J. exp. Biol. (1979), 81, 93–112
With 14 figures
Printed in Great Britain

MEMBRANE POTENTIAL OSCILLATIONS IN MOLLUSCAN "BURSTER" NEURONES

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SUMMARY

Membrane potential oscillations can be induced in molluscan neurones under a variety of artificial conditions. In the so-called 'burster' neurones oscillations are generated even in isolated cells. A likely mechanism for 'bursting' involves the following ionic currents:

1. A transient inward current carried by Na^+ and Ca^{2+}. This current is responsible for the upstroke of the action potentials.

2. A delayed outward current carried by K^+. This current is voltage-sensitive and is responsible for the downstroke of the action potential during the early part of the burst. It becomes progressively inactivated during the burst. Its amplitude depends on the intracellular pH.

3. A rapidly developing outward current carried by K^+ which is inactivated at potentials close to action potential threshold. This current tends to hold the membrane in the hyperpolarized state and is involved in spacing the action potentials.

4. A prolonged inward current which may not inactivate. It is probably carried by both Na^+ and Ca^{2+}. This current is responsible for the depolarizing phase of the burst but also contributes to the action potential.

5. A slowly developing outward current, carried by K^+. This current appears as a result of a slow increase in intracellular ionized calcium and is responsible for the hyperpolarizing phase of the burst. Note that a transient increase in this current may also contribute to the falling phase of the action potential during the later stages of the burst. It is also sensitive to intracellular pH.

One of the more significant features of this system of producing membrane potential oscillations is that the frequency of the bursts depends on the rate at which the intracellular ionized calcium returns to its resting level. This process depends on the metabolic state of the animal which can thereby exert a considerable influence on the electrical activity of burster neurones.

INTRODUCTION

The timing element or oscillator responsible for a centrally generated pattern of behaviour could be a network of cells or a single rate determining neurone. This review presents evidence for an ionic mechanism by which bursts of action potentials are generated in an exceptionally regular fashion in certain molluscan 'burster' neurones. The function of this particular oscillation is unknown but it may have some value as a model system.

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CHARACTERISTICS OF MOLLUSCAN 'BURSTER' NEURONES

Cell R_{15} in the abdominal ganglion of *Aplysia* is readily identifiable by its size, colour and position (Frazier *et al.* 1967). In isolated ganglia, it has a distinctive electrical rhythm which consists of trains, or bursts, of action potentials separated by periods of hyperpolarization (Chalazonitis, 1961; Strumwasser, 1965). The inter-burst hyperpolarization gives way to a slowly developing depolarization which culminates in the next parabolic burst of action potentials. During the burst, the action potentials at first increase in frequency. They reach a maximum rate and then the rate of firing declines. There is a general increase in the duration and size of the action potentials throughout the burst and there are changes in the action potential undershoot. Cells apparently homologous to R_{15} have been described in *Helix aspersa* (cell A; Kerkut & Meech, 1966) and *Otala lactea* (cell 11; Gainer, 1972*a*). Fig. 1 shows intracellular recordings from each of these cells showing typical patterns of activity. The oscillation appears to be an endogenous rhythm which persists even when the cell is totally isolated (Alving, 1968; Gainer, 1972*b*; Chen, von Baumgarten & Takeda, 1971).

Whether or not the oscillation has any physiological significance is not known. Typical 'bursting' is recorded in R_{15} only in isolated ganglia (Stinnakre & Tauc, 1969) and the cell fires irregularly when the connexion to the branchial ganglion remains intact. (But see Kater & Kaneko, 1972.)

The cells themselves appear to have a secretory function and each has an axon which terminates in the heart. It has been suggested that they are ideally situated for the release of an agent having a general effect on the animal. In *Aplysia* activation of osmoreceptors in the osphradium by dilute sea water inhibits R_{15} (Stinnakre & Tauc, 1969) and the cell is known to contain one or more polypeptide hormones apparently involved in ionic regulation or water balance. Injection of an R_{15} homogenate into the haemocele of *Aplysia* produces a 3–10% weight increase in 90 min even in 5% hyperosmotic sea water. Control injections of other neurones have no effect and the action of the R_{15} homogenate is destroyed by proteolytic digestion (Kupfermann & Weiss, 1976).

The rate of firing of spontaneous action potentials in R_{15} reaches a maximum at about 'dawn' each day. The time of this peak depends on the time of 'dawn' to which the animal has been conditioned (Strumwasser, 1965). In cell 11 of *Otala* the pattern of electrical activity changes from bursting to continuous firing for 2–3 h near 12 a.m. (Gainer, 1972*b*) and in *Helix* a similar change in pattern occurs in cell A at about 12 p.m. (Meech, 1969). However, in neither of these latter cases has it been possible to change the time of the transition period by altering the environment of the snails.

In *Helix*, cell A is spontaneously active for most of the year. However, during the summer this spontaneous activity is often absent and the membrane potential reaches a maximum level of about −63 mV (Kerkut & Meech, 1967). At this time the animals themselves are quiescent and are said to be in diapause. Animals in this state can be recognized by the calcarious epiphragm covering the shell aperture. Cell 11 from specimens of *Otala* in diapause have a lower membrane resistance and a higher potassium permeability than actively 'bursting' cells. The current–voltage

lationship of the cell membrane is linear rather than anomalously rectifying (Gainer, 1972*b*). Synthesis of a polypeptide specific to cell 11 is also inhibited.

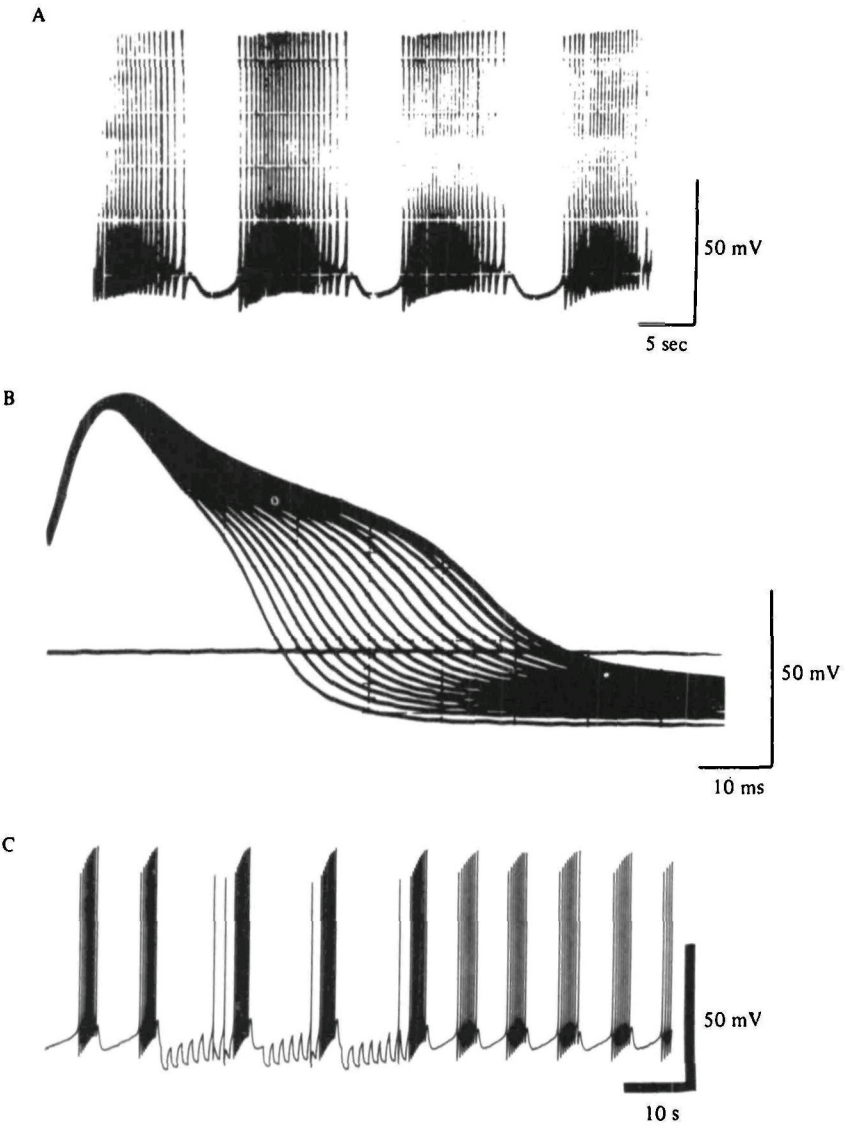

Fig. 1. Patterns of electrical activity in 'burster' neurones. (A) *Helix aspersa*, cell A; intracellular record from an active snail. (B) *Aplysia californica*, R_{15}; superimposed action potentials from a single burst, intracellular record. (From Strumwasser, 1967.) (C) *Otala lactea*, cell 11; pen-recording of electrical activity. The action potentials are slightly attenuated. Constant current hyperpolarizing pulses between bursts 2–5 indicate change in membrane resistance. Note the low membrane resistance at the end of each burst (from Gainer, 1972*c*).

IONIC MECHANISM OF 'BURSTING'

During membrane potential oscillations of the type recorded from 'burster' neurones (Fig. 1), there are repeated changes in the current flow across the neuronal membrane. A slowly developing inward current depolarizes the cell to its action potential threshold and produces a train of action potentials while a slowly developing

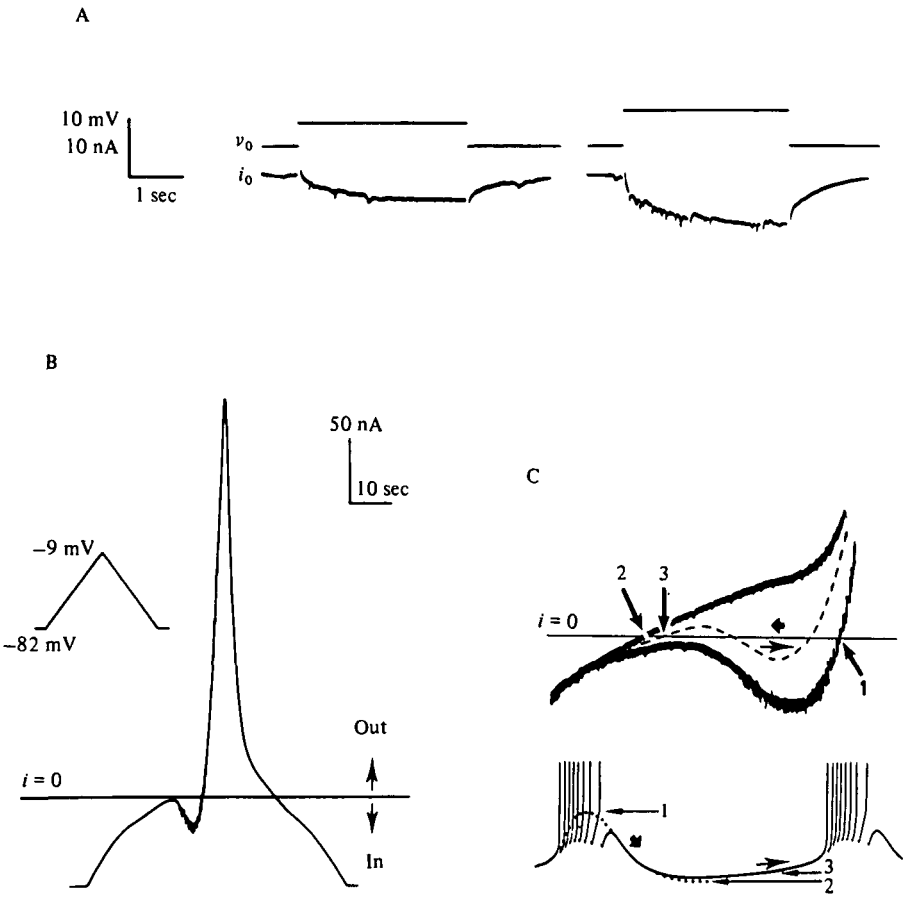

Fig. 2. Voltage-clamp records from cell R_{15}, *Aplysia fasciata*. (A) Slowly developing inward current (lower trace) generated in response to a step depolarization lasting 3 s; holding potential, −47 mV. (B) Membrane currents generated in response to a ramp change in membrane voltage from −82 to −9 mV and back to −82 mV over a period of 62 s. (C) Lower trace: typical electrical activity recorded intracellularly from R_{15}. Upper trace: membrane currents generated by a ramp change in membrane voltage. The currents during the depolarizing ramp have been superimposed on those recorded during the repolarizing ramp. See text for explanation. (From Gola, 1974*b*.)

outward current tends to terminate the burst. This simplified description implies that if the membrane voltage is held at a fixed potential, it should be possible to identify a membrane current rising and falling with the same frequency as the bursts of action potentials seen in the unclamped cell.

In reality, if the membrane is held steady at some slightly depolarized potential a long-lasting inward current develops as shown in Fig. 2A (Gola, 1974*b*; Eckert &

Lux, 1975; see also Wilson & Wachtel, 1974). At slightly higher potentials, an outward current eventually develops which opposes the inward current (Gola, 1974*b*). This current, which is responsible for the post-burst hyperpolarization, slowly declines when the membrane is repolarized to its resting level.

Since the changes in membrane current are bound up with changes in membrane potential a useful approach is to slowly depolarize and repolarize the cell in a ramp fashion (Gola, 1974*b*). Fig. 2B shows that the inward current develops as the

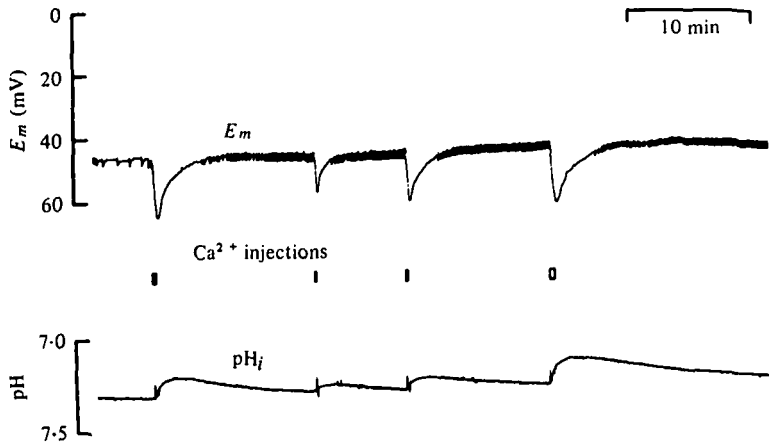

Fig. 3. Pen recording of an experiment to show the effect of calcium injection on the membrane potential of cell A (upper trace) and on intracellular pH (lower trace). The duration of each calcium injection is shown below the membrane potential trace (from Meech & Thomas, 1977).

membrane potential of the cell is slowly changed from −82 to −9 mV, but that it does not reappear as the cell is repolarized. Thus, if the membrane voltage is slowly changed back and forth over a sufficient range of potential, the net membrane current is at first inward and then outward.

A model presented by Gola (1974*b*) which summarizes these findings and which accounts for the potential oscillation is shown in Fig. 2C. The lower trace is the typical bursting pattern of action potentials. During the burst position 1 is the potential at which the net membrane current is zero (see upper trace). The membrane is stable at position 1 for a short time but as the characteristics of the membrane change the stable position moves to a more negative potential (position 2). The current voltage relationship then slowly returns to its former state and the membrane potential moves back from position 2 to position 1. Position 3 is an intermediate state.

Identification of currents

There is good evidence that the post-burst hyperpolarization is caused by an increase in the potassium conductance of the cell membrane. Fig. 1C shows that the conductance of the cell membrane (as judged by the voltage displacement produced by constant current hyperpolarizing pulses) declines to a minimum value just before the start of the next burst (Gainer, 1972*c*). In R_{15} Junge and Stephens (1973) were able to demonstrate that the value of the reversal potential of the post-

burst hyperpolarization depends on the concentration of potassium in the bathing medium. This increase in potassium conductance appears to be a direct result of an increase in the level of ionized calcium in the cell cytoplasm. There is a considerable increase in the level of ionized calcium during a train of impulses in R_{15} (Stinnakre & Tauc, 1973) and in bursting neurones, the calcium level increases during the burst and declines during the hyperpolarizing phase (see Fig. 4; Thomas & Gorman, 1977;

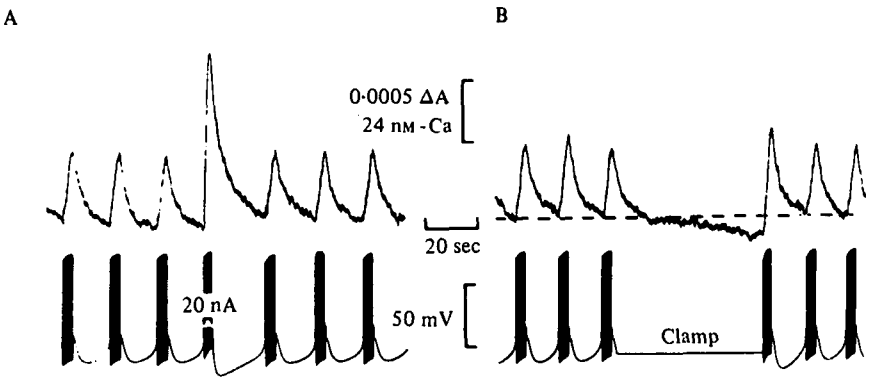

Fig. 4. Changes in free intracellular calcium and membrane potential in cell R_{15} (*Aplysia californica*) during bursts of activity. Changes in Arsenazo III dye absorbance (upper trace) indicate changes in the level of ionized calcium. (A) Effect of a 20 nA depolarizing current passed during a single burst. The membrane hyperpolarization is larger and the next burst delayed until the absorbance has declined almost to its normal baseline value. (B) Effect of period under voltage clamp. The dye absorbance declines below its normal base-line value at the time when the next burst is expected. The next burst occurs immediately upon release from voltage-clamp (from Thomas & Gorman, 1977).

Gorman & Thomas, 1978). If cells are injected with the calcium-chelating agent EGTA so that the increase in intracellular ionized calcium is prevented, they lose their post-burst hyperpolarization (see Fig. 5; Meech, 1974*a*). Injection of calcium, on the other hand, leads to a hyperpolarization of the cell membrane (see Fig. 3; Meech & Strumwasser, 1970; Meech, 1972; Meech, 1978). The abrupt initiation of the hyperpolarizing phase of the burst (see Fig. 1) is accounted for at least in part by the steep relationship between potassium conductance and intracellular calcium (Meech & Thomas, unpublished).

In summary, the available evidence suggests the following mechanism for the oscillations in membrane potential seen in burster neurones.

(1) A slowly developing inward current depolarizes the cell membrane. It is probably at least partly carried by calcium ions and contributes to the slow increase in intracellular calcium which occurs at the same time.

(2) The increase in intracellular calcium causes an increase in the potassium conductance of the cell membrane which hyperpolarizes the cell and terminates the burst.

(3) In the hyperpolarized state there is no longer an inward current and the level of ionized calcium returns to its resting level. This process is dependent on metabolism.

This description accounts for the oscillations in a general fashion, but the bursts of action potentials themselves have complex characteristics and so a more detailed

examination of the properties of the cell membrane is required. Five different current components appear to be involved.

INWARD CURRENTS

The importance of calcium ions in the generation of action potentials in molluscan neurones was first demonstrated by Oomura, Ozaki & Maéno (1961). Subsequently action potentials in many neurones of both *Helix* and *Aplysia* were shown to depend

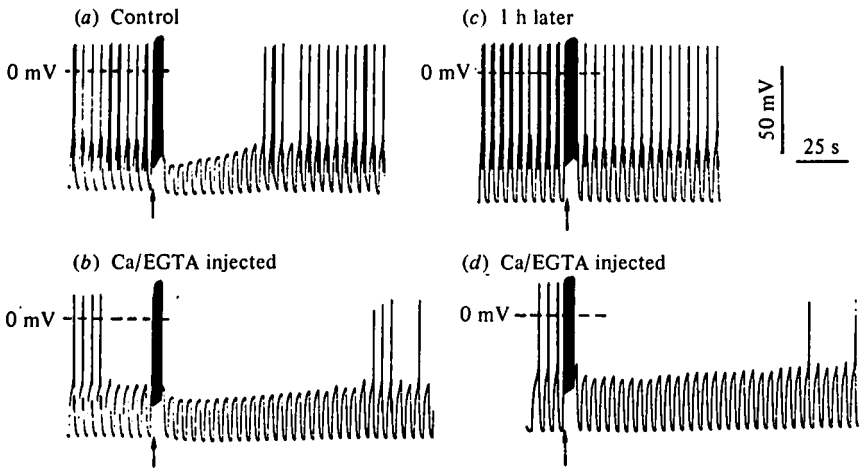

Fig. 5. Post-burst hyperpolarization in cell R_{15} (*Aplysia californica*). Pen recording of an experiment to show the effect of injecting calcium/EGTA. Constant current hyperpolarizing pulses provide an estimate of membrane resistance. The burst was generated by a 5 s depolarizing current (2×10^{-8} A) injected at the point indicated by the arrow. (*a*) Control; (*b*) after injection of a small volume of calcium/EGTA buffer containing 3×10^{-6} M free calcium ion at pH 7·26; (*c*) after 1 h to allow for sequestration of the injected calcium, the remaining EGTA buffers the calcium influx during the burst; (*d*) the post-burst hyperpolarization after injection of a further 5×10^{-10} l (approx.) calcium/EGTA solution. See Meech (1974*c*) for composition of solutions. (From Meech, 1974*a*.)

on both sodium and calcium ions (Kerkut & Gardner, 1967; Junge, 1967). Voltage-clamp experiments show that depolarization produces a transitory inward current which can be carried by calcium ions in sodium-free saline and sodium ions in calcium-free saline (Geduldig & Gruener, 1970; Krishtal & Magura, 1970; Standen, 1974).

By analogy with the early and late inward currents in squid axons (Baker, Hodgkin & Ridgway, 1971) it seemed likely that the transient inward current referred to above and the prolonged inward current described by Gola (1974*b*) and Eckert & Lux (1975) – see Fig. 2 – passed through two different populations of channels. In perfused *Helix* neurones, the calcium current decay is made up of at least two exponential processes, but it is apparently not possible to separate the currents into early and late components (Kostyuk & Krishtal, 1977). Akaike, Lee & Brown (1977) have suggested that the inward current inactivates only slowly during a small depolarization and that it is not necessary to postulate two distinct conductances for the transient and persistent channels (but see Connor, 1979).

The prolonged inward current studied by Eckert & Lux (1976) in cell A remains essentially unaffected until 95% or more of the NaCl in the medium is replaced by glucose. On the other hand, substitution of 30 mM-$CoCl_2$ for 10 mM-$CaCl_2$ abolishes the inward current in 2–5 min. These observations suggest that the prolonged inward current is primarily carried by calcium. However, the calcium blocking agent D-600 does not abolish the inward current in normal saline (Barker & Smith, 1978), although it is effective when NaCl and $CaCl_2$ are both replaced by $BaCl_2$. These conflicting observations may arise from species differences, but it is also possible that the prolonged inward current like the transient inward current, is a mixed current, both sodium and calcium ions being involved.

As shown Fig. 1, successive action potentials during a burst change in shape. One possible reason for this is that the characteristics of the inward current change in some way during the train. For example, it may be activated more rapidly with succeeding action potentials. A facilitating calcium current has been reported on the basis of aequroin experiments in *Helix* (Lux & Heyer, 1977) and *Anisodoris* (Eckert, Tillotson & Ridgway, 1977) and on the basis of an apparent discrepancy between outward currents and potassium flux in cell A (Heyer & Lux, 1976*b*), but this has not been confirmed with experiments with arsenazo III (Gorman & Thomas, 1978; Ahmed & Connor, 1979) or by experiments on perfused snail neurones (Akaike *et al.* 1978).

OUTWARD CURRENTS

Another possible reason for the increase in the duration of the action potentials during a train is that the outward potassium current responsible for the repolarizing phase may become progressively inactivated. As shown in Fig. 6, inactivation of the outward current either during a depolarizing pulse or with successive depolarizing pulses is a well-established phenomenon in molluscan neurones (Gola & Romey, 1971; Leicht, Neves & Wellhöner, 1971; Neher & Lux, 1971; Connor & Stevens, 1971*a*) and the gradual inactivation of the delayed outward current during a train of impulses seems likely (see also Aldrich, Getting & Thompson, 1979).

In molluscan neurones, a depolarizing pulse generates two different delayed outward currents. Both are carried by potassium ions but one depends on an increase in intracellular calcium during the pulse, while the other appears to be simply dependent on the voltage across the cell membrane (see Meech, 1978, for review). The two components are demonstrated by the experiment on cell A shown in Fig. 7A. The graph shows the outward current measured at the end of a 55 ms pulse plotted against the potential of the membrane during the pulse. Under normal conditions the curve is 'N-shaped' (Meech & Standen, 1975) and in Fig. 7A, this is shown by the filled circles. The open circles show that the effect of injecting EGTA into the cell is to reduce the potassium currents for depolarizing pulses up to about +150 mV. The remaining current, which is primarily the voltage-sensitive component, can be subtracted from the control curve leaving the calcium-dependent component (see Fig. 7B). A similar effect is produced by washing the cell in calcium-free saline (Meech & Standen, 1975). The current–voltage relationship for the calcium-dependent component closely resembles the relationship between membrane potential and

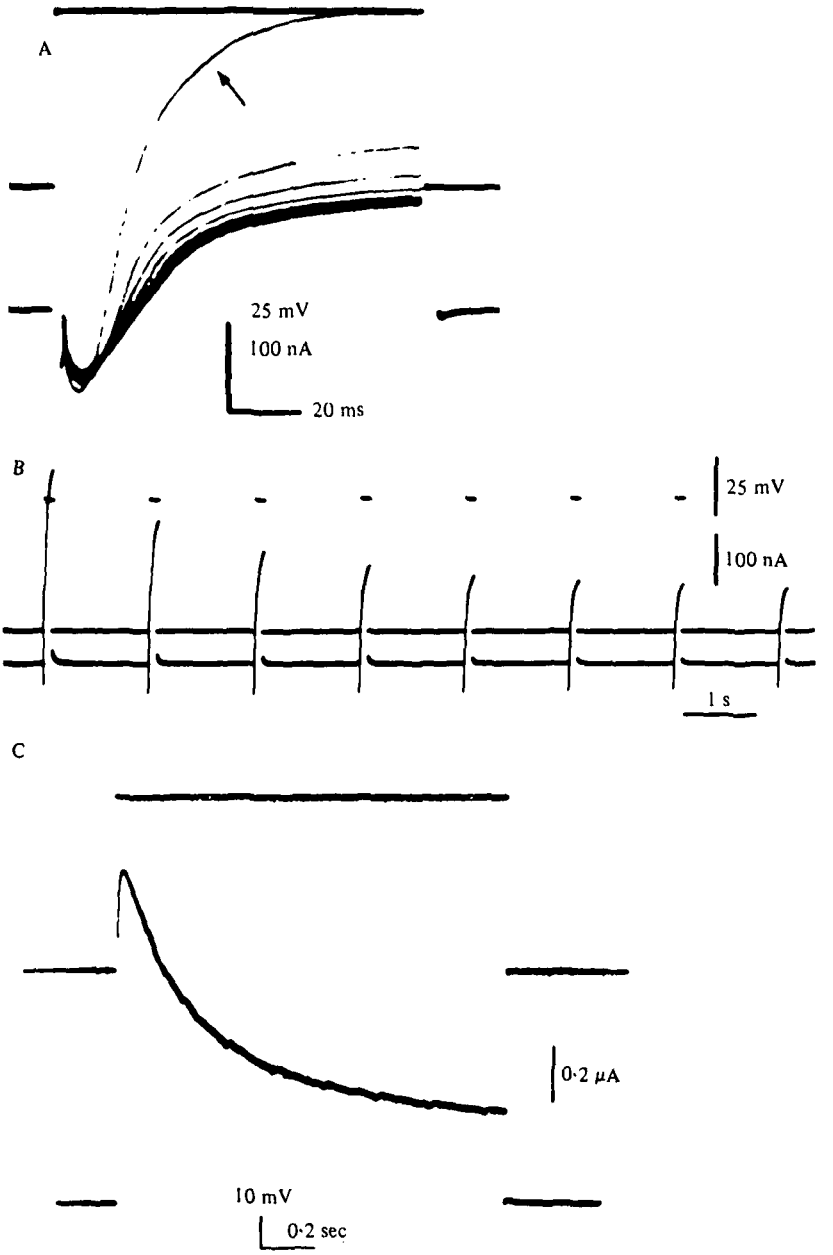

Fig. 6. Inactivation of outward current in cell R_{15}, *Aplysia fasciata*. (A) Arrowed trace: transient inward and delayed outward current recorded under voltage-clamp in response to a 50 mV step depolarization. Superimposed records show the currents recorded in response to subsequent 50 mV pulses at a frequency of 1·5 c/s. Note the depression in outward current. Holding potential, −54 mV. (B) Depression in outward currents with repetitive depolarizing pulses shown on a longer time scale. Holding potential, −48 mV. (C) Outward current recorded in response to a prolonged 55 mV depolarizing pulse. Note that the outward current reaches a maximum within 0·1 s and then declines to a new steady level (A, B from Gola, 1974*a*; C from Gola & Romey, 1971).

light output measured in aequorin-injected cells (Lux & Heyer, 1977). The amplitude of the calcium-dependent component is thus directly related to the level of intracellular calcium.

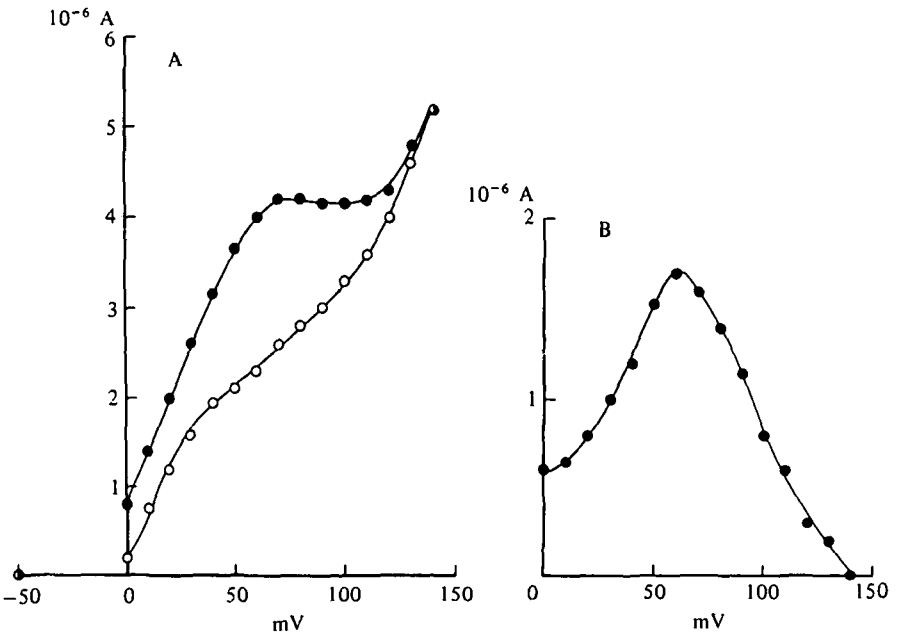

Fig. 7. (A) The relationship between outward membrane current and membrane potential in cell A, before (filled circles) and after (open circles) injection with EGTA. Abscissa: command potential; ordinate: membrane current measured 55 ms after the beginning of the command pulse. For EGTA solution and voltage-clamp circuit see Meech (1974*c*). Cell bathed in normal Tris buffered saline, see Meech & Standen (1975). Holding potential −50 mV; cell diameter 220 μm; temperature 23 °C (replotted from Meech, 1978). (B) The calcium-dependent component of outward current, the difference between the two curves shown in (A).

Except for small depolarizations (see Fig. 2) the inward current responsible for the increase in intracellular calcium is concealed by the much larger outward current. However, in EGTA-injected cells the outward current inactivates sufficiently during a prolonged pulse for the maintained inward current to be seen (see Fig. 8). Such EGTA-injected cells produce prolonged action potentials which have a plateau on their falling phase (Meech, 1974*b*).

The calcium-dependent potassium current inactivates slowly if at all (Meech & Standen, unpublished; Aldrich *et al.* 1979). However, the inactivation undergone by the voltage-dependent component (see Fig. 8) is sufficient to account for the broadening of the action potentials during a train.

An alternative hypothesis has been presented by Eckert & Lux (1977), who have identified a voltage-dependent inactivation of the outward current which has the appearance of being calcium-dependent. They find that a 1 s prepulse has the effect of depressing currents produced by a second pulse presented 1 s later. The effect

of the prepulse is to abolish the 'N-shape' of the *I–V* curve in a similar fashion to the effect of injecting EGTA shown in Fig. 7. Eckert and Lux conclude that it is primarily the calcium-dependent currents which are depressed and that an influx of calcium is responsible for this depression. The hypothesis is supported by the observation that injection of calcium into cell A can reduce the size of the time-dependent outward currents recorded under voltage-clamp (Heyer & Lux, 1976*b*).

Fig. 8. Currents recorded from an EGTA-injected cell A under voltage clamp. Three superimposed records are shown; membrane voltage at the top and membrane current at the bottom. Pulse duration 1 s. As in Fig. 6, the outward current reaches a maximum in less than 0·1 s and then declines. For 50 and 60 mV command pulses the final current is inward. This long-lasting inward current has a reversal potential at about +15 mV. Cell bathed in normal Tris buffered saline (Meech & Standen, 1975). Holding potential −50 mV; cell diameter 200 μm; temperature 21–23 °C.

In this scheme the calcium dependency of the outward current arises from the influx of calcium across the membrane rather than from the increase in intracellular calcium. Consequently, Eckert and Lux propose that the influx of calcium during a train of impulses leads to a progressive reduction in the amplitude of the outward current and to a prolongation of the action potential. This proposal seems unlikely for the following reasons.

(1) In cell A, inactivation of outward current occurs in EGTA-injected cells (see Fig. 8) and in calcium-free saline (Meech & Standen, unpublished).

(2) Injection of a small volume of calcium chloride into cell A has the effect of reducing the net inward current measured during a depolarizing pulse. It has no effect on the net outward current (see Fig. 9) and so this should shorten rather than prolong the action potentials in a train.

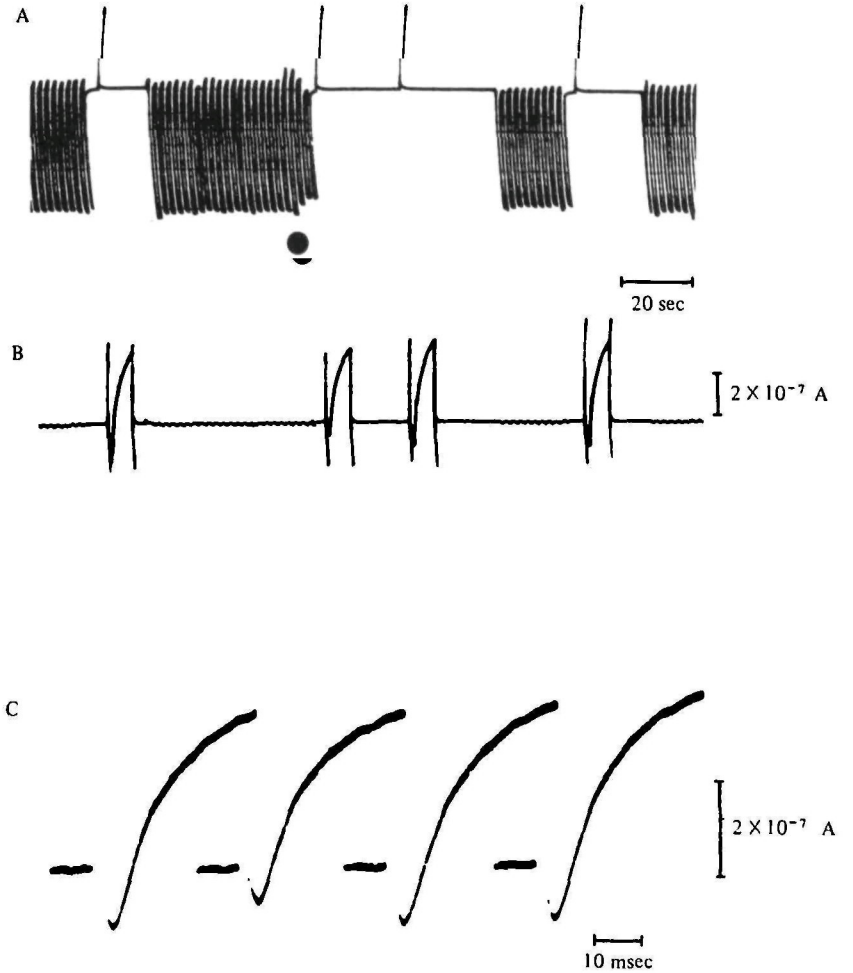

Fig. 9. Effect of calcium injection on membrane currents in cell A. (A) Pen-recording of the complete experiment. Except for the periods when voltage-clamp was imposed, hyperpolarizing constant-current pulses were injected into the cell to provide an estimate of membrane resistance. At the point indicated by the filled circle the cell was pressure-injected with calcium chloride (see Meech, 1974*c* for conditions). About 5 s later the cell was voltage-clamped and then briefly depolarized to 0 mV by a 37·5 ms command pulse. Cell bathed in Tris buffered saline (Meech & Standen, 1975). Holding potential −50 mV; cell diameter 200 μm; temperature 21–23 °C. (B) Pen-recording of the membrane currents generated by the sequence of four 50 mV depolarizing command pulses shown in A. The trace was made by replaying the stored current records at a slow speed. (C) Superimposed oscilloscope records of the traces shown in B. Note that the net inward current during the depolarizing pulse is smaller after the calcium injection while the net outward current is approximately the same.

EFFECT OF CALCIUM INJECTION ON MEMBRANE CURRENTS UNDER VOLTAGE-CLAMP

Injection of calcium into cell A increases the potassium conductance of the cell membrane and hyperpolarizes the cell (see Fig. 3). Under voltage-clamp the injection has the effect of increasing the instantaneous or time-independent current measured during a step change in voltage. This is not obvious in Fig. 9 because of the small

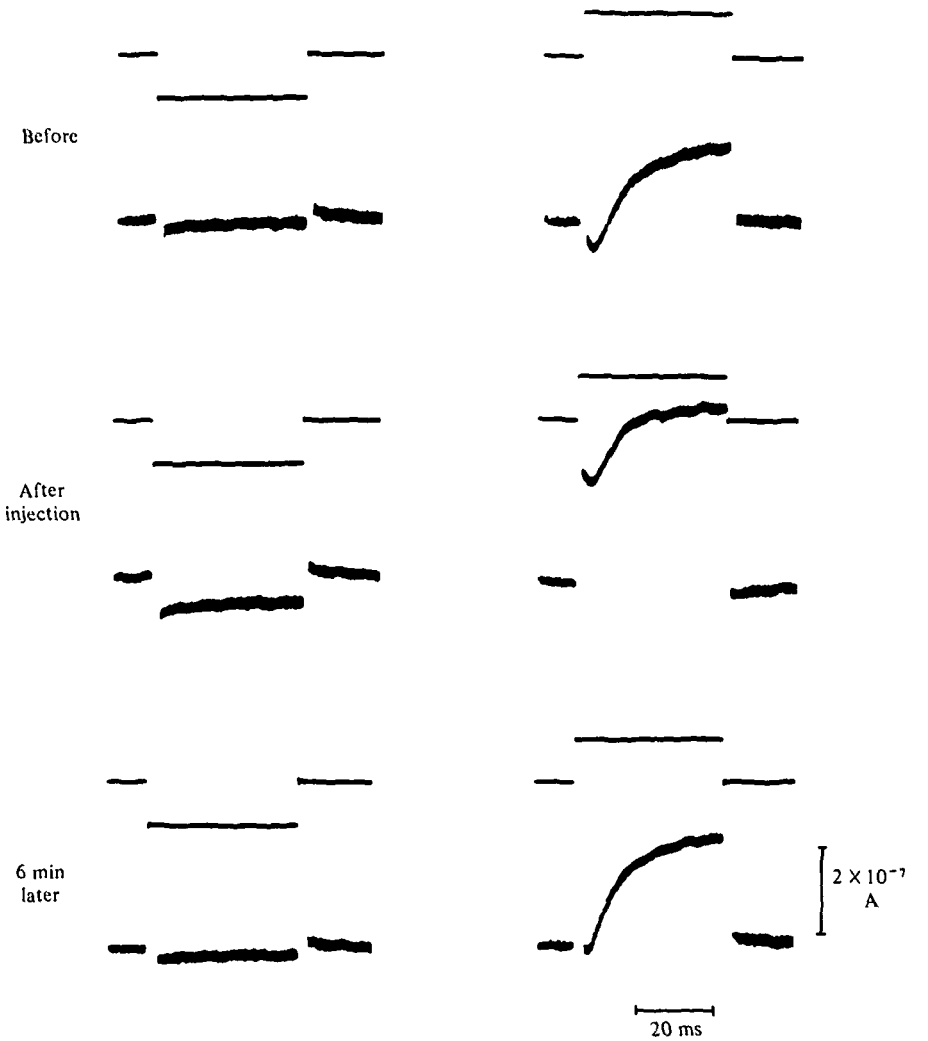

Fig. 10. Voltage-clamp experiment on cell A to show the effect of calcium injection on membrane currents. Depolarizing command pulses are shown on the right and hyperpolarizing pulses are shown on the left. The voltage traces are shown above the recorded currents. The membrane currents recorded before injection are shown at the top. After calcium injection (middle records) there is a large increase in the instantaneous current which has largely disappeared 6 min later (lower record). Cell bathed in saline containing 23 mM HCO_3^- equilibrated with 2·5 % CO_2. Holding potential −50 mV; cell diameter 200 μm; temperature 21–23 °C.

amount of calcium injected. The effect of a higher calcium injection is shown in Figure. 10. These voltage-clamp records show (on the right) the currents generated by 50 mV depolarizing pulses and (on the left) 50 mV hyperpolarizing pulses. After injection (middle records) the inward current generated by a hyperpolarizing pulse is larger than in the control (top records) because of the increase in the resting potassium conductance of the membrane. This increase in the resting conductance also accounts for the large immediate step change in the outward current generated by the depolarizing pulse. It appears to behave in a simple ohmic fashion with an

equilibrium potential at about −75 mV. Subtraction of this time-independent current shows that calcium injection produces a significant reduction in the time-dependent delayed outward current while leaving the transient inward current unaffected.

Another way in which it is possible to investigate the effect of injected calcium is to examine 'tail' currents. These currents represent the closing of potassium channels following a depolarizing pulse. In Fig. 11 the membrane was first depolarized

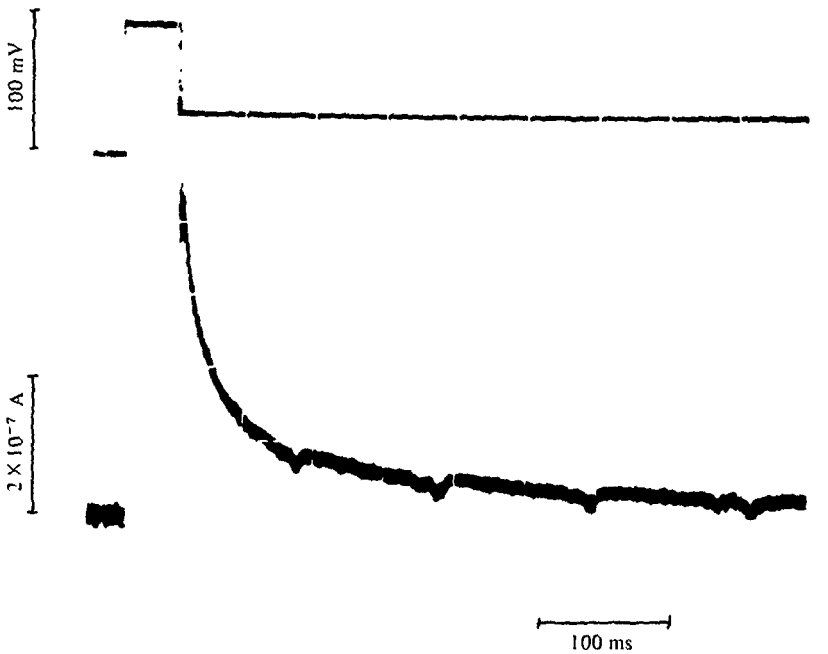

Fig. 11. 'Tail' current recorded from cell A. Upper trace, membrane voltage; lower trace, membrane current. Cell depolarized to +50 mV for 45 ms and repolarized to −20 mV. The currents generated during the first 100 mV step are not shown but there is a slowly declining outward current 'tail' during the second step. Cell bathed in normal Tris buffered saline (Meech & Standen, 1975). Holding potential, −50 mV; cell diameter 200 μm, temperature 21–23 °C.

by 100 mV to +50 mV for 45 ms and then repolarized to −20 mV. The tail of outward current shown is made up of two exponentially declining components which can be separated as in Fig. 12. The fast component is known to be unaffected by calcium-free saline or EGTA injection, while the slower component is abolished by EGTA injection and greatly reduced in calcium-free saline. It presumably represents the closing of the calcium-dependent potassium channels. Analysis of 'tail' currents after $CaCl_2$ injection shows that both fast and slow components are affected.

The effect of calcium injection on the delayed outward current appears to be brought about by a fall in intracellular pH (pH_i). Fig. 3 shows that calcium injection not only hyperpolarizes the cell membrane but also reduces the intracellular pH of the cell cytoplasm. In fact since each injected calcium is exchanged for a proton, the effect of injecting $CaCl_2$ on pH_i is equivalent to injecting HCl (see Meech & Thomas, 1977). Note, however, that HCl injection does not hyperpolarize the cell membrane,

i.e. the increase in potassium conductance does not depend on the fall in pH_i. Fig. 12 shows that injection of HCl into cell A like $CaCl_2$ injection reduces the amplitude of the 'tail' currents. A comparison of the effects of HCl and $CaCl_2$ injection on the 'tail' currents recorded from cell A is shown in Fig. 13. In part A, the recovery of the 'tail' over a period of 25 min is shown. In B the fast component has been separated and C shows the recovery of the slow component. The filled

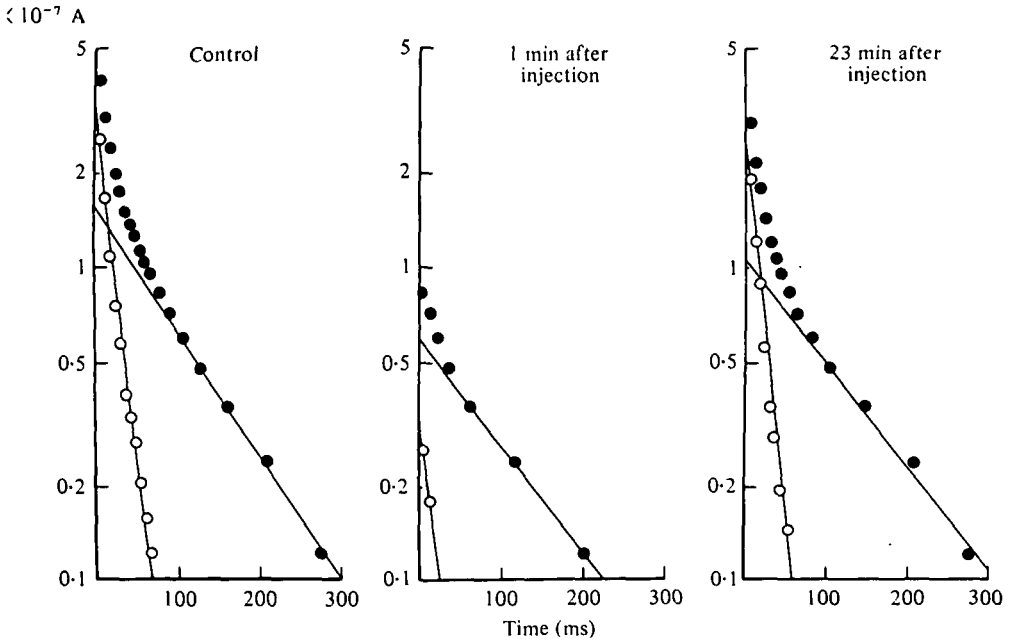

Fig. 12. Effect of HCl injection on 'tail' currents in cell A. Tail generated as shown in Fig. 11. Abscissa, time after repolarization; ordinate, membrane current (logarithmic scale). ●, Total current; ○, fast component obtained by subtraction of extrapolated slow component from total current. (A) Control; (B) after brief (approx. 1 s) injection of solution containing 100 mM-HCl and 450 mM-KCl. Injection pipette diameter 1·5 μm; (C) 23 min after injection. Cell bathed in normal Tris saline (Meech & Standen, 1975). Holding potential −50 mV; cell diameter 200 μm; temperature 21–23 °C.

circles are from an HCl injection experiment and the open circles show the effect of a $CaCl_2$ injection. In both cases the fast component is the most affected and recovers most quickly. Inhibition of the slow component appears to develop with time and recovers more slowly. Brief injections of a solution containing 500 mM-PIPES buffer and 500 mM-KCl at pH 6·8 also give a reversible reduction in 'tail' current while control injections of a solution of 500 mM-HEPES and 500 mM-KCl at pH 7·4 give an increase in tail current amplitude.

In summary, the effect of injecting calcium into cell A is to increase the resting potassium conductance of the cell membrane. However, a secondary fall in pH_i caused by calcium/hydrogen exchange in the cell cytoplasm reduces the time-dependent outward currents seen under voltage clamp. The voltage-sensitive component of the outward current is the most affected.

FAST OUTWARD CURRENT

The action potential frequency during a train or burst is greatly influenced by an outward current component which is inactivated at potentials near the action potential threshold (Hagiwara, Kusano & Saito, 1961; Connor & Stevens, 1971*b*, *c*;

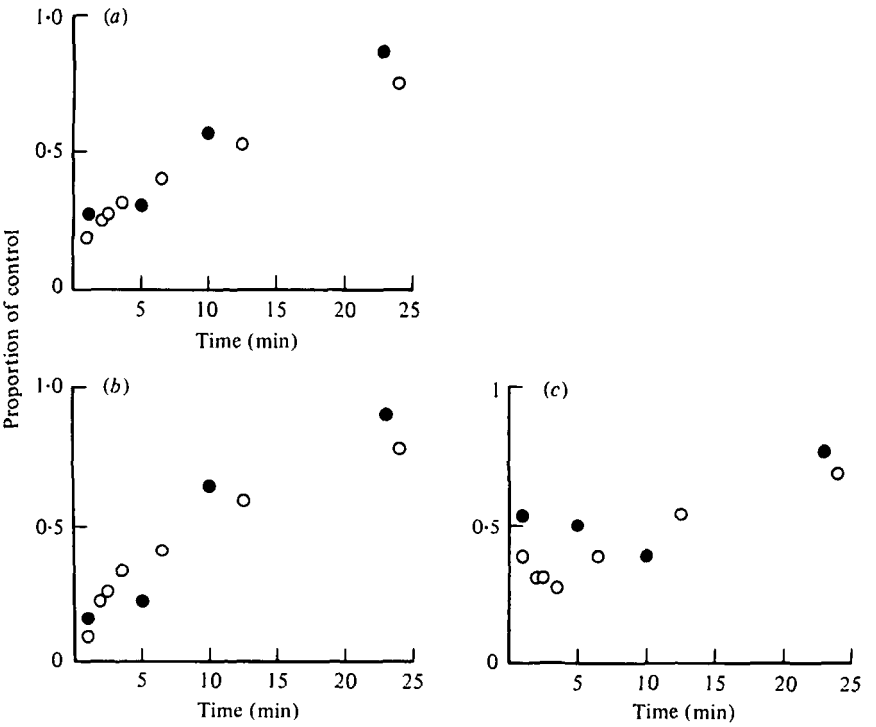

Fig. 13. Recovery of 'tail' currents after injection of $CaCl_2$ (open circles) and HCl (filled circles) into cell A. (*a*) Abscissa, amplitude of tail current 1 msec. After repolarization expressed as a proportion of control; ordinate, time after brief (approx. 1 s) injection of, (●) solution containing 100 mM-HCl and 450 mM-KCl, (○) solution containing 100 mM-$CaCl_2$ and 450 mM-KCl. (*b*, *c*) Recovery of tail current components separated as shown in Fig. 12. Rapidly declining component (*b*) and slowly declining component (*c*). Two different cells were used for the two experiments. Both cells bathed in normal Tris buffered saline (Meech & Standen, 1975). Holding potential in each case, −50 mV; cells' diameter approximately 200 μm; temperature approximately 22 °C.

Neher, 1971). When inactivation is removed by hyperpolarization, a depolarizing pulse generates a rapidly developing outward current which opposes the fast inward current responsible for the rising phase of the action potential. Thus, the effect of the fast outward current is to reduce the frequency of firing of the action potentials (Connor & Stevens, 1971*c*). Early in the burst the action potential undershoot becomes smaller, possibly because of inactivation of the voltage-dependent delayed outward current. This means that the fast outward current is partially inactivated and the frequency of firing of action potentials increases. Later in the burst the increasing level of intracellular calcium tends to oppose the inward current and to slow the rate of firing.

ARTIFICIALLY INDUCED OSCILLATORS

One of the main difficulties in accepting the ionic mechanisms for 'bursting' presented above is that oscillations can be recorded in calcium-free saline (Carpenter & Gunn, 1970; Mathew & Roberge, 1971; Strumwasser, 1973), although it is

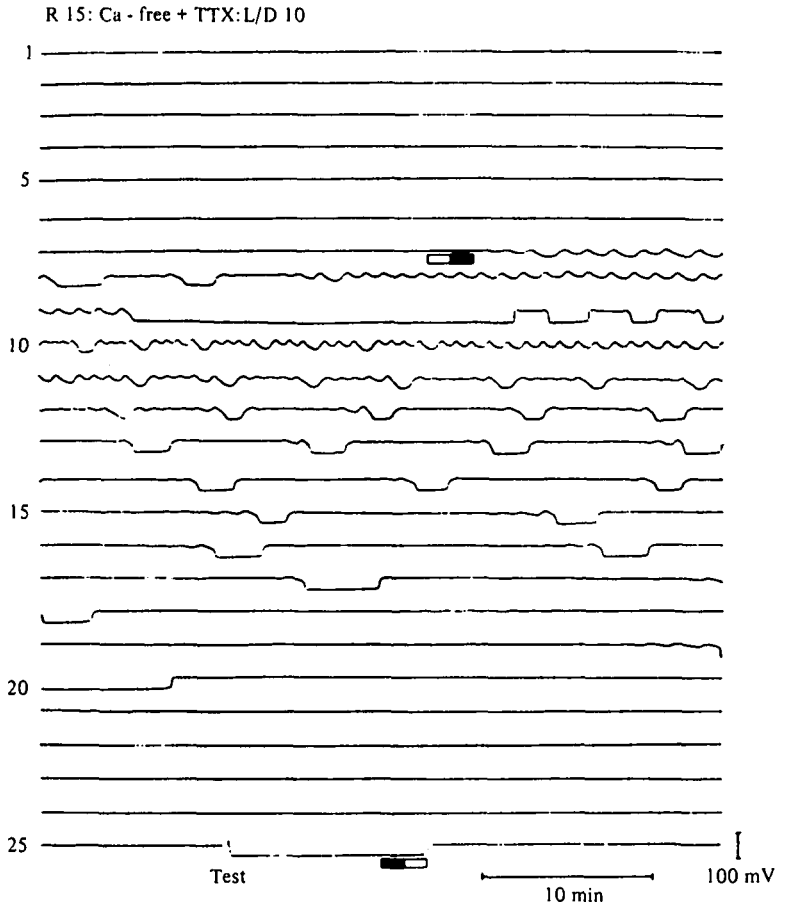

Fig. 14. Intracellular record of membrane potential oscillations in cell R_{15} (*Aplysia californica*) in calcium-free tetrodotoxin (TTX) containing saline. The change from light to dark conditions is shown under trace 7. (From Strumwasser, 1974.)

sometimes necessary to apply a constant hyperpolarizing current. Fig. 14 shows an experiment showing oscillations recorded from R_{15} in calcium-free saline containing TTX (tetrodotoxin). Strumwasser has suggested that the oscillations (which occur in the absence of action potentials) are the result of an electrogenic sodium pump. However, the hyperpolarizing phase of these cycles appear to have a normal reversal potential and they are not abolished by ouabain (Junge & Stephens, 1973). Bursting is abolished if all the divalent cations in the external saline are replaced by 50 mM cobalt chloride (Barker & Gainer, 1975*a*) and it is likely that the 10^{-5} M calcium normally present in nominally calcium-free saline is sufficient to support bursting.

The effect of inhibitors however, must be treated with caution. Cell R_{15} can produce bursts in the presence of the calcium blocking agent D600 (Barker & Gainer, 1975*a*) or in the presence of verapamil (Meech, unpublished), but bursting in the presence of verapamil is unlike that of normal cells because the membrane resistance is highest at the most negative potential.

Bursting may be induced in other molluscan neurones in a variety of ways (Ducreux, 1978). In barium-containing salines, bursts of action potentials are generated which have a prolonged plateau on the falling phase. These bursts are blocked by 10 mM cobalt. In this experimental situation, barium carries the inward current and because it does not itself activate the potassium conductance (Meech & Thomas, unpublished), repolarization is delayed. The drug pentamethylene tetrazol (PTZ) also induces bursting, but in this case both TTX and Na-free saline block the bursts, although cobalt does not.

ADDENDUM

Although calcium influx appears to be necessary for the post-burst increase in potassium conductance observed in these neurones, it is possible that at least part of the increase in intracellular ionized calcium during the burst results from 'calcium induced calcium release' from some intracellular site. An argument against this possibility is that calcium injected into cell A appears to be taken up almost exclusively by mitochondria. Isolated preparations of mitochondria are known to take up Ca^{2+} and release H^+ in a ratio of 1:1. Experiments on cell A in which the effects of injecting measured quantities of $CaCl_2$ or HCl were studied show that the Ca:H ratio is 1:1 under these conditions. It is unlikely, therefore, that calcium injection induces any further release of calcium (see Meech & Thomas, 1977, for references).

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