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THE CALCIUM-ACTIVATED POTASSIUM CONDUCTANCE IN GUINEA-PIG MYENTERIC NEURONES

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SUMMARY

1. Intracellular recordings were made from guinea-pig myenteric neurones *in vitro*.

2. From one to sixty action potentials were followed by an afterhyperpolarization, the amplitude and duration of which increased with the number of preceding action potentials.

3. The afterhyperpolarization reversed its polarity at a membrane potential of −91 mV. This value changed by 58 mV when the potassium concentration of the perfusing solution was changed ten-fold.

4. The afterhyperpolarization was abolished in calcium-free solutions. It was shortened in low calcium (1·2 mM) solutions and prolonged in solutions which contained high (5·0 mM) calcium concentrations, TEA (1 mM) or caffeine (1 μM).

5. The conductance increase during the afterhyperpolarization ($g_{K,Ca}$) was calculated from the amplitude of electrotonic potentials, taking advantage of the lack of membrane rectification in the range −60 to −90 mV. Peak $g_{K,Ca}$ increased as the number of action potentials was increased, but was relatively independent of membrane potential in this range.

6. $g_{K,Ca}$ declined with a time course which was single exponential (time constant 1·5–5 s) following one to six action potentials, and double exponential (time constants about 3 and 12 s) following fifteen to sixty action potentials.

7. It is concluded that the calcium which enters the neurone during the action potential elevates the membrane potassium conductance. The time course of this conductance increase probably reflects the free intracellular calcium concentration, and therefore describes the calcium sequestration or extrusion process.

INTRODUCTION

One class of neurone in the myenteric plexus of the guinea-pig ileum has a prominent afterhyperpolarization following each action potential (Nishi & North, 1973; Hirst, Holman & Spence, 1974). Earlier work has indicated that this is due to an activation of the membrane potassium conductance by calcium entry during the action potential (North, 1973; Hirst & Spence, 1973). There is increasing evidence that such a calcium-activated potassium conductance contributes significantly to the control of discharge frequency (Meech, 1978). Furthermore, it offers a sensitive

mechanism by which circulating drugs and hormones or locally released substances may modify neuronal firing (Tokimasa, Morita & North, 1981). The present experiments were performed to characterize more fully than heretofore the mechanism of generation of the afterhyperpolarization which follows the action potential in myenteric neurones.

METHODS

Intracellular recordings were made from neurones of the myenteric plexus of the guinea-pig ileum by techniques described previously (Nishi & North, 1973). The isolated ganglia were superfused with a heated (37 °C) Krebs solution of the following composition (mM): NaCl, 117; KCl, 4·7; $CaCl_2$, 2·5; NaH_2PO_4, 1·2; $MgCl_2$, 1·2; $NaHCO_3$, 25; glucose, 11; gassed with 95 % O_2–5 % CO_2. Recordings were made with electrodes containing KCl (2 M) (d.c. resistance 50–100 MΩ), inserted into neurones under visual control. Both voltage recording and current injection were made through the same electrode by use of an active bridge circuit. Micro-eletrode resistance was nulled prior to cell impalement; the bridge balance was checked after withdrawal of the electrode using currents as great as those passed during the intracellular recording. Membrane potential and current injected were recorded on an oscilloscope and a pen recorder (pen response 50 mm (usually 50 mV) in less than 5 ms). Action potentials were evoked either by passing brief depolarizing currents across the cell membrane (WP instruments M701 amplifier) or by electrical stimulation of the neuronal process by a second larger micro-electrode, the tip of which was placed on the surface of the ganglion some 50–100 μm from the impaled neurone soma.

Membrane input resistance was measured from the amplitude of small hyperpolarizations (50–80 ms duration) evoked by passing known currents across the cell membrane. The conductance increment during the afterhyperpolarization was calculated from $(R/R')-1$, where R is the input resistance at the resting potential and R' is the input resistance during the afterhyperpolarization. This we call the fractional conductance increase and denote it by $g_{K,Ca}$. Part of the current crossing the membrane during the electrotonic pulse may be carried by chloride ions; this would cause our estimate of $g_{K,Ca}$ to be somewhat low. In the present experiments, as in previous studies (Nishi & North, 1973; Hirst *et al.* 1974), we found that the voltage–current relation was usually linear in the membrane potential range −60 to −90 mV (see Fig. 1).

Solutions of different ionic composition were made by adjusting the sodium chloride concentration to maintain the same osmolarity. Low chloride solutions contained sodium isethionate or glutamate. Drugs used were ouabain, caffeine and tetraethylammonium bromide (Sigma).

RESULTS

The present results are based on intracellular recordings from more than eighty Type 2 or afterhyperpolarizing myenteric neurones. These cells did not show nicotinic fast excitatory post-synaptic potentials (e.p.s.p.s) (Nishi & North, 1973; Hirst *et al.* 1974).

Peak amplitude of afterhyperpolarization

From one to sixty action potentials were followed by a prolonged afterhyperpolarization (Fig. 1). This was associated with a fall in neurone input resistance which was still apparent when the afterhyperpolarization was prevented by passing current through the recording electrode (Fig. 1*B*). A similar afterhyperpolarization followed action potentials induced by depolarizing current pulses or by focal stimulation of a cell process which conducted to the soma, although in most experiments the afterhyperpolarization caused failure of invasion of the soma after the first few spikes (North & Nishi, 1974).

The peak amplitude of the afterhyperpolarization increased with the number of

action potentials used to evoke it. Fig. 2 shows the increase in peak amplitude, which reached 23·5 ± 1·1 mV (mean ± S.E. of mean, $n = 13$) when sixty action potentials were used. There was no significant difference between the peak amplitudes evoked by thirty or sixty action potentials. The peak conductance increment ($g_{K,Ca}$) grew linearly during the first three action potentials, and continued to increase even between thirty and sixty action potentials (Fig. 2).

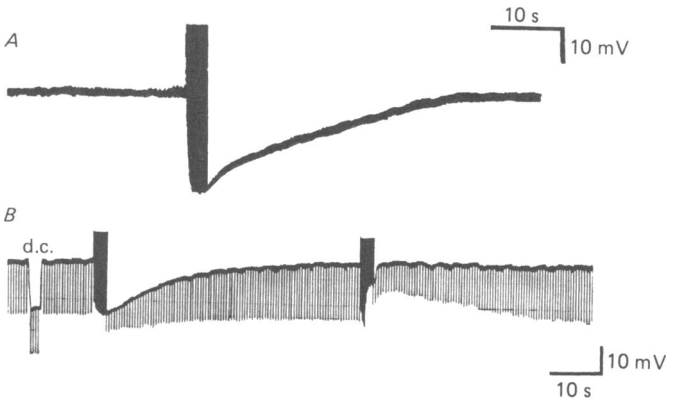

Fig. 1. *A*, typical afterhyperpolarization lasts for more than 30 s following thirty action potentials (10 Hz/3 s). *B*, fall in resistance during afterhyperpolarization (5 Hz/3 s). Left side (d.c.) shows effect on input resistance of shifting membrane potential by passing outward current. Right side shows fall in resistance when afterhyperpolarization was prevented by manual-clamp technique. In this cell, a small part of the resistance fall is due to rectification.

Time course of afterhyperpolarization

The total duration of the afterhyperpolarization varied considerably from cell to cell; even following a single action potential, the hyperpolarization lasted from 2 to 30 s. However, in all cells an increase in the number of action potentials progressively increased the total duration of the afterhyperpolarization. The underlying conductance increase declined according to a single exponential function, the time constant of which increased as the number of action potentials used to induce the afterhyperpolarization was increased. With fifteen to sixty action potentials, the time course of the conductance change was better fitted by two exponentials. The dependence of the time constant(s) of decay on the number of action potentials was evident on a single neurone, and also when measurements were averaged from many neurones (Fig. 3). The mean time constants of decay were: one spike, 1·5 s; two spikes, 2·4 s; three spikes, 3·7 s; six spikes, 5·0 s; fifteen spikes, 2·5 and 10·5 s; thirty spikes, 2·6 and 12·6 s; sixty spikes, 2·8 and 14·8 s. The increase in time constant of decay of the fractional conductance increase could arise from the participation of more than one mechanism in the process by which the neurone reduces its free intracellular calcium concentration following a period of activity.

Effect of membrane polarization on the afterhyperpolarization

The amplitude of the afterhyperpolarization was dependent on membrane potential (Fig. 4) and reversal occurred at approximately −91 mV. The afterhyperpolarization following thirty action potentials was measured at a series of membrane potentials ranging from −30 to −120 mV (Fig. 4*C*). In each of thirteen neurones, the regression of afterhyperpolarization amplitude on membrane potential was highly linear in the

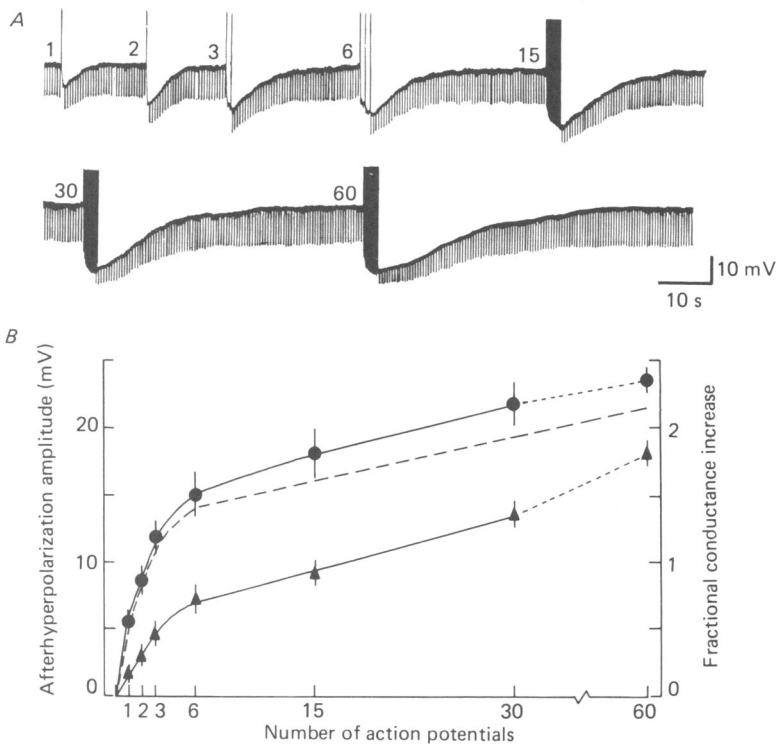

Fig. 2. *A*, afterhyperpolarization amplitude and duration is dependent on number of action potentials used to evoke it (indicated beside each trace). A single depolarizing pulse, upward deflection, sometimes elicited two spikes. This was monitored on an oscilloscope. *B*, peak afterhyperpolarization amplitude as a function of the number of action potentials used to evoke it. ●, observed amplitude (mV); ▲, observed conductance change expressed as fractional conductance increase (see Methods). As the number of action potentials was increased, the peak amplitude reached a maximum at 23·5 mV, but the conductance change continued to rise. The dashed line is the peak amplitude predicted from the observed mean conductance change (eqn. 5, Appendix; $E_{K,Ca} = -91{\cdot}2$ mV, $E_r = -57{\cdot}8$ mV). The peak amplitude predicted from the conductance was similar to the observed peak amplitude. Points are means (±S.E. of mean) of 7–13 observations.

range −60 to −120 mV ($P < 0{\cdot}05$) and the reversal potential was −91·2±0·65 mV (mean±S.E. of mean, range −87·5 to −94·9). The reversal potential for the hyperpolarization which followed a single action potential (−91·1±1·7, mean±S.E. of mean, $n = 8$) was not different from the reversal potential of the hyperpolarization which followed thirty action potentials (see also Fig. 4*B*).

The conductance change which accompanied the afterhyperpolarization was much

reduced by membrane depolarization in the range −60 to −30 mV (Fig. 5), and this may be responsible for the marked nonlinearity of Fig. 4*B* in this range of potentials. However, the voltage dependence of the peak fractional conductance increase was relatively small in the range −60 to −100 mV (Fig. 5). In contrast, the time constant of decay of the conductance change was shortened by membrane depolarization and

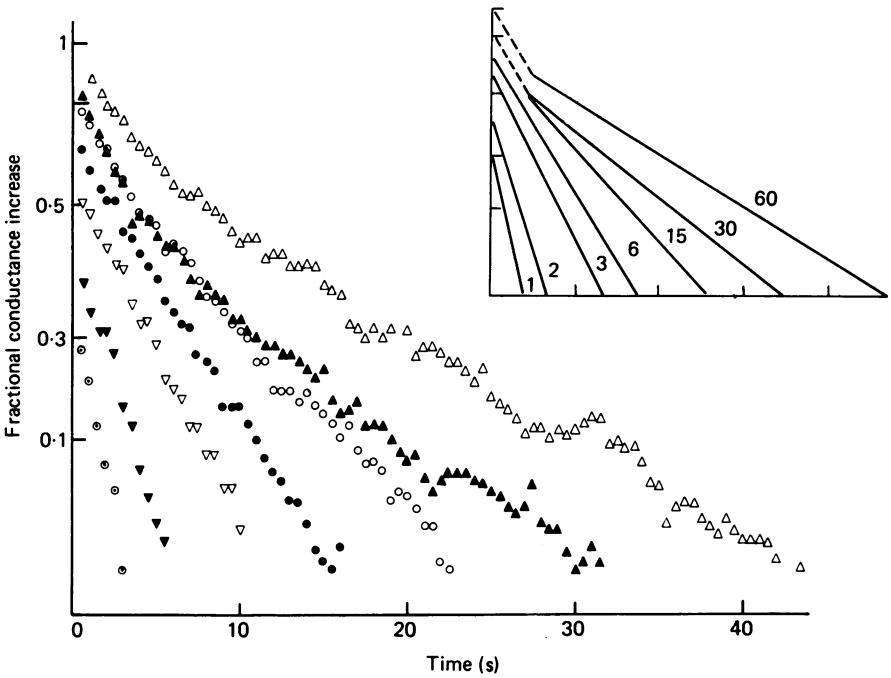

Fig. 3. Relative conductance increase as a function of time for different amplitudes of afterhyperpolarization. The conductance change evoked by a single spike (⊙) was compared with that following two (▼), three (1 Hz) (▽), six (3 Hz) (●), fifteen (5 Hz) (○), thirty (10 Hz) (▲) and sixty (20 Hz) (△) spikes. Inset shows lines fitted by least squares to later components of decay. Points are means of seven to thirteen observations (membrane potential −58·1 ± 4·1 mV). The lines for fifteen, thirty and sixty spikes are best fitted by two exponentials with initial time constants of 3 s and later time constants of 11, 13 and 15 s.

considerably increased by hyperpolarization; this may indicate a voltage dependence of the process or processes by which the intracellular calcium ion concentration is restored to normal following the action potentials.

Comparison of conductance increase with potential change

If the afterhyperpolarization is generated entirely by activation of the membrane potassium conductance, then its amplitude at any time should be related to the conductance change in a predictable manner. The amplitude (v) was calculated from the conductance change according to (see Appendix):

$$v = \left(1 - \frac{R'}{R}\right)(E_{K,Ca} - E_m),$$

where R' and R are the input resistances during and prior to the afterhyperpolarization, $E_{K,Ca}$ is the equilibrium potential for the potassium ion channels opened during the afterhyperpolarization and E_m is the membrane potential set by passage of steady current. When no current was used, $E_m = E_r$ (the combined equilibrium potential for all channels open at rest). Fig. 2*A* shows that the peak amplitude of the

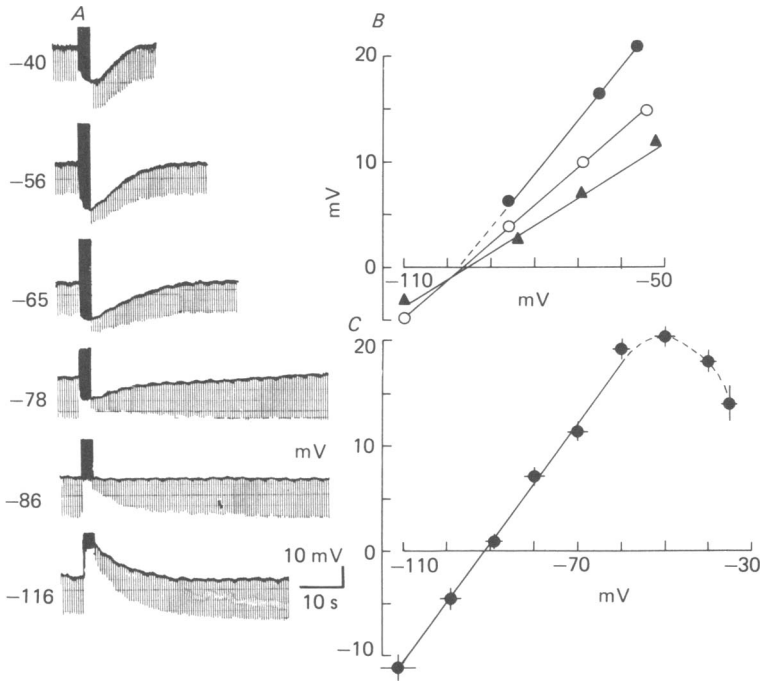

Fig. 4. Afterhyperpolarization amplitude as a function of membrane potential. *A*, reversal of afterhyperpolarization evoked by thirty spikes (10 Hz/3 s). Resting potential, −56 mV. Note the lack of rectification in this neurone and the relative insensitivity of the peak conductance change to membrane potential (see Fig. 5). *B*, reversal of afterhyperpolarizations evoked by one (▲), five (○) or ten (●) spikes. The reversal potential was not dependent on the initial amplitude of the afterhyperpolarization. Resting potential, −56 mV. *C*, data from experiments such as that shown in part *A*. Afterhyperpolarizations evoked by thirty spikes (10 Hz/3 s). Bars indicate S.E. of mean for the number of observations indicated. The amplitude is rather linearly related to the membrane potential in the range −60 to −110 mV.

afterhyperpolarization could be well predicted from the conductance change for various numbers of action potentials. Fig. 6 shows that the entire time course of the afterhyperpolarization was similar to that predicted from the conductance increase. When polarizing currents were used, the amplitude of the afterhyperpolarization was linearly related to the membrane potential (Fig. 4*C*). A further test for the underlying assumption that the potassium activation mediates the afterhyperpolarization is the comparison of the slope of the line of Fig. 4*C* with the theoretical value of $(1-R'/R)$. In the experiments of Fig. 4*C* the slope was 0·580. The mean value of $(1-R'/R)$ for the peak conductance change induced by thirty action potentials was 0·574.

Effects of changing ion concentrations

These experiments were carried out on the afterhyperpolarization following thirty action potentials (10 Hz/3 s).

Potassium. The amplitude of the afterhyperpolarization became larger in low potassium-containing solutions and smaller when the potassium concentration was elevated (Fig. 7*A*, *B*). The reversal potential also changed in solutions of different

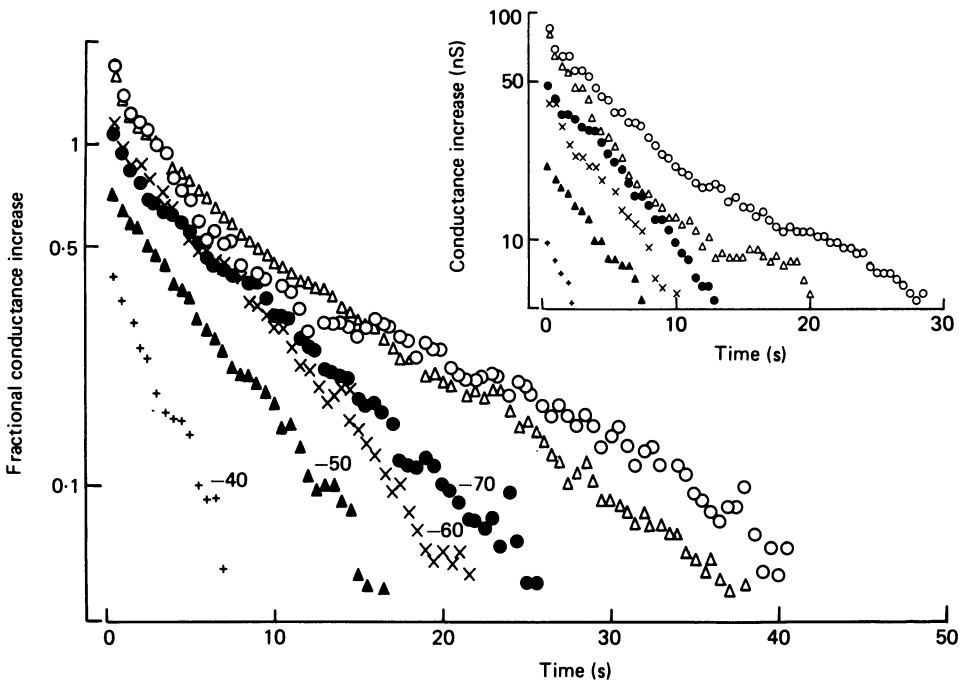

Fig. 5. Effect of membrane potential on peak amplitude and time course of conductance increase underlying the after hyperpolarization. Points are means of observations from four to eight neurones. Actual membrane potentials were as follows (±S.E. of mean, number of cells in parentheses) +, −40·0±0·8 (5); ▲, −50·0±1·6 (5); ×, −58·2±1·3 (6); ●, −68·6±1·7 (5); △, −78·5±0·7 (5); ○, −90·0±2·0 (3). There was not a great effect of membrane potential on peak fractional conductance increase in the range −60 to −90 mV. Measurements made at −100 and −116 mV are not shown for clarity, but closely overlapped with those for −70 mV. Inset: the absolute conductance increase (nS) as a function of time for a single myenteric neurone. A similar voltage dependence of the conductance increase was apparent in all the individual neurones studied.

potassium content, by 57·7 mV for one decade change in potassium concentration (Fig. 7*B*). This is close to the value which would be expected for a hyperpolarization generated by an increase in conductance to potassium ions.

Chloride. Reduction of the chloride concentration to 9 mM (by substituting the isethionate or glutamate salts for sodium chloride) did not significantly change the reversal potential of the afterhyperpolarization (control, −86·5±3·1 mV, mean±S.E. of mean; in low chloride −84·7±0·8 mV, $n = 4$).

Calcium. Reduction of the calcium concentration from 2·5 to 1·2 mM reduced the

peak amplitude and duration of the afterhyperpolarization to 51·4±12·0% and 40·0±7·3% respectively of their control values (Fig. 8). Increasing the calcium concentration to 5·0 mM caused no significant change in amplitude of the afterhyperpolarization (98·6±6·3% of control) but greatly prolonged it (184·2±15·4%, mean±S.E. of mean, $n = 5$) (Fig. 8). Complete removal of calcium ions abolished the

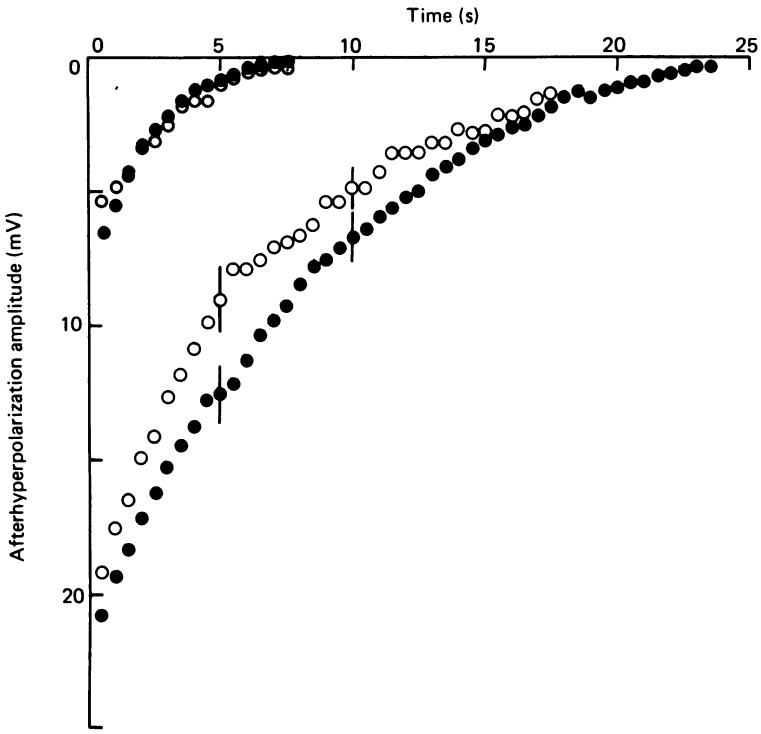

Fig. 6. Amplitude of afterhyperpolarization compared with that predicted from conductance change. ●, observed afterhyperpolarization amplitude. ○, amplitudes predicted from the observed conductance change (eqn. 5, Appendix). All points are means of eight observations in different neurones; vertical bars are S.E. of means. The amplitude of the afterhyperpolarization of a single action potential agrees with that expected from the conductance change, but exceeds the predicted value following thirty spikes (10 Hz/3 s). The difference is significant from about 5–15 s.

afterhyperpolarization induced by one or thirty action potentials (Fig. 8*A*, *B*). Halving or doubling the calcium concentration caused changes in membrane potential of less than 5 mV. Complete removal of calcium caused a depolarization of 5–20 mV; however, the removal of calcium ions combined with an increase in the magnesium concentration largely prevented this depolarization but still completely abolished the afterhyperpolarization.

Effects of drugs

Addition of cobalt chloride (1 mM) shortened the afterhyperpolarization without greatly affecting the peak conductance increase (Fig. 8*D*), but higher concentrations (3 mM) applied for more than 10 min completely abolished the afterhyperpolarization and the associated conductance increase.

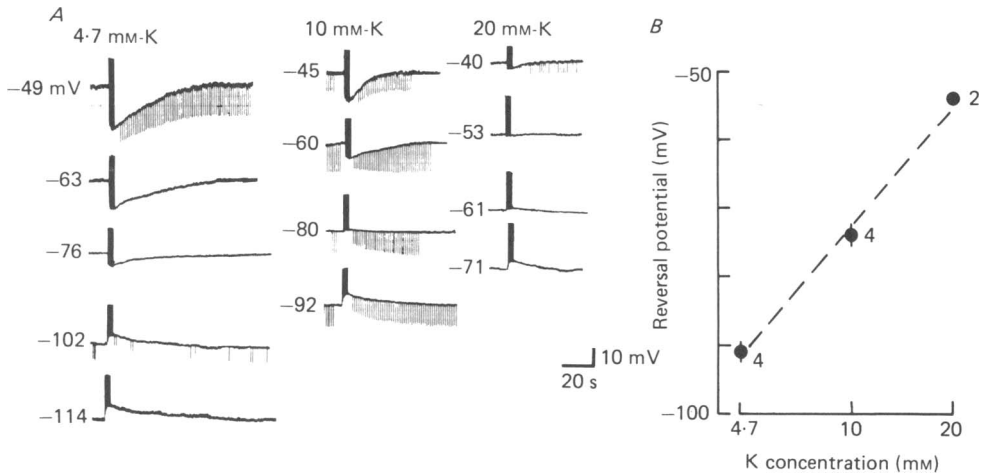

Fig. 7. Effect of changing extracellular potassium concentration on afterhyperpolarization evoked by thirty pulses (10 Hz/3 s). *A*, recording from a neurone at different membrane potentials and three different potassium concentrations. *B*, reversal potential was related to potassium concentration. Line is least square fit to raw data ($r = 0{\cdot}982$, $n = 10$). Slope is $-57{\cdot}7$ mV/$\log_{10}$ [K]. All bars are S.E. of mean.

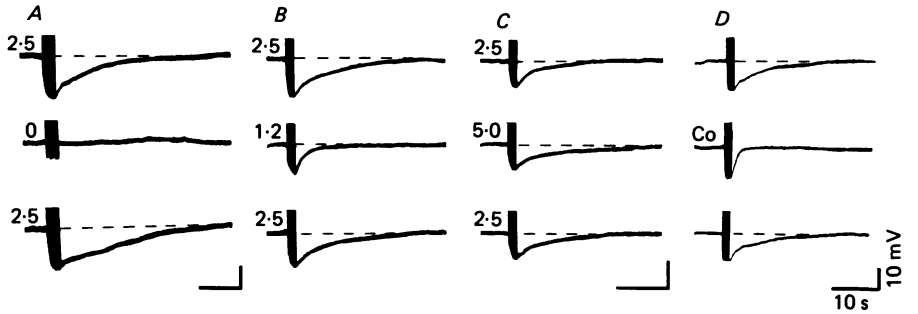

Fig. 8. Effect of changing calcium concentration or adding cobalt on the afterhyperpolarization. Afterhyperpolarizations were evoked by thirty action potentials (10 Hz/3 s). Three different cells. *A*, afterhyperpolarization was abolished in calcium-free solution. Resting potentials were −60 mV (control) and −56 mV (calcium-free). *B* and *C*, afterhyperpolarization was much shortened in low calcium solution and prolonged in high calcium solution with no change in resting membrane potential (−56 mV). *D*, addition of cobalt (3 mM) first shortened the afterhyperpolarization (middle trace, 5 min in cobalt) but after 10 min abolished it altogether (not shown). Resting potential −58 mV (control) and −53 mV (in cobalt).

Tetraethylammonium bromide (TEA) (1 mM) and caffeine (1 μM) both prolonged the afterhyperpolarization with little effect on its peak amplitude (Fig. 9). Neither TEA nor caffeine affected the membrane potential at these concentrations. The mean increase in duration by TEA was $138 \pm 36\%$ ($n = 5$), and by caffeine was $61 \pm 5{\cdot}6\%$ ($n = 5$). TEA may exert its effect by increasing the amount of calcium which enters during the action potential. Higher concentrations are known to block $g_{K,Ca}$ (Meech & Standen, 1975). Caffeine did not change the action potential configuration,

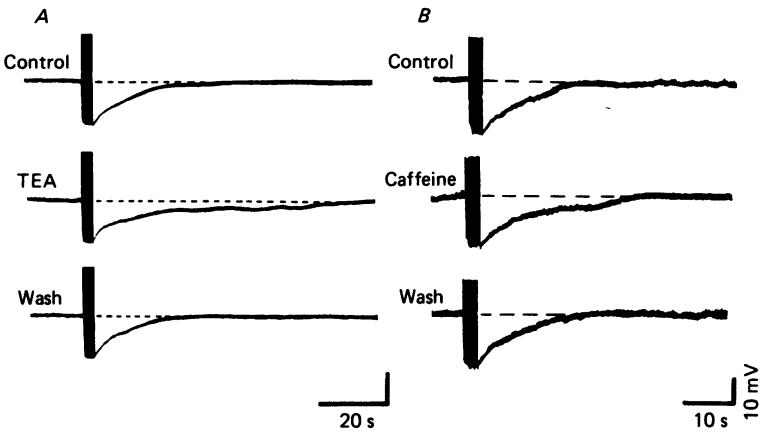

Fig. 9. TEA and caffeine prolong the afterhyperpolarization evoked by thirty spikes (10 Hz/3 s). *A*, effect of 5 min exposure to TEA (1 mM). *B*, effect of 2 min exposure to caffeine (1 μM). Both effects reversed after 10 min wash. Neither TEA nor caffeine changed membrane potential (−60 mV).

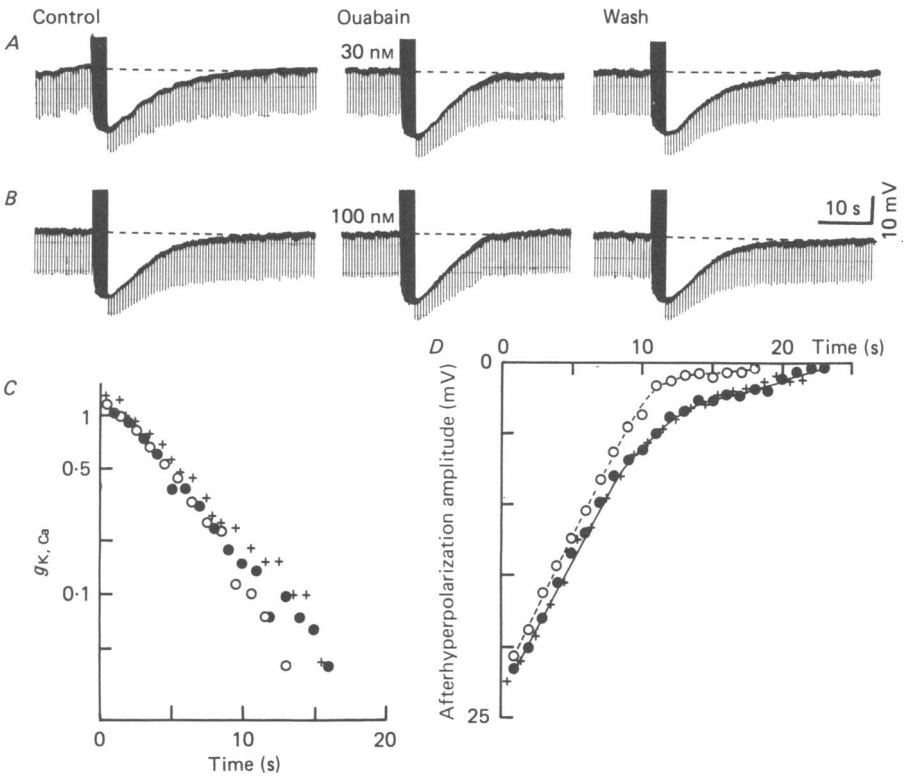

Fig. 10. Ouabain reduces late component of afterhyperpolarization evoked by thirty spikes (10 Hz/3 sec). *A*, effect of ouabain (30 nM). *B*, effect of ouabain (100 nM). *C* and *D*, ●, control; ○, in the presence of ouabain (100 nM); +, washout. Ouabain had little effect on the time course of the conductance change (*C*) but depressed the late component of the potential change. Date in (*C*) and (*D*) from (*B*). Resting membrane potential (−55 mV) was not changed by ouabain.

but may act by promoting release of calcium from intracellular storage sites (Kuba, 1980).

Ouabain (30–500 nM) significantly reduced the later part of the afterhyperpolarization induced by thirty action potentials (Fig. 10) but had no detectable effect on the afterhyperpolarization which followed a single action potential. The reduction in the late component of the afterhyperpolarization was reflected by a decrease in duration of $57{\cdot}7 \pm 7{\cdot}8\%$ ($n = 6$). This concentration did not affect the resting membrane potential.

DISCUSSION

The results leave little doubt that the hyperpolarization following one or several action potentials is due to an activation of g_K secondary to calcium entry. This confirms and extends earlier observations made on one or a few action potentials (North, 1973; Hirst & Spence, 1973; North & Hishi, 1974; North & Nishi, 1976). The principal evidence which substantiates the g_K activation is the complete reversal of the afterhyperpolarization at a potential which varied with the logarithm of the external potassium concentration, and the generally good agreement between the amplitude of the potential change and the amplitude of the underlying conductance change.

The evidence which supports the claim that calcium ion entry triggers the activation of g_K is the complete abolition of the afterhyperpolarization following from one to sixty action potentials in calcium-free or cobalt containing solutions, and its prolongation by calcium, TEA and caffeine. If one assumes that an equal amount of calcium enters with each action potential, then the number of spikes should be linearly related to the conductance increase, so long as calcium sequestration systems can be ignored. Fig. 2 shows that from zero to three action potentials the estimate of $g_{K,Ca}$ did increase linearly, whereas larger numbers of spikes showed a progressively smaller conductance increment. In view of the observed time constants of decay of the fractional conductance increase, which is presumed to be a measure of $g_{K,Ca}$, it would be reasonable to suppose that the cell is already sequestering or extruding its calcium load by the time the peak measurement is made after six to sixty action potentials. High calcium and TEA will increase calcium entry into the neurone by increasing the driving force and prolonging the action potential respectively. Caffeine may limit the binding of intracellular calcium to an internal sequestration site and thereby prolong the rise in $g_{K,Ca}$ (Kuba, 1980).

We considered the possibility that electrogenic pumping may contribute to the amplitude of the afterhyperpolarization which follows many action potentials, as it does in the leech (Jansen & Nicholls, 1973). Two pieces of evidence suggest a small contribution by active ion pumping. The first is the finding that a deviation between observed amplitude and amplitude predicted from the conductance change became larger between 5 and 15 s after thirty action potentials (Fig. 6). The second is the finding that low concentrations of ouabain, which had no effect on resting potential, shortened the duration of the afterhyperpolarization following thirty spikes (Fig. 10). On the other hand, two findings argue against any significant active pumping. These are the complete abolition of the response by hyperpolarization with symmetrical reversal (Fig. 4) and the finding that no hyperpolarization whatsoever remained after removal of calcium ions. One might conclude that any activation of electrogenic pumping by one to sixty action potentials is rather small.

In invertebrate neurones, g_K follows the intracellular free calcium concentration with great fidelity (Gorman & Thomas, 1980*a*, *b*). This affords some confidence in

interpreting the decay of the fractional conductance increase ($g_{K,Ca}$) as reflecting the time course of sequestration of the calcium which enters the neurone during activity. At the resting potential (about −60 mV) the mean time constant of decay increased from 1·5 to 6 s as the number of action potentials was increased whereas larger amounts of calcium entry evoked by fifteen to sixty spikes caused an increase in $g_{K,Ca}$ which decayed biphasically with time constants of approximately 3 s and 12 s. Taken together, the results may indicate two calcium binding sites within the cell, one of which sequesters calcium rapidly but can be readily saturated, and a second one which removes free calcium with a slower time constant. In *Aplysia* neurones, the time course of decay of $I_{K,Ca}$, or intracellular calcium measured by Arsenazo III absorbance, was also double exponential, with time constants of about 6 and 28 s. This is true either for calcium injection close to the membrane (Gorman & Hermann, 1979) or for calcium entry in response to step depolarization under voltage clamp (Gorman & Thomas, 1980*a*). It is difficult to compare the values directly with our own findings because of the temperature difference (17 °C *vs.* 37 °C) and the difference in cell diameter (approximately 300 μm *vs.* 30 μm). It would be desirable, but unfortunately is not possible, to ascribe identitites to the two calcium sequestration sites: the sensitivity of the later component to caffeine suggested that it may represent the submembrane cisternae (Kuba, 1980). The more rapid component which is readily saturated may represent sites on the membrane itself. We can not discount the possibility that the later component reflects extrusion of calcium from the cell across the plasma membrane.

The significance of the calcium-activated potassium conductance in neuronal function has been stressed by several workers (e.g. Meech, 1978). In myenteric neurones, its long time course following even a few action potentials establishes it as a very important regulator of cell firing. The hyperpolarization occurring in the soma readily fractionates the incoming action potentials (North & Nishi, 1974) and such propagation block may also occur on cellular processes (see Morita & North, 1981). Furthermore, the potassium channels which are activated by calcium entry may be the same channels which close during the slow e.p.s.p. (Johnson, Katayama & North, 1980; Grafe, Mayer & Wood, 1980). Indeed, the slow afterhyperpolarization is greatly reduced during the slow e.p.s.p. (Grafe *et al.* 1980; K. Morita & K. A. North, unpublished observations), and this provides for a strong form of synaptic interaction. A detailed report on the interactions between slow synaptic potentials and the afterhyperpolarization is in preparation (K. Morita & R. A. North). The possibility that drugs and neurotransmitter substances primarily affect the activation of $g_{K,Ca}$ must be considered. Both clonidine and opiates (and their naturally occurring counterparts noradrenaline and enkephalin) prolong the afterhyperpolarization (Tokimasa *et al.* 1981) under concentrations in which resting membrane potential is unaffected. The present study of the mechanism of the afterhyperpolarization may provide a framework in which to interpret the effects of such substances which may directly alter the disposition of intracellular calcium ions or the ability of calcium to activate $g_{K,Ca}$.

APPENDIX

The relation between the amplitude of the afterhyperpolarization at any membrane potential and the associated conductance change was determined. The circuit

diagram is shown in Fig. 11. g_r is the resting membrane conductance ($= 1/R$) and E_r is the combined equilibrium potential for all channels open at rest. $E_{K,\,Ca}$ is the equilibrium potential for potassium ions moving through channels of conductance $g_{K,\,Ca}$ opened by intracellular calcium ion accumulation. I represents an external current source used to pass constant current across the membrane.

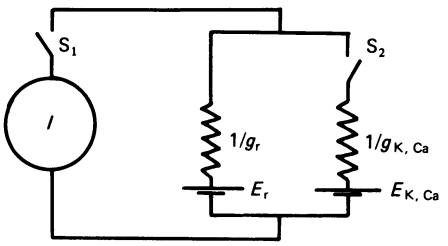

Fig. 11. Equivalent circuit for calculation of predicted value of afterhyperpolarization from amplitude of electrotonic potentials (see Appendix).

By Kirchoff's law, with S_1 open and S_2 closed

$$i = \frac{E_r - E_{K,\,Ca}}{\frac{1}{g_r} + \frac{1}{g_{K,\,Ca}}}. \tag{1}$$

The membrane potential E is given by

$$E = E_r - \frac{1}{g_r}. \tag{2}$$

Therefore

$$E = \left(\frac{g_r}{g_r + g_{K,\,Ca}}\right) E_r + \left(\frac{g_{K,\,Ca}}{g_r + g_{K,\,Ca}}\right) E_{K,\,Ca}. \tag{3}$$

The amplitude of the afterhyperpolarization is v, where $v = E - E_r$; hence

$$v = \left(\frac{g_{K,\,Ca}}{g_r + g_{K,\,Ca}}\right)(E_{K,\,Ca} - E_r). \tag{4}$$

Since $R = 1/g_r$ and $R' = 1/(g_r + g_{K,\,Ca})$ (see Methods), then

$$v = \left(1 - \frac{R'}{R}\right)(E_{K,\,Ca} - E_r). \tag{5}$$

Eqn. 5 was used to determine the amplitude of the afterhyperpolarization which would be expected for a given conductance change on the assumption that it caused only an increase in conductance to potassium ions (see Figs. 2 and 6).

In some experiments constant currents were used to polarize the membrane. Let E_m be the membrane potential which is set by passage of a polarizing current i_m. Then, with S_1 closed and S_2 open (i.e. before induction of the afterhyperpolarization) we have

$$i_m = (E_r - E_m)\, g_r. \tag{6}$$

During the afterhyperpolarization, with S_1 and S_2 closed, the same applied current continues to flow but the proportion passing across g_r falls to $(E_r - E_m) \cdot g_r(g_r/g_r + g_{K,Ca})$. This adds to the current flowing as a result of closing S_2, giving a total current across g_r of

$$i^* = \frac{(E_r - E_{K,Ca})}{\frac{1}{g_r} + \frac{1}{g_{K,Ca}}} + \frac{(E_r - E_m) g_r^2}{g_r + g_{K,Ca}}. \quad (7)$$

The potential across the membrane becomes $E = E_r - i^*/g_r$, and the amplitude of the afterhyperpolarization is $v = E - E_m$. Appropriate substitution leads to

$$v = \left(1 - \frac{R'}{R}\right)(E_{K,Ca} - E_m). \quad (8)$$

This equation indicates that the amplitude of the afterhyperpolarization should be linearly related to the membrane potential at which it was evoked.

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