6}M) or atropine (1×10^{-6}M) failed to lower tone significantly. The absence of e.j.ps, in spite of the presence of adrenergic nerves, and the ability to obtain phentolamine-sensitive contractions to field stimulation was surprising. Several explanations can be advanced: e.j.ps could have been masked, even in low tone, by the powerful i.j.ps, a situation evident elsewhere in the intestine (Furness, 1969; Cheung & Daniel, 1980), which may arise from the indiscriminate and simultaneous activation of all nerve fibres by field stimulation. It is also true that there is considerable variation in the size of the membrane response to adrenergic nerve stimulation among different smooth muscles. In both the rat annococcygeus (Creed *et al.*, 1975) and BRP (Byrne & Muir, 1984) field stimulation during low tone gave distinct e.j.ps and contractions. However, in the rabbit ear artery, the adrenergic component responsible for the contraction is a small, slow membrane depolarization (Allcorn *et al.*, 1985); such a response, had it occurred, could have been obscured easily by the spontaneous membrane response of the sphincter. It is unlikely that the method of measurement in the present experiments could have obscured e.j.ps. Although electrical events were recorded from a single cell and tension from the entire muscle, impalement of several hundred of cells throughout the course of the experiments, even in the continued absence of spikes, failed to reveal e.j.ps.

I.j.ps were accompanied by a change in G_K. Thus, lowering of E_K by raising $[K^+]_o$ reduced, and increasing E_K by lowering $[K^+]_o$ enhanced, the i.j.p. Also, the i.j.p. was abolished at membrane potential values (−94 mV) close to those for E_K in spontaneously active smooth muscle. In contrast, failure of either Na^+-deficient or Cl^--free solutions to affect i.j.p. amplitude significantly, argues against their involvement in the inhibitory response. These results indicate that the i.j.p. in response to NANC nerve stimulation is produced by an increase in G_K as occurs in taenia coli (Den Hertog & Jager, 1975).

When the relationship between electrical and mechanical inhibitory responses was examined using TEA, two components of the relaxation were identified. The major one was voltage-dependent, sensitive to TEA and closely linked to the i.j.p.; the other much smaller component was insensitive to TEA and independent of the electrical event. These results suggest that, in the i.a.s., the major mechanical component may be mediated by membrane hyperpolarization and that only a very small part of the relaxation is independent of membrane electrical change. In the non-spontaneously active BRP on the other hand, where TEA abolished the i.j.p. but not the relaxation

to NANC inhibitory nerve stimulation (Byrne *et al.*, 1984), electrical and mechanical components are independent of one another, the latter being accompanied by a rise in cyclic GMP (Bowman & Drummond, 1984).

NANC-mediated responses in sphincteric and non-sphincteric smooth muscle (e.g. taenia coli) resemble each other closely. In both, the inhibitory response is accompanied by an increase in G_K and a fall in membrane resistance; the electrical and mechanical events appear inter-dependent. In non-spontaneously active tissues, e.g. dog fundus (Morgan *et al.*, 1981), rat anococcygeus (Creed *et al.*, 1975) and BRP (Byrne & Muir, 1984), the i.j.p. accompanying the relaxation is smaller. In the BRP, relaxation is accompanied by an increase in membrane resistance with K^+ having no apparent major role in the i.j.p. (Byrne & Muir, 1985). Clearly different receptor mechanisms operate; whether or not these derive from different transmitters must await identification of the latter.

The authors gratefully acknowledge the support of Glasgow University, their Medical Research Funds and the Lee Foundation.

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(*Received March 11, 1985.*
Revised April 29, 1985.
Accepted May 24, 1985.)