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Mechanisms underlying electrical and mechanical responses of the bovine retractor penis to inhibitory nerve stimulation and to an inhibitory extract

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1 The response of the bovine retractor penis (BRP) to stimulation of non-adrenergic, non-cholinergic (NANC) inhibitory nerves and to an inhibitory extract prepared from this muscle have been studied using intracellular microelectrode, sucrose gap and conventional mechanical recording techniques.

2 Both inhibitory nerve stimulation and inhibitory extract hyperpolarized the membrane potential and relaxed spontaneous or guanethidine (3×10^{-5} M)-induced tone. These effects were accompanied by an increase in membrane resistance.

3 Following membrane potential displacement from an average value of -53 ± 7 mV ($n = 184$; Byrne & Muir, 1984) inhibitory potentials to nerve stimulation were abolished at approximately -30 mV; there was no evidence of reversal. Displacement by inward hyperpolarizing current over the range -45 to -60 mV increased the inhibitory response to nerve stimulation and to inhibitory extract; at more negative potential values (above approximately -60 mV) the inhibitory potential decreased and was abolished (approximately -103 mV). There was no evidence of reversal.

4 Removal of $[K^+]_o$ reversibly reduced hyperpolarization to nerve stimulation and inhibitory extract. No enhancement was observed. Increasing the $[K^+]_o$ to 20 mM reduced the inhibitory potential to nerve stimulation but this was restored by passive membrane hyperpolarization. Inhibitory potentials were obtained at membrane potential values exceeding that of the estimated E_K (-49 mV).

5 $[Cl^-]_o$-free or $[Cl^-]_o$-deficient solutions reduced and abolished (after some 20–25 min) the hyperpolarization produced by inhibitory nerve stimulation or inhibitory extract. The inhibitory potential amplitude following nerve stimulation was not restored by passive displacement of the membrane potential from -26 to -104 mV approximately. Ouabain ($1-5 \times 10^{-5}$ M) reduced then (45–60 min later) abolished the inhibitory potential to nerve stimulation. The effects of this drug on the extract were not investigated.

6 It is concluded that the inhibitory response to nerve stimulation and extract in the BRP may involve several ionic species. However, unlike that in gastrointestinal muscles the NANC response in the BRP is accompanied by an increased membrane resistance and does not primarily involve K^+.

7 The underlying mechanisms for the inhibitory response to both NANC nerve stimulation and inhibitory extract appear to be similar, compatible with the view that the latter may contain the inhibitory transmitter released from these nerves in this tissue.

Introduction

The mechanical responses of the bovine retractor penis (BRP) to an inhibitory extract prepared from this tissue (Gillespie *et al.*, 1981) have been shown to resemble, sufficiently, those to stimulation of inhibitory non-adrenergic non-cholinergic (NANC) nerves to suggest that the extract may contain the transmitter released from these nerves (Bowman & Gillespie, 1982). If so, the electrical as well as the mechanical response to the extract and to inhibitory nerve stimulation should be the same in any given tissue. Accordingly, in order to study the mechanisms underlying the relaxation, the electrical and mechanical responses to the inhibitory extract and inhibitory (NANC) nerve stimulation have been compared in the BRP. Preliminary accounts of these results have been communicated previously (Byrne & Muir, 1982).

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Methods

BRP muscles from fresh (not more than 2 h after slaughter) abattoir specimens were set up for microelectrode and sucrose gap recording as described previously (Byrne & Muir, 1984). The results obtained with intracellular and sucrose gap recording methods were qualitatively similar. Inhibitory junction potentials (i.j.ps) refer to responses obtained to field stimulation of intramural (NANC) nerves using intracellular recording; excitatory and inhibitory potentials to responses from the sucrose gap. Tension was measured simultaneously by means of a Grass FT03 isometric transducer and displayed, with the electrical responses, on a u.v. recorder and stored on an instrumentation tape recorder (Racal 4D). Displacement of the membrane potential was carried out by the Abe & Tomita (1968) partition method or by two chlorided Ag rings in the double sucrose gap as described previously (Byrne & Muir, 1984). Values of the displaced membrane potentials were obtained by subtraction of the voltage transient recorded outside the cell during the current pulse (Bywater & Taylor, 1980).

The Krebs perfusing solution had the following composition (mM): NaCl 111.8, $NaHCO_3$ 25.0, NaH_2PO_4 1.13, KCl 4.7, $CaCl_2$ 2.7, $MgCl_2$ 1.3, glucose 5.5. The bath temperature was maintained at 37 ± 0.5°C and gassed with 95% O_2 and 5% CO_2. To ensure effective mixing of injected and perfusing solutions, the latter were pumped through the bath inlets (3 ml min^{-1}) by a Watson-Marlow flow inducer. The bore of each inlet was narrowed to 0.1 mm i.d. by a piece of drawn out polythene tubing. This arrangement provided turbulence in the bath fluid which (a) helped to equilibrate the bath contents rapidly as confirmed in preliminary experiments by injecting methylene blue solution (0.01%), (b) avoided any drop in temperature and associated relaxation which followed injection (>0.1 ml) of cold (0–4°C) fluid.

The inhibitory extract was provided by Professor J.S. Gillespie. It was prepared from BRP muscles and assayed on this tissue as described previously (Bowman *et al.*, 1979; Gillespie *et al.*, 1981; Crossley & Gillespie, 1983). The dry powdered inhibitory extract so prepared was impure. To ensure, as far as possible, that the inhibitory factor assayed in the BRP was responsible for the effects observed, two procedures were adopted. First the extract was cleaned of known contaminants such as adenine nucleotides and formate ions by passing it through an alumina column at pH 9. Removal of the nucleotides was confirmed by spectrophotometric measurement at 260 nm on a Unicam SP 8000 spectrophotometer. Secondly, since the extract provided was inactive (cleaned unactivated extract (CUE), Bowman & Gillespie, 1982) it was activated by adjustment of the pH to 2.0 with 1 M HCl for 10 min. The acidified extract was then neutralized with 1 M NaOH to pH 6.8 then diluted with distilled water to a concentration equivalent to 1 g net weight per ml. Only those relaxant effects which were present in the activated extract but absent in the unactivated product, were attributed to the material which had relaxed the BRP in the assay procedure. The term 'inhibitory extract' refers to the cleaned activated material (CAE). Solutions of CAE were unstable and kept on ice; they were added to the tissue bath in volumes not exceeding 0.1 ml in intracellular experiments and 0.02 ml in the sucrose gap to avoid the relaxation which accompanied the addition of larger volumes of cold fluid. Similar volumes of CUE, kept on ice, served as controls.

$[Cl^-]_o$-free solutions were prepared by replacing NaCl in the Krebs solution with an equivalent amount of sodium benzenesulphonate, and $MgCl_2$, $CaCl_2$ and KCl by an equivalent amount of $MgSO_4$, $CaSO_4$ and K_2SO_4 respectively. $[Cl^-]_o$-deficient solutions, in which the $[Cl^-]_o$ was reduced from 111.8 mM to 12.7 mM, were prepared by replacing only the NaCl by an equivalent amount of sodium glutamate. $[K^+]_o$-free solutions were prepared by replacing KCl in the Krebs solution with an equivalent amount of NaCl.

The following drugs were used: atropine sulphate (BDH), guanethidine sulphate (Ciba), ouabain (BDH), phentolamine mesylate (Ciba), tetrodotoxin (Sankyo). Concentrations in the bath refer to the salt with the exception of ouabain and tetrodotoxin where concentrations refer to the base. Except where otherwise stated, drugs were dissolved in saline (0.9% w/v NaCl solution) and this stock solution added to Krebs solution to achieve the final dilution. Oxyhaemoglobin was prepared by Dr D. Pollock, Department of Pharmacology, from human red corpuscles and contained glucose 2% dry weight as stabilizer; it was dissolved in distilled water.

Results

Response of the bovine retractor penis to inhibitory extract (CAE)

The muscle had no resting tone when first set up; tone developed (up to 25 g) spontaneously within 1 h or was induced by guanethidine (10^{-5} M). This drug also abolished the excitation produced by stimulation of adrenergic nerves. The electrical and mechanical responses to field stimulation of inhibitory nerves in the BRP have been described recently (Byrne & Muir, 1984). CAE hyperpolarized the membrane and relaxed tone in a dose-dependent manner (Figure 1). However, no clear relationship emerged between hyperpolarizations and the amount of CAE added. The threshold relaxant dose (0.001–0.01 ml) varied but was similar

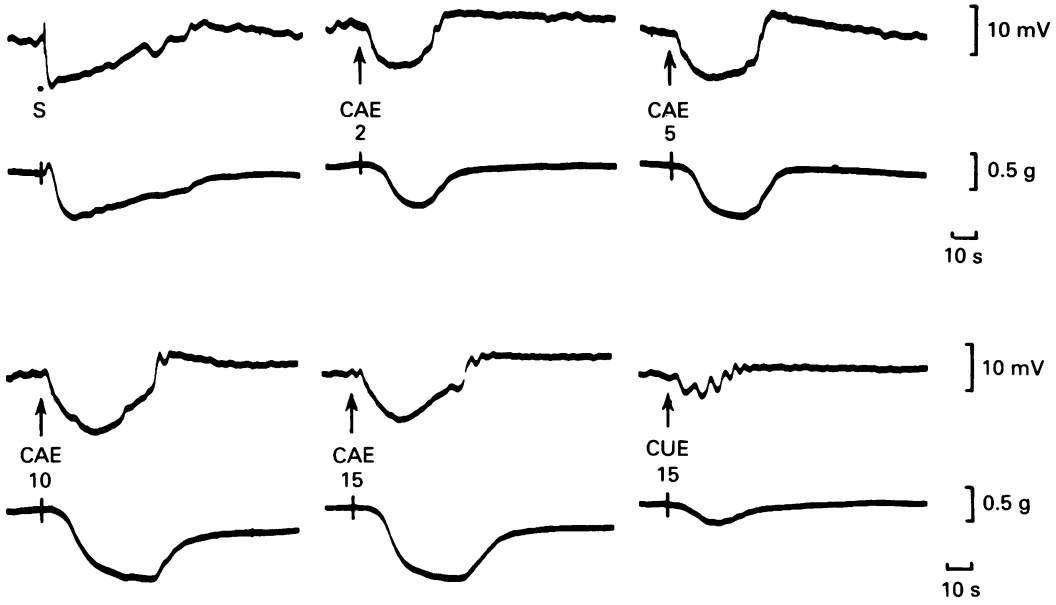

Figure 1 A comparison of the response to increasing concentrations (0.002(2), 0.005(5), 0.01(10) and 0.015(15) ml) of inhibitory extract (CAE) with that to field stimulation of intramural inhibitory nerves (S; single pulse, 0.5 ms, supramaximal voltage) and unactivated extract (CUE; 0.015 ml) in the bovine retractor penis in the presence of tone. In each set of records (in this and subsequent figures), the upper shows the electrical and the lower the mechanical response. CAE and stimulation of inhibitory nerves hyperpolarized the membrane and relaxed tone; CUE was comparatively ineffective, sucrose gap recording.

in range to that previously reported for this tissue for identically prepared material (Bowman & Gillespie, 1982; Crossley & Gillespie, 1983); CUE was comparatively ineffective. The maximum hyperpolarization obtained to either CAE or inhibitory nerve stimulation (supramaximal voltage, 5–10 Hz, 0.1 ms) was approximately 10–15 mV. For comparable degrees of membrane potential change, the onset and duration of the hyperpolarization produced by CAE were more prolonged than those to nerve stimulation. Significantly, in the presence or absence of guanethidine, the hyperpolarization to CAE was often followed by a rebound depolarization and oscillation in membrane potential, characteristics of responses to stimulation of NANC nerves in this tissue (Byrne & Muir, 1984). Tachyphylaxis to repeated injections of CAE or to recurrent trains of inhibitory nerve stimulation was not observed. As with the response to nerve stimulation (Byrne & Muir, 1984) the hyperpolarization and relaxation produced by CAE (0.001–0.002 ml) was antagonized by oxyhaemoglobin ($4–8 \times 10^{-6}$ M) which blocks guanylate cyclase (Figure 2) but unaffected by either tetrodotoxin (3×10^{-6} M), atropine (5×10^{-7} M) or phentolamine (5×10^{-6} M).

Effects on membrane resistance

(a) *Inhibitory junction potential* Resistance was determined, intracellularly, at the peak of the i.j.p. and at the plateau of the electrotonic response some 2 s after application of inward and outward current (1 s) pulses by external electrodes (Abe & Tomita, 1968). The amplitude of the current pulses was increased by up to 125% during the i.j.p.; (mean $74 \pm 22\%$, $n = 14$, during i.j.ps of 4 mV) causing a shift of the V/I curve to the right (Figure 3) and indicating a decrease in membrane conductance during the inhibitory response.

(b) *Inhibitory extract* Membrane resistance was also determined during application of CAE by the Abe & Tomita (1968) method. Consistent results were difficult to obtain, probably as a result of impurities in the extract. In approximately 40% cells ($n = 26$) CAE (0.02–0.1 ml) enhanced the amplitude of electrotonic

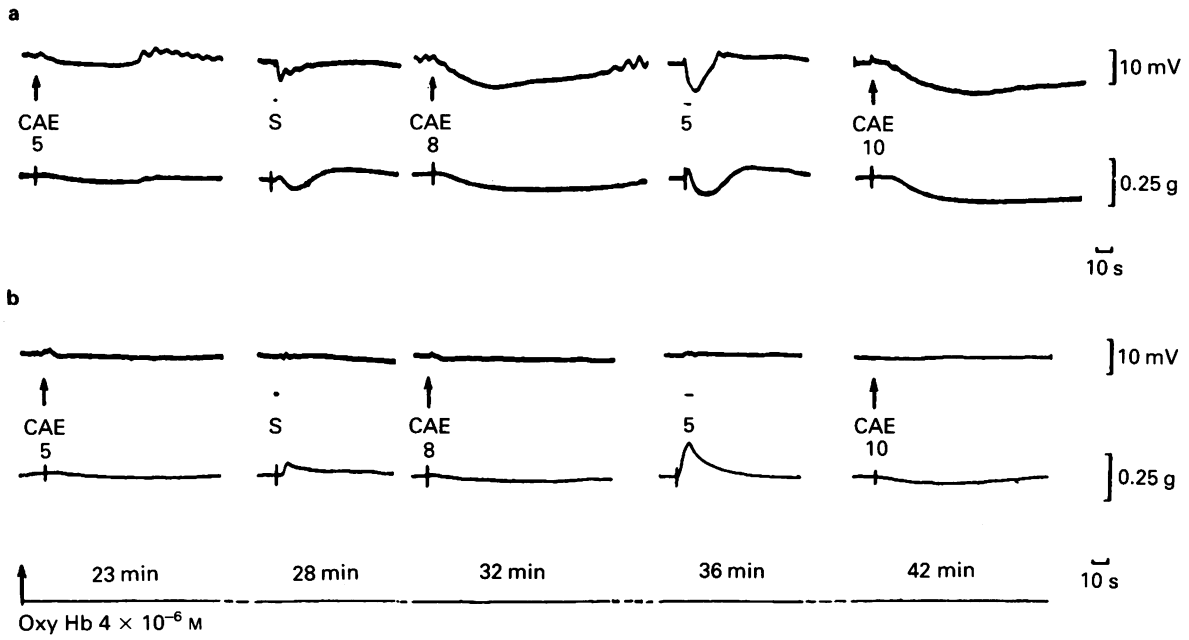

Figure 2 The antagonism by oxyhaemoglobin (↑Oxy Hb; 4×10^{-6} M), superfused for the times indicated, of the hyperpolarization (upper trace in each set of records) and relaxation of the bovine retractor penis to inhibitory extract (CAE; 0.005(5), 0.008(8) and 0.010(10) ml and to field stimulation of inhibitory nerves (single pulse(S) and 5 Hz, 0.5 ms, supramaximal voltage (5)) in the presence of tone. (a) Shows responses in absence of oxyhaemoglobin. (b) Oxyhaemoglobin (4×10^{-6} M) abolished the electrical and mechanical inhibitory responses to both CAE and nerve stimulation. The excitatory mechanical component to nerve stimulation was revealed though the accompanying electrical change was insignificant. Sucrose gap recording.

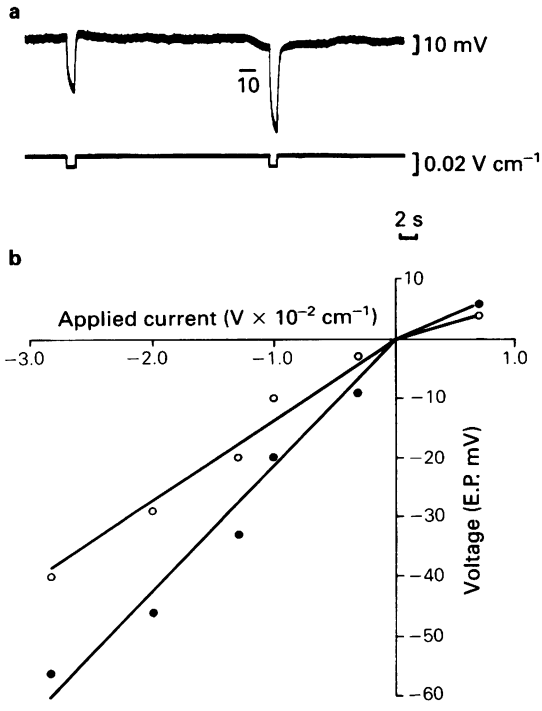

Figure 3 The increase in membrane resistance as indicated by an increased amplitude of the electrotonic pulse before and during the inhibitory junction potential (i.j.p.) recorded intracellularly from a single cell in the bovine retractor penis. In the experimental record (a), the electrotonic pulse was increased during the i.j.p. (10 pulses, 5 Hz, supramaximal voltage, 0.5 ms at bar). In the V/I plot (b) taken from a single cell in another experiment the amplitude (E.P. mV) of the electrotonic pulse relative to the applied current and expressed as a voltage gradient (abscissa) was increased ($P < 0.0025$, paired t test). The V/I plot was shifted to the right during the i.j.p. (●——●), (○——○) control values taken before i.j.p., confirming the increase in membrane resistance. Guanethidine (2×10^{-6} M) was present throughout.

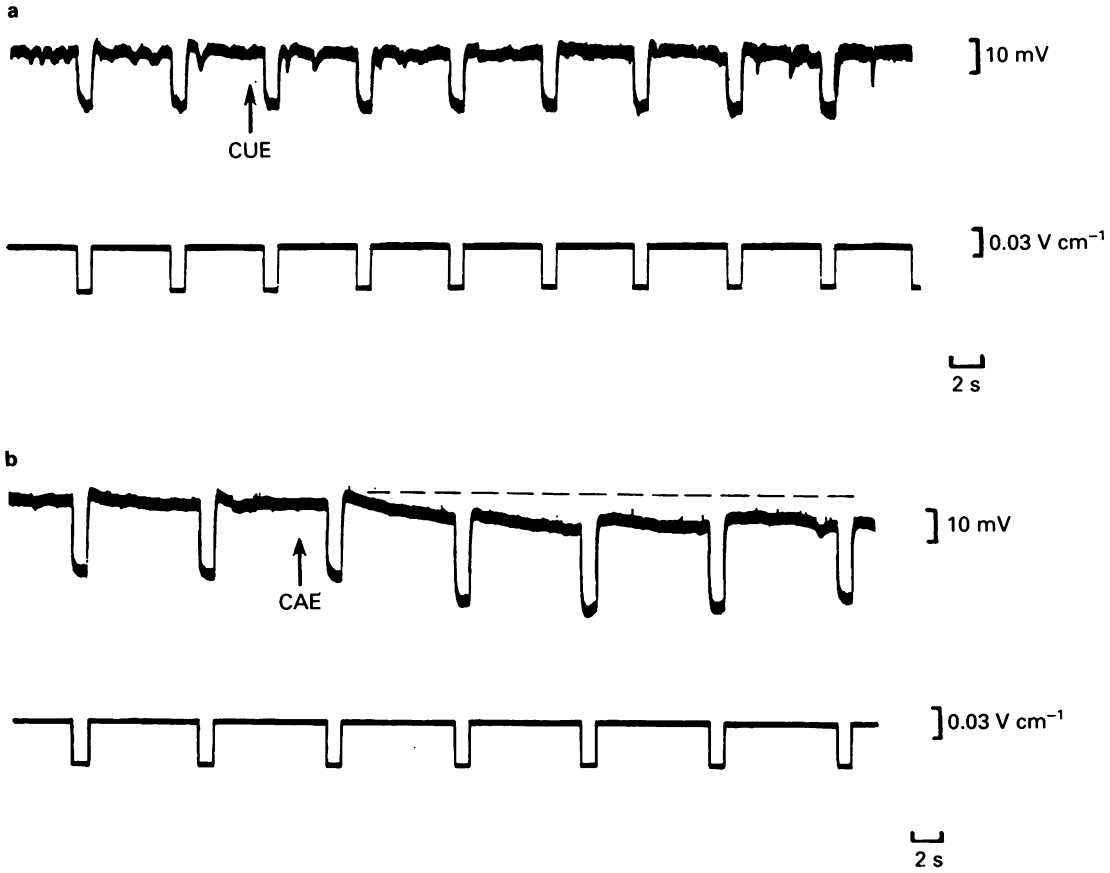

Figure 4 The membrane hyperpolarization and increase in membrane resistance, as indicated by an increase in the amplitude (mV) of electrotonic pulses (1 s, current intensity V cm^{-1} as shown in each lower trace), produced by inhibitory extract (CAE; 0.05 ml). Records are from two cells (a and b) in the same bovine retractor penis in the presence of tone. Unactivated extract (CUE; 0.05 ml) was ineffective.

pulses (1 s every 8 s) significantly, indicating an increase in membrane resistance (Figure 4). However in an equal number of experiments no significant increase in amplitude was observed and in some (10%) the amplitude was decreased. CUE (0.02–0.1 ml) produced no change in pulse amplitude.

Effect of membrane potential displacement on inhibitory junction potential amplitude and the response to inhibitory extract

Displacement (Abe & Tomita, 1968) from rest (− 40 to − 45 mV) to more positive values by outward depolarizing current, in the presence of guanethidine (0.2×10^{-5} M) to abolish excitation, reduced i.j.p. amplitude. The i.j.p. was abolished at approximately − 30 mV. Displacement by inward hyperpolarizing current pulses produced two distinct effects on i.j.p. amplitude. First, over the range − 45 to − 60 mV approximately, the amplitude was increased; secondly at values exceeding − 60 mV, the i.j.p. decreased in proportion to the change in membrane potential (Figure 5). To obtain the average values of membrane potential at which the i.j.p. was abolished, regression analysis of the combined results from nine tissues was carried out. Data from the range of membrane potentials − 55 to − 65 mV were omitted. The two linear plots so obtained had opposite slopes of different magnitude; over the membrane potential range − 27 mV to − 55 mV the i.j.p. showed proportionately larger changes in amplitude than between − 65 mV and − 103 mV. Respective correlation coefficients and degrees of freedom for the lines obtained from regression analysis from the nine experiments were 0.598 and 43 for membrane potentials of − 55 mV or less and 0.613 and 21 for membrane potentials − 65 or

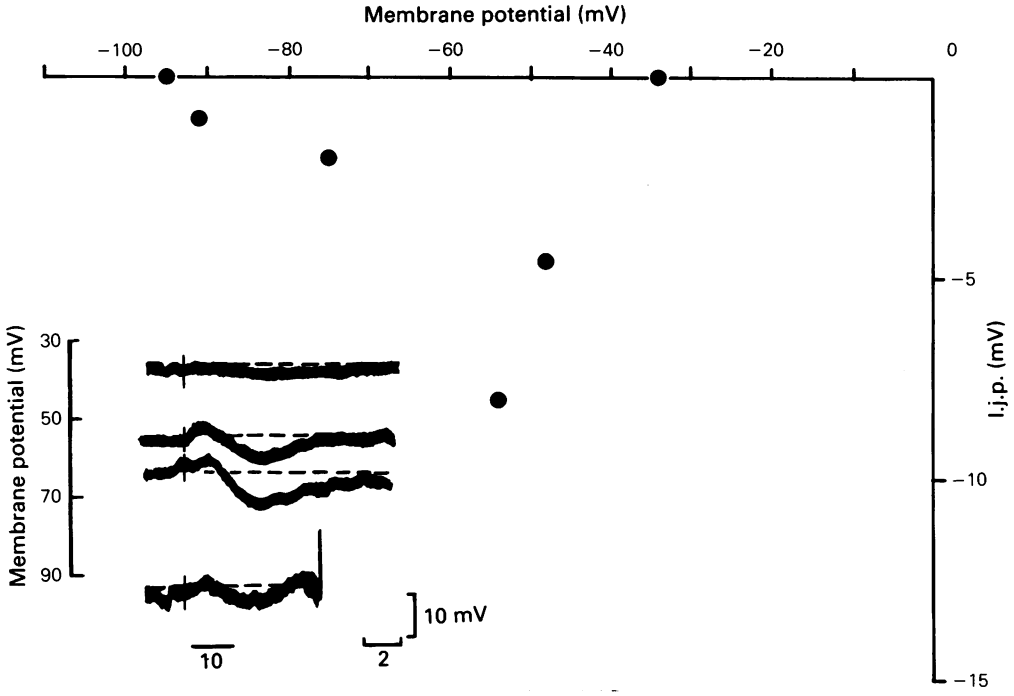

Figure 5 The relationship between the amplitude of the inhibitory junction potential (i.j.p.) to field stimulation (10 pulses, 5 Hz, 0.5 ms, supramaxiamal voltage) and membrane potential recorded intracellularly from the bovine retractor penis in the presence of guanethidine (0.2×10^{-5} M). The graph shows the results from one experiment and the inset an experimental record from another cell in a different preparation. From the graph, displacement of the membrane potential from rest to more positive values reduced i.j.p. amplitude. Displacement to more negative values produced two distinct changes in i.j.p. amplitude; over the range − 40 to − 60 mV it was enhanced. With further displacement to values more negative than approximately − 60 mV, i.j.p. amplitude declined. No reversal of the i.j.p. was observed.

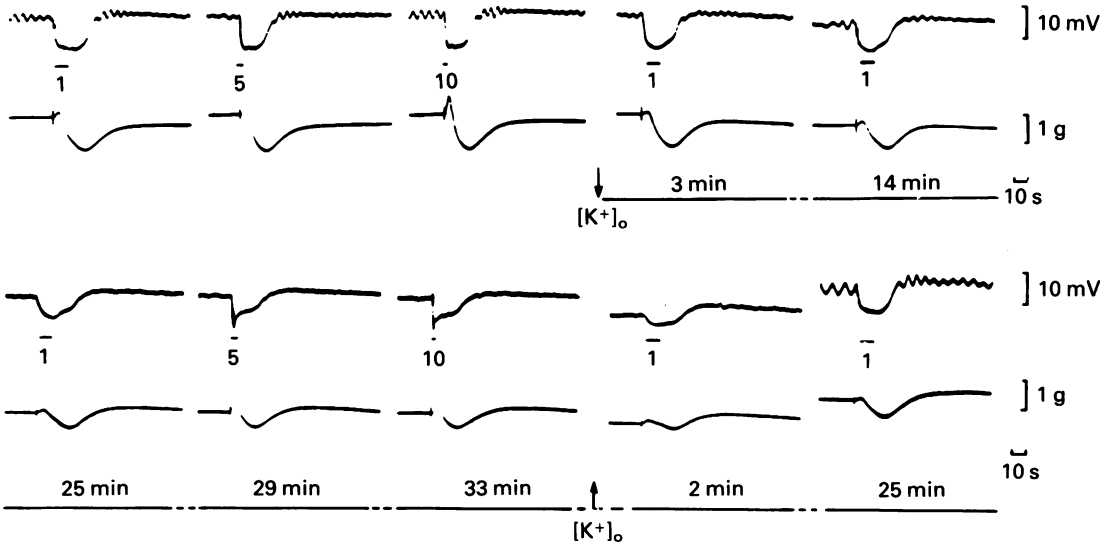

Figure 6 The effect of removal of K^+ ($\downarrow[K^+]_o$) on the inhibitory potential (upper record) induced by stimulation and relaxation (lower record) 10 pulses at 1, 5 and 10 Hz, 0.2 ms supramaximal voltage) of the bovine retractor penis. The duration of the perfusion with K^+-free Krebs is indicated above each bar. Initially, removal of K^+ produced a slight membrane hyperpolarization and reduction in tone, and with it a reduction in the response to field stimulation. There was no initial enhancement of the inhibitory potential during K^+-withdrawal. After some 25 min the inhibitory potential and tone were further reduced. These effects were reversed on readmission of K^+ ($\uparrow[K^+]_o$). Sucrose gap recording.

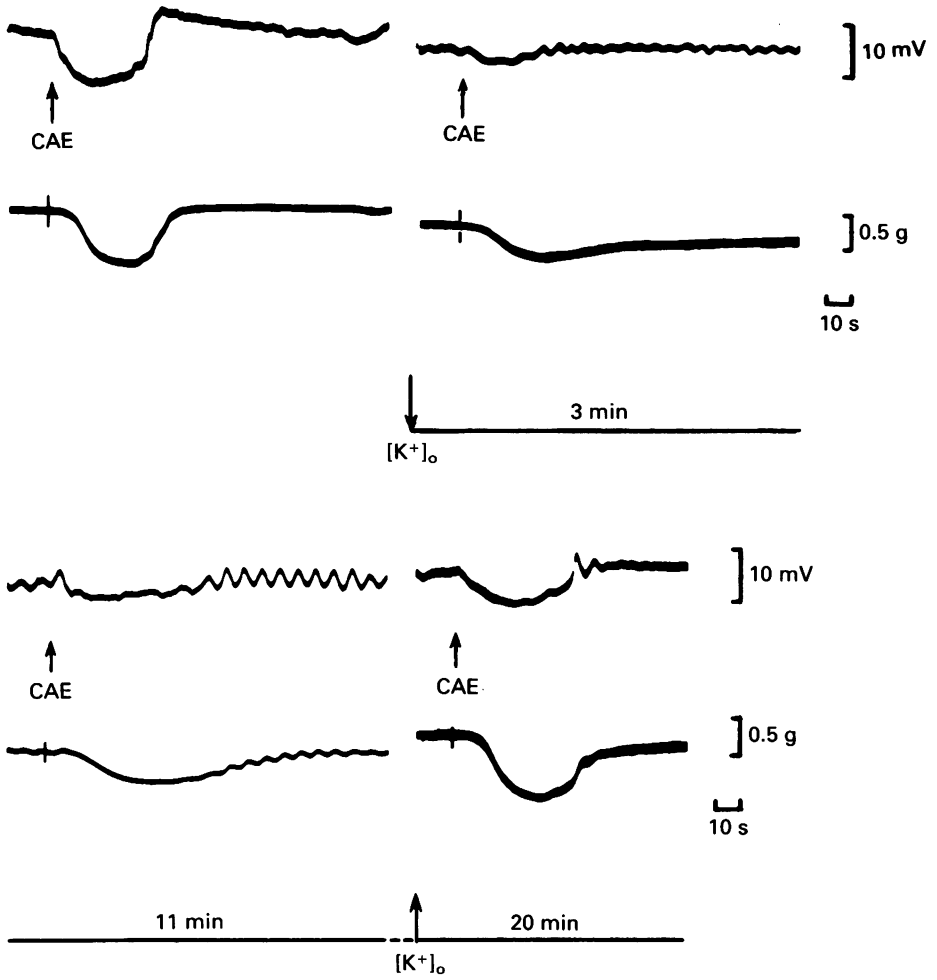

Figure 7 The effect of removal of K^+ ($\downarrow[K^+]_o$), for the times indicated, on the response of the bovine retractor penis to inhibitory extract (CAE; 0.005 ml) in the presence of tone. The hyperpolarization and relaxation to CAE was inhibited within 3 min of K^+ withdrawal and oscillations in membrane potential observed. Continued exposure to K^+-free solution hyperpolarized the membrane and reduced the resting tone. Oscillations in membrane potential and tone increased and the electrical and mechanical responses to extract were reduced. These effects were reversed on readmission of K^+ ($\uparrow[K^+]_o$). Sucrose gap recording.

greater. The values of membrane potential at which the i.j.p. was abolished, obtained by extrapolation of the plots, were − 103 mV and − 27 mV. There was no reversal of the i.j.p. when the membrane was depolarized to −20 mV or hyperpolarized to approximately − 120 mV.

Because of the long latency and duration of the response to CAE, long (40 s) current pulses were required to displace passively the membrane potential long enough to enable the extract response to be observed. These pulses caused the response to both inhibitory extract and the i.j.ps to decline. Intracellular impalement during passage of long current pulses was difficult to maintain and the number of experiments carried out was small. However hyperpolarization of the membrane potential (from − 40 to − 60 mV approximately) enhanced the effect of CAE. CUE was unaffected.

Effect of change of external ions

(a) *K^+* Removal of $[K^+]_o$ initially produced slight membrane hyperpolarization and a decline in tone in the sucrose gap. The electrical and mechanical respon-

ses to inhibitory nerve stimulation were not enhanced and were indeed reduced slightly during this period. Some 25–35 min later in the continued absence of $[K^+]_o$ a significant reduction in the response to inhibitory nerve stimulation took place, and tone was further inhibited. These changes were reversed eventually (after some 25 min) on readmission of K^+ (Figure 6). Reduction in the inhibtory potential to nerve stimulation was not the consequence of the hyperpolarization produced by K^+ withdrawal. In a separate intracellular study following $[K^+]_o$ withdrawal the i.j.p. was restored during passive hyperpolarization of the membrane to − 60 mV. Nor was the inhibitory potential abolished because of a de-

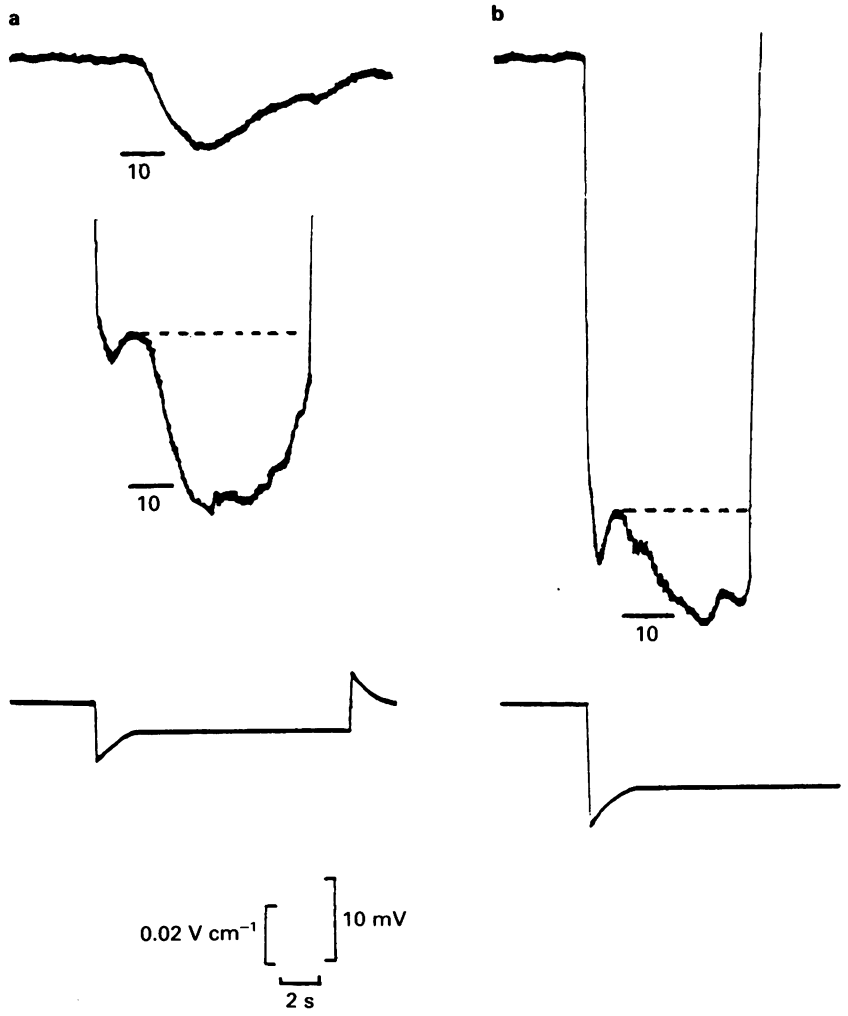

Figure 8 The effects of increasing $(K^+]_o$ from 4.7 mM (a) to 20 mM (b) on the changes produced by passive membrane displacement (current intensity expressed as V cm^{-1} bottom trace) on the inhibitory junction potential (i.j.p.) to field stimulation (10 pulses, 5 Hz, 0.5 ms, supramaximal voltage) recorded intracellularly in the bovine retractor penis preparation by the method of Abe & Tomita (1968) in the presence of guanethidine (2×10^{-6} M). In (a), the i.j.p. in normal Krebs (resting membrane potential − 42 mV) was enhanced during passive displacement of the membrane potential to − 73 mV (broken line). (b) After some 20 min in 20 mM $[K^+]_o$ (resting membrane potential − 33 mV) in a different cell of the same preparation, an i.j.p. was recorded at a membrane potential of − 83 mV (broken line) i.e. 34 mV more negative than the estimated equilibrium potential for K^+. In both (a) and (b) the values of the displaced membrane potentials were obtained following subtraction of the voltage transient recorded outside the cell during the current pulse.

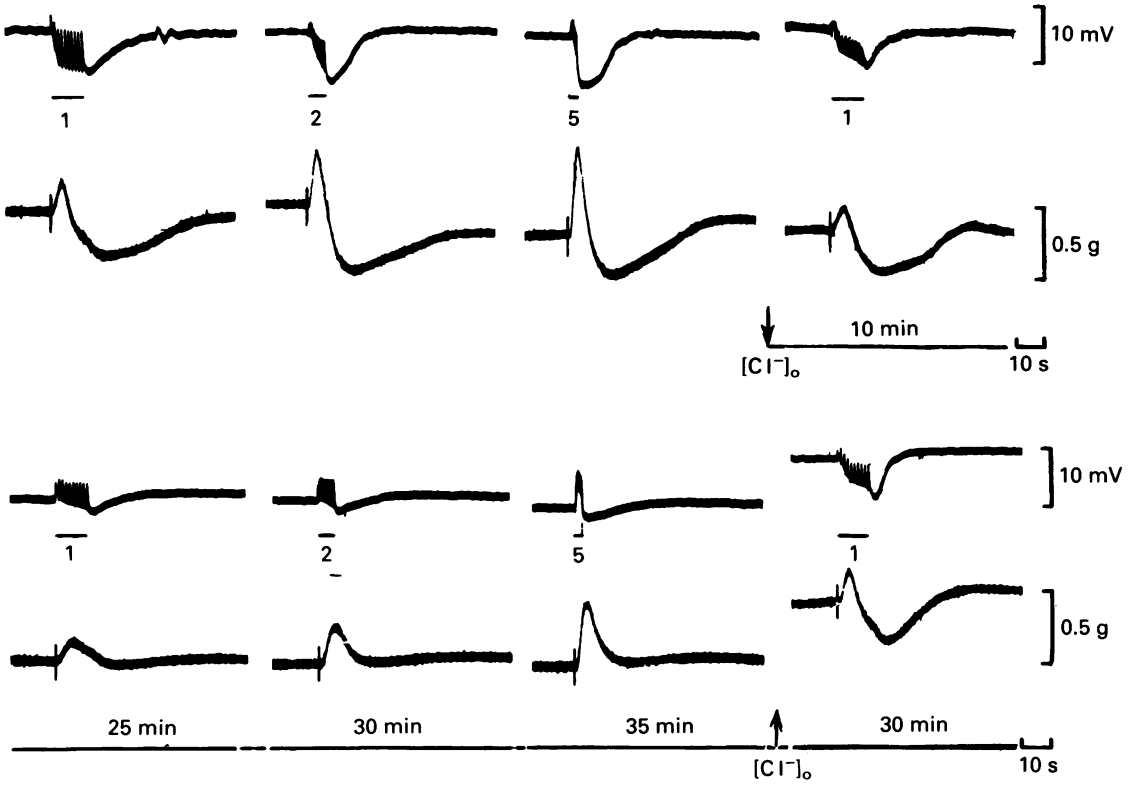

Figure 9 The effect of Cl^--deficient Krebs solutions ($\downarrow[Cl^-]_o$), for the times indicated, on the response of the bovine retractor penis to field stimulation (10 pulses at 1, 2 and 5 Hz, 0.5 ms supramaximal voltage, as indicated below bars) in the presence of tone. In each series, the upper trace represents the electrical and the lower the mechanical response. Perfusion with Cl^--deficient solution initially depolarized then hyperpolarized the membrane and reduced tone. The inhibitory potential was reduced both during the initial depolarization and subsequent hyperpolarization and reduction in tone. Small excitatory potentials and contractions were observed following prolonged perfusion with Cl^--deficient solutions. These arose from the activity of the excitatory transmitter, noradrenaline, in the absence of tone and guanethidine. Readmission of Cl^--containing Krebs solution ($\uparrow[Cl^-]_o$) restored the responses to field stimulation. Sucrose gap recording.

creased membrane resistance since the V/I plot in the presence and absence of K^+ over a period of 15 min was unchanged. The hyperpolarization produced by CAE (0.002–0.02 ml) was reduced within 5 min of K^+ withdrawal, and the maximum inhibition was reached some 20 min later (Figure 7). No enhancement of the extract response occurred. The accompanying relaxation was also reduced, at least in part on account of the loss of tone upon which the inhibitory response was displayed. The lack of enhancement of the hyperpolarization to CAE and to field stimulation during K^+ withdrawal suggested that the inhibitory response was not mediated by an increase in K conductance.

Increase in $[K^+]_o$ to 20 mM by replacement of an equivalent amount of NaCl, depolarized the cells significantly (-38 ± 6 mV to -30 ± 5 mV, $P < 0.0001$, $n = 28$ and 29 respectively). The i.j.p. was abolished but recovered during passive hyperpolarization (up to -83 mV) of the membrane potential. If the intracellular concentration of K^+ ($[K^+]_i$) in the BRP is similar to that (127 mM; Creed & Pollock, unpublished) in the rat anococcygeus, in the absence of values for the BRP itself, E_K in 20 mM $[K^+]_o$ would be some -49 mV, as calculated from the Nernst equation. I.j.ps could be obtained at membrane potentials exceeding this value, suggesting that the inhibitory nerve response is not mediated primarily by an increase in K^+ conductance (Figure 8).

(b) *Cl^-* Cl^--free solutions hyperpolarized and Cl^--deficient solutions initially depolarized then hyperpolarized the membrane in experiments which

employed the sucrose-gap method. In both solutions, the inhibitory potential was reduced (within 5 min exposure) and abolished 25–30 min later (Figure 9). Initially, the accompanying relaxation was enhanced by the increased tone (Cl^--deficient solutions) but was reduced or abolished by prolonged (25–30 min) exposure to either solution. The inhibitory potential was restored by readmission of normal Krebs solution but not by passive displacement of the membrane potential from −26 to −104 mV by externally applied current. In Cl^--free solutions the hyperpolarization and relaxation produced by CAE (0.002–0.02 ml, Figure 10) were also abolished but restored on readmission of Cl^- to the perfusing Krebs solution.

Effect of ouabain

Ouabain ($1-5 \times 10^{-1}$ M) which inhibits Na^+/K^+-ATPase, initially depolarized the membrane potential (by some 2–4 mV) and increased tone. The inhibitory potential (1–10 pulses, 1–10 Hz) was reduced by some 30–40% during this period and was usually accompanied by an enhancement of the relaxation (presumably due to the increased tone). After 6–10 min exposure the membrane potential was hyperpolarized, tone declined and the inhibitory potential and relaxation further reduced, being abolished 45–60 min later. The effects of ouabain on the response to CAE were not investigated.

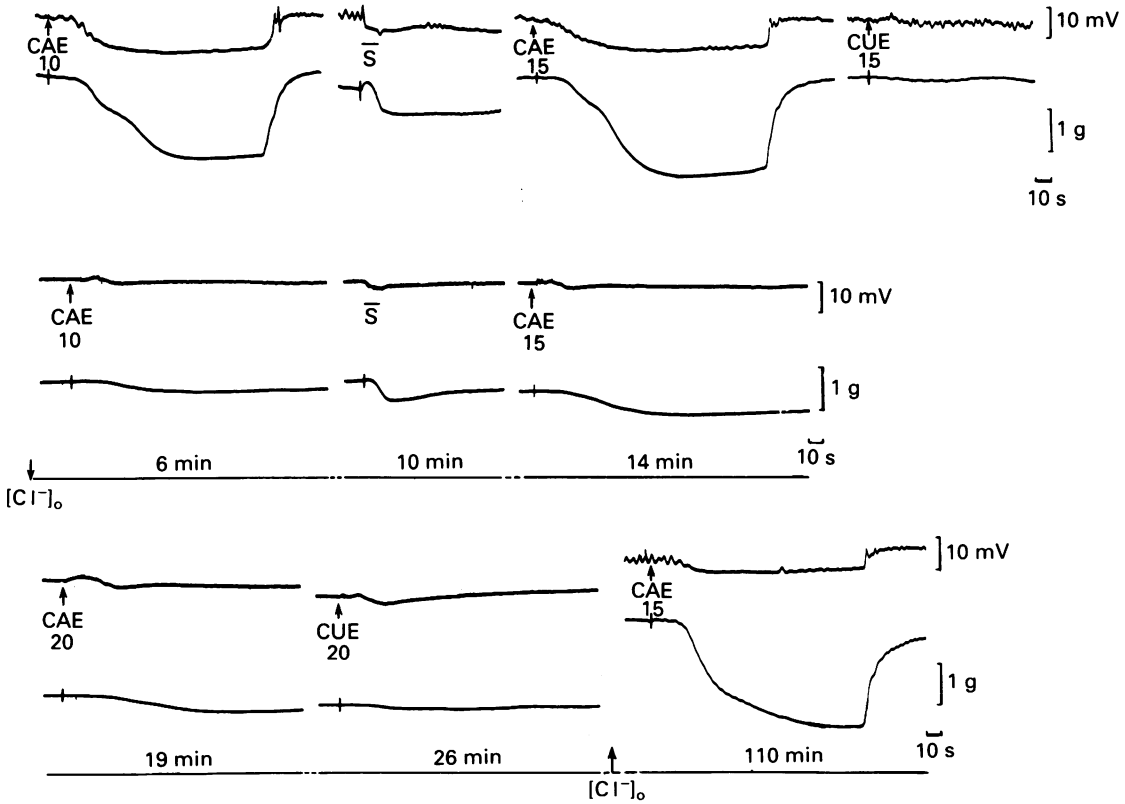

Figure 10 The effect of Cl^--free solution (↓$[Cl^-]_o$ (for the times indicated on the bars) on the response of the bovine retractor penis to inhibitory extract and to field stimulation(s) of inhibitory nerves (1 Hz, 0.5 ms, supramaximal voltage) in the presence of tone. In each set of records the upper represents the electrical and the lower the mechanical responses. In Cl^--free solution the membrane was slightly hyperpolarized, tone reduced. The electrical and mechanical response to inhibitory extract (CAE; 0.001(10), 0.0015(15), 0.002(20) ml) was virtually abolished; the inhibitory junction potential was significantly reduced though a mechanical relaxation persisted. The response to the unactivated extract (CUE) was unaffected. The response to CAE was restored following readmission of Cl^- ($[Cl^-]_o$). Sucrose gap recording.

Discussion

The effects of a semi-purified mixture of biological origin may not be attributed readily to a single component (see discussion by Crossley & Gillespie, 1983). However, the removal of contaminants and the use of unactivated, but otherwise identical, extracts as controls, permit the assumption that the inhibitory effects observed are due to the same factor responsible for relaxing the BRP as found previously in this laboratory (Gillespie *et al.*, 1981; Bowman & Gillespie, 1982). There are also problems unique to the investigation of the effect of the extract on membrane electrical characteristics. The instability of the material precluded its being perfused along with the warmed Krebs solution and it had to be kept on ice. The volume of added extract was restricted; volumes of cold solution above 0.1 ml inhibited tone and hyperpolarized the muscle, albeit slightly. Inhibitory activity was sometimes present in unactivated extracts though to a very small degree compared with that of the activated material. The potency of the extract also varied. The reasons for these anomalies are unclear. Residual contaminants are one possible source of inhibitory activity; of these, the contribution of adenine nucleotides is likely to be small, at least in the BRP, which is relatively insensitive to these compounds (Crossley & Gillespie, 1983). These factors restricted the investigation of the extract's electrical effects.

Both stimulation of inhibitory nerves and CAE hyperpolarized the membrane and relaxed the BRP. The mechanical responses obtained to each had similar characteristics, identical to those previously described for this muscle (Gillespie *et al.*, 1981; Bowman & Gillespie, 1982).

The electrical responses to inhibitory nerve stimulation and CAE were also similar; they were associated with a decrease in membrane conductance, enhanced by membrane hyperpolarization (to approximately -60 mV) and antagonized by oxyhaemoglobin which blocks guanylate cyclase. These similarities are compatible with the view that the extract may contain the inhibitory transmitter.

The electrical characteristics of the response in the BRP contrast with those produced by inhibitory, NANC nerve stimulation in other smooth muscle tissues. For example in the guinea-pig taenia coli (Den Hertog & Jager, 1975; Jager & Scheivers, 1980) and internal anal sphincter (Lim & Muir, 1983) the relationship between i.j.p. amplitude and the membrane potential is linear and the i.j.p. accompanied by an increased membrane conductance (to K^+). Indeed the non-linear relationship between i.j.p. amplitude and membrane potential over the range -30 to -103 mV in the BRP more closely resembles that of the slow i.p.s.p. of certain ganglia (Hartzel *et al.*, 1977).

A decreased membrane conductance during both inhibitory (Engberg & Marshall, 1971; Weight & Padjen, 1973) and excitatory potentials (Dudel & Kuffler, 1960; Shuba, 1977) in nerve and muscle has been shown. However, the proposed explanation, at least for excitatory potentials (Dudel & Kuffler, 1960), – a non-linear V/I relationship in the membrane – seems unlikely to apply here. Thus in the BRP, the V/I relationship is linear for inward currents (Byrne & Muir, 1984). Alterations in ionic conductance by the inhibitory transmitter seem a more likely explanation. The evidence, though indirect, suggests that more than one ionic species may be involved in the conductance change which accompanies the i.j.p.. The non-linearity between amplitude and membrane potential, the associated increase in membrane resistance and the failure to increase amplitude by K^+ removal or to abolish it by increasing $[K^+]_o$ to 20 mM suggest that K^+ is not the principal ion mediating the inhibitory potential to NANC nerve stimulation. This may be so despite the abolition of the i.j.p. at membrane potential values close to E_K and by prolonged withdrawal of $[K^+]_o$ and does not preclude its involvement together with other ions in the inhibitory potential (*vide infra*). This conclusion is consistent with the ineffectiveness of apamin in the BRP (Byrne & Muir, 1984), in contrast to its ability to block K^+ channels activated by NANC inhibitory nerves in other tissues (Maas, 1981; Bauer & Kuriyama, 1982).

The decreased membrane conductance could have arisen from a decreased resting G_{Cl^-} produced by the inhibitory transmitter. While G_{Cl^-} may contribute only about 4% of the total membrane conductance (Aickin & Brading, 1982; 1983), inactivation of G_{Cl^-} could account for the small i.j.ps observed, especially if G_{Cl^-} increases following the onset of tone. The reduction in i.j.p. amplitude at membrane potentials ranging from -60 to -27 mV would be explained if E_{Cl^-} were close to -27 mV. Membrane rectification due to an increase in G_K could have prevented i.j.p. reversal at more positive values. A decreased G_{Cl^-} (i.e. a reduction in the amount of chloride leaving the cell) is compatible not only with the consequences of Cl^- withdrawal on the inhibitory potential but with the effects of ouabain and K^+-free solutions. Both treatments block the Na-pump and so reduce Cl efflux as a result of this ion becoming passively distributed. Alternative explanations for the ability of Cl^- deficient or Cl^--free solutions to depress the inhibitory potential seems less likely. Reduction in transmitter output by Cl^- withdrawal seems unlikely in view of the persistence of inhibitory potentials in Cl^--deficient solutions in both the taenia coli (Bennet *et al.*, 1963; Tomita, 1972) and jejunum (Hidaka & Kuriyama, 1969). Transmitter-induced stimulation of Cl^- uptake, as an alternative to a decreased Cl^- loss, fails to account for the decreased membrane conductance.

The voltage dependence of the i.j.p. was measured in the presence of guanethidine-induced tone where the amount of excitatory transmitter being released was minimal. The voltage dependence of the i.j.p. over the range − 60 to − 103 mV is compatible with a decrease in the resting conductances to Cl^- or Na^+. Transmitter-induced inactivation of G_{Na^+} could explain the inhibitory effect of ouabain. A voltage-dependent decline in G_{Cl^-} with membrane hyperpolarization has been reported for skeletal muscle (Hutter & Noble, 1960). The ability of the inhibitory transmitter to reduce G_{Cl^-} and its apparent effects would therefore be diminished at hyperpolarized potential values. The individual role of the potential ionic participants awaits further study.

In conclusion it is clear that, on the basis of their electrical characteristics, inhibitory responses to NANC nerve stimulation in smooth muscle can be divided into two classes. In spontaneously active gastrointestinal muscle, e.g. guinea-pig taenia coli and internal anal sphincter, relaxation is accompanied by membrane hyperpolarization associated with increased membrane conductance to K^+. These responses are sensitive to apamin and the mechanical relaxation appears to be a consequence of membrane electrical change. On the other hand in the BRP and rat anococcygeus muscle (Creed *et al.*, 1975) relaxation to stimulation of NANC nerves is accompanied by small membrane hyperpolarizations which, in the BRP, are inhibited by oxyhaelmoglobin (Byrne & Muir, 1984). In the BRP, the relationship between the amplitude of the inhibitory potential and membrane potential over the range − 30 to − 120 mV is not linear. The underlying conductance change may be mediated by several ions in particular a decrease in G_{Cl^-}.

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