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ACTION POTENTIALS IN *RHODNIUS* OOCYTES: REPOLARIZATION IS SENSITIVE TO POTASSIUM CHANNEL BLOCKERS

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SUMMARY

Depolarization of *Rhodnius* oocytes evokes action potentials (APs) whose rising phase is calcium-dependent. The ionic basis for the repolarizing (i.e. falling) phase of the AP was examined.

Addition of potassium channel blockers (tetraethylammonium, tetrabutylammonium, 4-aminopyridine, atropine) to the bathing saline increased the duration and overshoot of APs. Intracellular injection of tetraethylammonium had similar effects. These results suggest that a voltage-dependent potassium conductance normally contributes to repolarization. Repolarization does not require a chloride influx, because substitution of impermeant anions for chloride did not increase AP duration. AP duration and overshoot actually decreased progressively when chloride levels were reduced.

Current/voltage curves show inward and outward rectification, properties often associated with potassium conductances. Outward rectification was largely blocked by external tetraethylammonium. Possible functions of the rectifying properties of the oocyte membrane are discussed.

INTRODUCTION

The developing oocytes of *Rhodnius* have recently been shown to be electrically excitable (O'Donnell, 1985). Action potentials (APs) can be elicited by injection of depolarizing current sufficient to change the membrane potential from a resting value of between −50 and −60 mV to a threshold value of about −35 to −40 mV. In control saline containing 2 mmol l^{-1} Ca^{2+}, APs overshoot zero to reach a peak potential (E_p) of about +16 mV, and last for 2–3 s. APs can be inhibited reversibly by calcium channel blockers such as Co^{2+}, La^{3+} or verapamil, and changes in E_p can be described by a Nernst equation for calcium. These results suggest that a voltage-dependent calcium conductance is responsible for the rising phase of the action potential. A sodium influx does not appear to contribute to the generation of the AP, because E_p is unaffected by removal of sodium from the bathing saline or by the addition of tetrodotoxin, a potent sodium channel blocker.

Key words: calcium action potential, K^+ channels, tetraethylammonium, 4-aminopyridine, rectification.

Although changes in E_p can be predicted by a Nernst equation for Ca^{2+}, the magnitude of E_p is less than the equilibrium potential for calcium (E_{Ca}), about +100 mV. Similar differences are common in other cells with calcium-dependent action potentials (Reuter, 1973), and it has been suggested that E_p is more negative than E_{Ca} because an outward current is present along with an inward calcium current. The outward current could be produced by an efflux of potassium or an influx of chloride. For example, chloride channels are involved in repolarization after action potentials in frog oocytes (Schlicter, 1983).

Some information as to the nature of the outward current has been gained through the use of salines containing barium (O'Donnell, 1985). Ba^{2+} is known to move through calcium channels as well as, or better than, Ca^{2+} in many tissues, and in *Rhodnius* oocytes bathed in calcium-free saline containing 20 mmol l^{-1} Ba^{2+}, APs lasted as long as 120 s and E_p was as high as +46 mV. These increases in overshoot and duration of APs in barium salines suggest that barium might not only permeate through calcium channels, but might also block a voltage-dependent potassium conductance which normally contributes to the termination of the action potential and the restoration of the resting potential (see Hagiwara & Naka, 1964). However, AP duration and overshoot might also be increased in Ba^{2+} salines because barium is less effective at promoting the inactivation of calcium channels, or because it blocks a passive (i.e. voltage-independent) potassium conductance.

This paper provides further information on the repolarizing phase of the *Rhodnius* oocyte action potential. Two tests for a voltage-dependent potassium conductance have been performed. First, agents known to block potassium channels in other excitable cells have been applied to oocytes. Second, current/voltage records have been examined for evidence of outward and inward rectification, properties often associated with potassium conductances. Outward rectification changes during development in *Periplaneta* (Blagburn, Beadle & Sattelle, 1985), and is the first non-linear electrical property of grasshopper embryos (Goodman & Spitzer, 1981). It is worth noting, however, that outward rectification is not a universal property of excitable cells. During parthenogenetic differentiation in the egg of the polychaete *Chaetopterus pergamentaceus*, calcium channels appear before potassium or sodium channels (Hagiwara & Miyazaki, 1977).

MATERIALS AND METHODS

Ovarioles from mated females were dissected under saline and the sheaths surrounding the developing follicles were removed. Follicles were secured in an experimental chamber as described in an earlier paper (O'Donnell, 1985). The chamber contained about 1 ml of saline and was perfused at 4–8 ml min^{-1}. The control saline contained (in mmol l^{-1}): NaCl, 129; KCl, 8·6; $MgCl_2$, 8·5; $CaCl_2$, 2; glucose, 34, and bis-(2-hydroxyethyl)imino-tris(hydroxymethyl)methane, 15. pH was adjusted to 6·8 by addition of 5 mmol l^{-1} HCl. Salines containing tetraethylammonium chloride (TEA) or tetrabutylammonium chloride (TBA) were prepared by substitution for an equimolar amount of NaCl. Salines containing atropine

(1 mmol l^{-1}) or 4-aminopyridine (5 or 10 mmol l^{-1}) were prepared by addition of these compounds to control saline. A saline in which chloride concentration was reduced to 9 mmol l^{-1} was prepared by substituting sodium isethionate, 129 mmol l^{-1}, K_2SO_4, 4·3 mmol l^{-1}, and $MgSO_4$, 8·5 mmol l^{-1}, for NaCl, KCl and $MgCl_2$, respectively.

Standard techniques for intracellular recording were employed. Oocytes were impaled with two microelectrodes, one to pass current and one for potential measurement. Each microelectrode was positioned with a micromanipulator until the tip contacted the follicle surface. The micromanipulator was then advanced a further 30–40 μm so that the microelectrode tip would traverse the follicular epithelium upon impalement. Impalement was accomplished by tapping the baseplate of the micromanipulator, or by using the capacitance compensation circuit of the associated amplifier to pass a short burst of alternating current through the electrode tip. Voltage recording microelectrodes pulled from borosilicate glass (1 mm o.d., 0·60 mm i.d.) had resistances of 20–40 MΩ when filled with 3 mol l^{-1} KCl. Microelectrodes that were used for current injection were pulled from thin-walled borosilicate glass (1 mm o.d., 0·75 mm i.d.) and had resistances of 2–4 MΩ when filled with 3 mol l^{-1} KCl. Injected current was measured through a current to voltage converter inserted in the ground circuit and connected to the perfusion chamber through $Ag^+/AgCl$ electrodes and agar bridges. Current and voltage were displayed on a two-channel recording oscilloscope and permanent records were made with a Polaroid oscilloscope camera and a two-channel pen recorder. In experiments involving changes in the chloride concentration of the bathing media, corrections were made for the small changes (<3–5 mV) in the ground potential.

Techniques for intracellular injection were modified from Gillette, Gillette & Davis (1982). Pressure was applied to a hand-held plastic syringe filled with 0·5 mol l^{-1} KCl and connected to an electrode by tightly fitting polyethylene tubing. A chlorided silver wire entering the tubing near the electrode allowed intracellular recordings to be taken from the injection electrode. The volumes injected were not measured precisely, but were never more than 0·1 % of the oocyte volume.

RESULTS

Effects of agents which reduce potassium conductance

Quaternary ammonium compounds are known to block voltage-dependent K^+ channels in a one-for-one manner (Hermann & Gorman, 1981*a*; Stanfield, 1983). Exposure of oocytes to saline containing 100 mmol l^{-1} TEA for 30 min produced a four-fold increase in the duration, and a doubling of the overshoot, of action potentials (Figs 1, 2). The resting potential either increased a few millivolts (Fig. 1) or did not change (Fig. 2). Exposure to 50 mmol l^{-1} TBA also increased duration and overshoot, but produced a significant depolarization of the resting potential (Fig. 3). The latter effect may be due to a decline in potassium permeability in TBA salines, because current injection experiments indicated that slope resistances within 5–10 mV of the resting potential increased significantly from 68 ± 9 kΩ ($N = 6$) in

control saline to 92 ± 17 kΩ in TBA saline. It is worth noting that the effects of TBA developed progressively over the 6-min test period, and decayed only slowly upon return to control saline. Increases in AP duration and overshoot remained significant

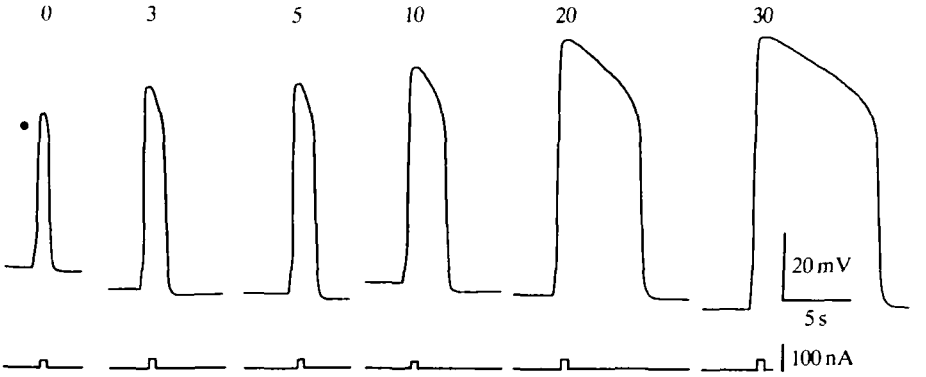

Fig. 1. Effects of tetraethylammonium chloride (TEA) on action potentials. The action potential at t = 0 was recorded in control saline. Subsequent action potentials were recorded at the times (in min) indicated after addition of saline containing 100 mmol l^{-1} TEA. In this and subsequent figures, the dot (●) adjacent to the first action potential indicates the zero potential.

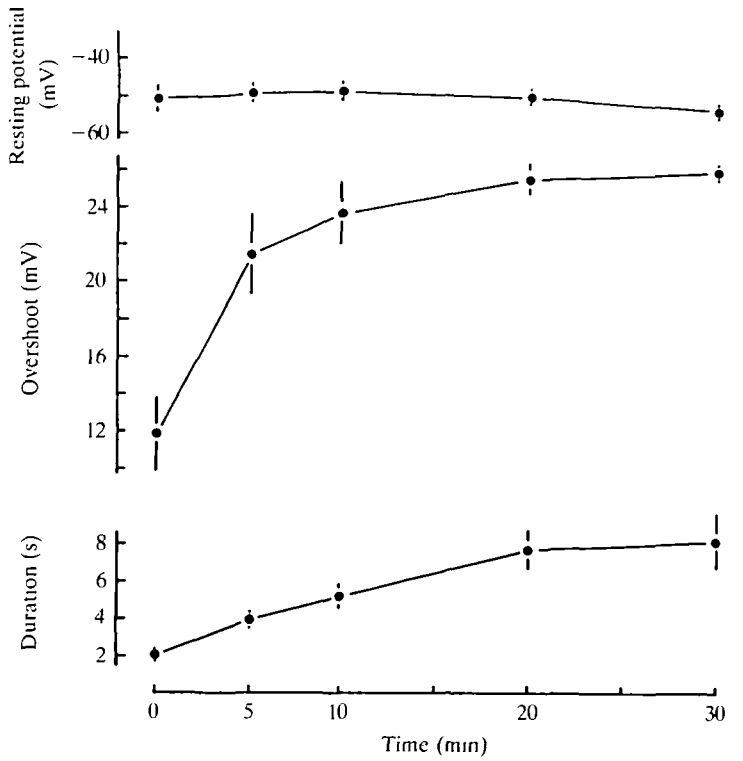

Fig. 2. Summary of the effects of 100 mmol l^{-1} tetraethylammonium chloride (TEA) on resting and action potentials. Data at t = 0 were recorded in control saline. Each point represents the mean ± S.E. for six oocytes.

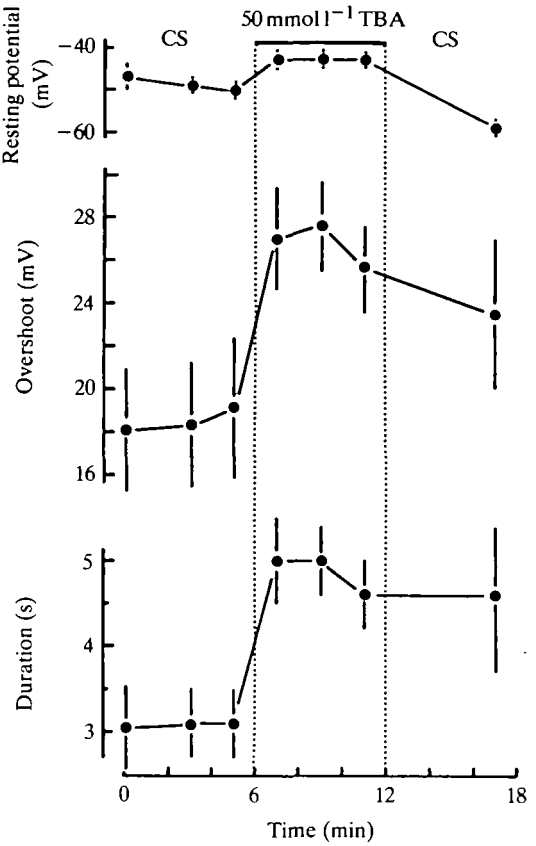

Fig. 3. Effects of 50 mmol l^{-1} tetrabutylammonium chloride (TBA) on resting and action potentials. Oocytes were bathed in control saline (CS) before and after exposure for 6 min to saline containing TBA. Mean ± S.E., $N = 6$.

even after the resting potential had returned to values more negative than those prior to TBA exposure, indicating that changes in resting conductances were not primarily responsible for the increase in excitability. Moreover, similar results were obtained with 50 mmol l^{-1} TEA, which did not significantly alter the resting potential. These results are consistent with the suggestion that a voltage-dependent K^+ conductance contributes to the restoration of the resting potential.

Increases in excitability outlasted the duration of exposure to TEA. For example, a 10-min exposure to saline containing 100 mmol l^{-1} TEA increased overshoot and duration from +11 ± 2 mV and 2·9 ± 0·3 s ($N = 5$), respectively, to +21 ± 1 mV and 4·9 ± 0·3 s, respectively. Ten minutes after the return to control saline, overshoot and duration remained elevated, at +16 ± 2 mV and 3·6 ± 0·3 s, respectively. The effects of lower concentrations of TEA were more readily reversible. After 10 min in saline containing 20 mmol l^{-1} TEA, overshoot and duration increased by 22% and 44%, respectively ($N = 6$); within 5 min of TEA removal these parameters did not differ significantly from their values prior to exposure to TEA.

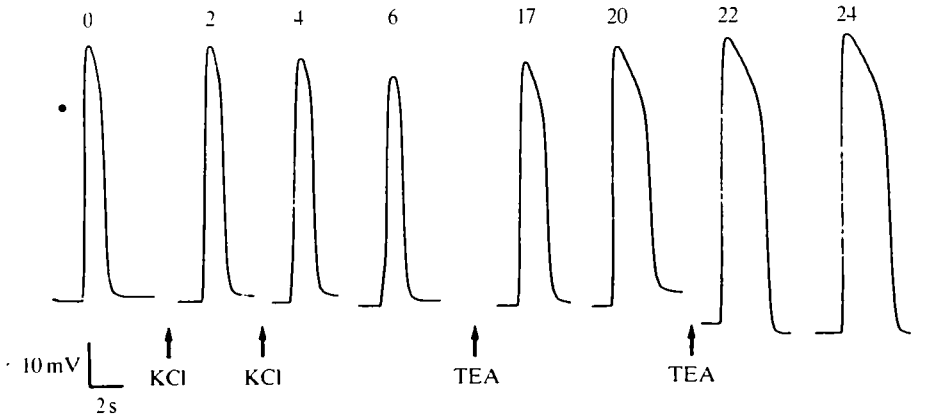

Fig. 4. Effects of intracellular injection of 3 mol l^{-1} KCl or 1 mol l^{-1} tetraethylammonium chloride (TEA). KCl or TEA were injected 1 min prior to the subsequent recording by applying 20 1-s pulses of pressure to the micropipette. Pulses were separated by 1 s. The time (in min) after the start of the experiment is indicated above each recording, and the zero potential (●) is indicated to the left of the first recording.

The results of intracellular TEA injection (Fig. 4) suggest that TEA can also exert its effects from inside the oocyte. Action potential overshoot and duration continued to increase for at least 5 min after TEA injection, and often for as long as 20–30 min. In experiments in which amaranth dye was added to 1 mol l^{-1} TEA in the injection electrode, the progressive increase in AP duration after one injection was coincident with the observed spread of the dye within the oocyte. For nine oocytes in control saline, overshoot and duration increased from $+9 \pm 2$ mV and $1{\cdot}7 \pm 0{\cdot}3$ s prior to TEA injection to $+15 \pm 2$ mV and $2{\cdot}2 \pm 0{\cdot}4$ s, respectively, 4–7 min after injection. Subsequent injections led to further increases, so that duration increased two- to four-fold after 2–4 injections of TEA at 5- to 10-min intervals. KCl injections decreased excitability slightly (Fig. 4), possibly through localized elevation of intracellular potassium activity.

In molluscan neurones, TEA is known to block K^+ channels which are dependent upon voltage (Thompson, 1977), or both voltage and calcium (Hermann & Gorman, 1981*a*). Aminopyridines also block voltage-dependent K^+ channels, but either increase or do not affect the calcium-activated K^+ channels, depending on their concentration (Hermann & Gorman, 1981*b*). These compounds can be used, therefore, to provide a pharmacological separation of the two types of K^+ currents.

The effects of 4-aminopyridine (4-AP) upon action potentials were consistent with the presence of voltage-dependent K^+ channels in *Rhodnius* oocytes. Exposure of oocytes to 5 mmol l^{-1} 4-AP produced a 40 % increase in duration and a 30 % increase in the overshoot of action potentials after 5 min ($N = 6$), with no significant change in the resting potential. Higher concentrations of 4-AP depolarized the resting potential by several millivolts. To eliminate any effects of altered resting conductances upon excitable properties, oocytes were manually voltage clamped at the resting potential measured in control saline prior to exposure to 10 mmol l^{-1} 4-AP. Under these conditions AP overshoot and duration increased from $+16 \pm 4$ mV ($N = 5$) and

2·6 ± 0·5 s, respectively, to +21 ± 2 mV and 3·8 ± 0·3 s, respectively, after 10 min in 4-AP. Overshoot and duration remained 25 % above control values 10 min after removal of 4-AP. Molluscan neurones also show incomplete recovery from prolonged exposure to 4-AP (Thompson, 1977). Therefore, the effects of shorter periods of exposure to 4-AP upon oocyte APs were examined. Two minutes of perfusion with saline containing 10 mmol l^{-1} 4-AP increased the overshoot and duration of action potentials by 26 % and 42 %, respectively ($N = 5$); these parameters returned to within 10 % of their pretreatment values within 2 min of removal of 4-AP.

Tropine esters are also known to decrease potassium conductance, and to cause a K^+ channel inactivation (Pelhate & Sattelle, 1982). Atropine (1 mmol l^{-1}) rapidly increased overshoot and duration of action potentials, with no effect upon resting potential (Fig. 5). These effects were completely reversible.

Rectification in Rhodnius *oocytes*

Fig. 6 shows representative current/voltage relationships for *Rhodnius* oocytes in control saline and in saline containing 10 mmol l^{-1} cobalt, which blocks calcium-

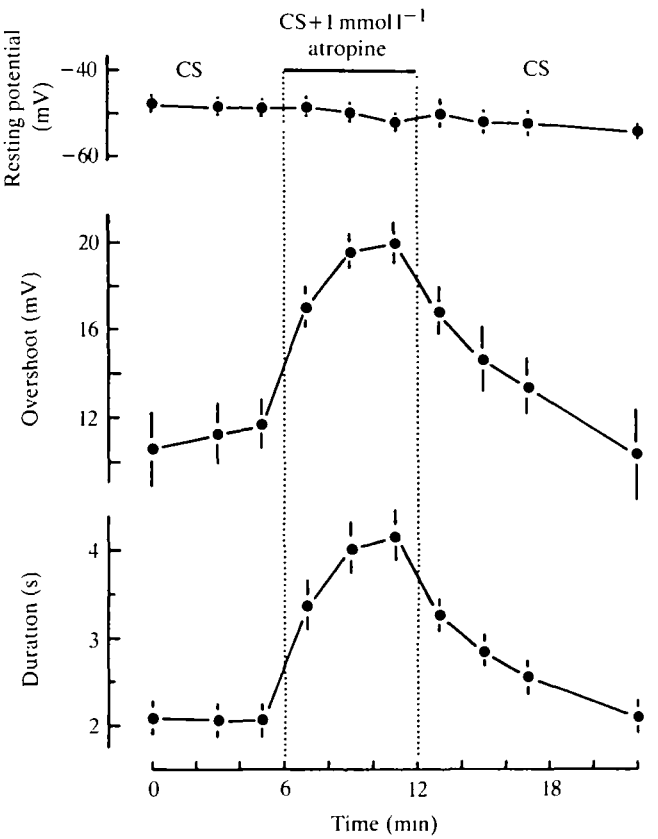

Fig. 5. Effects of atropine on resting and action potentials. 1 mmol l^{-1} atropine was added to control saline (CS) for the 6-min period indicated between the dotted lines. Mean ± S.E., $N = 6$.

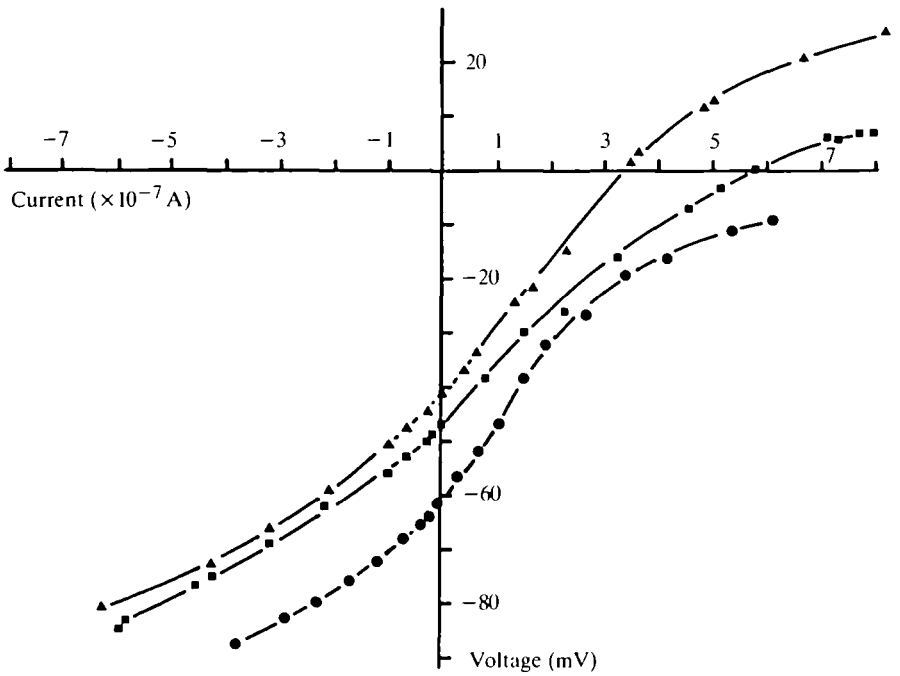

Fig. 6. Current/voltage relationships for three oocytes. Each data point represents the potential measured at the end of a 3- to 5-s current pulse in control saline (▲, ●) or in control saline containing 10 mmol l^{-1} Co^{2+} (■).

dependent action potentials in these cells (O'Donnell, 1985). The potentials plotted are those measured 4–5 s after the initiation of the current pulse. Steady-state I/V curves with and without Co^{2+} were similar, suggesting that the Ca^{2+} permeability increase at the peak of the action potential decays with time and reaches a small, steady-state amplitude after 4–5 s. All oocytes showed evidence of both inward and outward rectification ($N = 11$ control, $N = 3$ with cobalt). Moreover, the steady-state conductance at positive or near zero membrane potentials is much higher than the conductance at the resting potential, suggesting that there is a conductance increase which may accelerate the falling phase of the action potential, as in many other excitable cells. The outward and inward rectification of the oocyte cell membrane is probably due to K^+, as in the egg cells of other animals (Hagiwara & Jaffe, 1979). Time-dependent aspects of I/V relationships, such as delayed activation of outward rectification, were difficult to ascertain because the time constant of the oocyte (>100 ms) is much longer than the delay before the onset of outward rectification in other cells.

The effects of external TEA upon current/voltage relationships were consistent with a reduction in outward rectification (Fig. 7). In saline containing 1 mmol l^{-1} La^{3+} to block Ca^{2+} action potentials (O'Donnell, 1985) the minimum resistance determined from the slope of the I/V curve during depolarization increased over 100%, from 84 ± 20 kΩ ($N = 5$) in control saline to 172 ± 45 kΩ after 10 min in saline containing 100 mmol l^{-1} TEA. There was also a slight but significant

reduction in inward rectification. The minimum slope of the I/V curve in response to hyperpolarizing current injection increased from 77 ± 13 kΩ in control saline to 90 ± 14 kΩ in TEA saline. Fig. 7B also shows that there was no inactivation of outward or inward rectification in response to current pulses of 2–3 s.

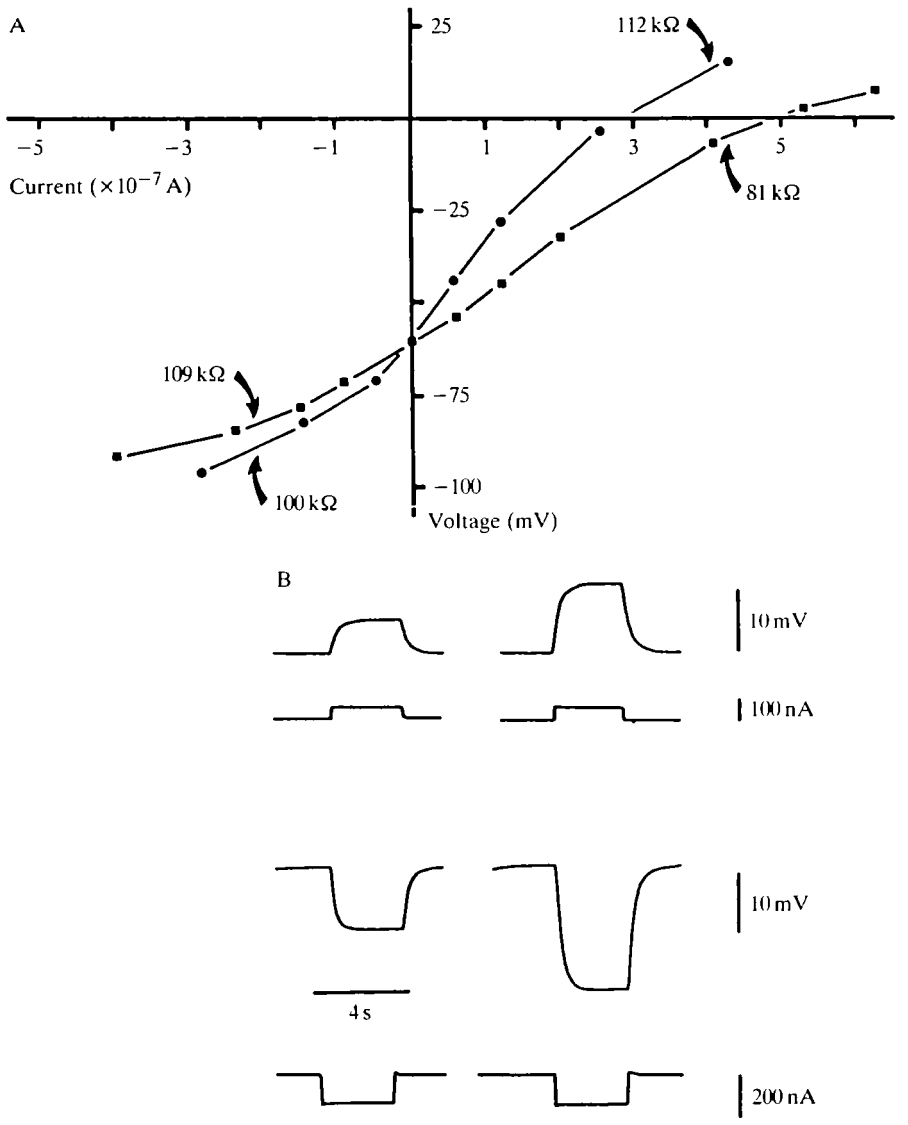

Fig. 7. (A) Current/voltage records for the same oocyte in control saline (squares) and after 10 min in saline containing 100 mmol l^{-1} tetraethylammonium chloride (TEA) (circles). Both salines contained 1 mmol l^{-1} La^{3+}. The resistances noted on the figure were determined from the slope of the curves at the points indicated by the arrows. (B) Individual voltage recordings in response to depolarizing (upper records) or hyperpolarizing (lower records) current in control saline (left) and after 10 min in saline containing 100 mmol l^{-1} TEA (right). Both salines contained 1 mmol l^{-1} La^{3+}.

In summary, a number of pharmacological agents known to reduce potassium conductance prolonged the action potential and increased the overshoot. TEA has also been shown to reduce outward rectification. These results suggest that a voltage-dependent potassium conductance contributes to the restoration of the resting potential after depolarization has initiated a regenerative calcium influx. However, a chloride influx through passive or voltage-gated channels would also tend to restore the resting potential. A reduction in the chloride concentration of the bathing saline would reduce the latter type of influx, and would be associated, therefore, with an increase in action potential duration.

Low-chloride salines reduce excitability

The effects of low-chloride salines upon action potentials were, in fact, opposite to the effects to be expected if a chloride influx were involved in restoring the resting potential. In salines in which chloride was partially replaced by isethionate, APs elicited by depolarization declined progressively in overshoot and duration, and a transient hyperpolarizing afterpotential appeared (Fig. 8). The afterpotential may be due to the displacement of the resting potential from E_K due to the positive shift of E_{Cl} in low-chloride salines. When the chloride concentration of the bathing saline was restored, the recovery of AP duration and overshoot to control values was prolonged, requiring more than 15 min (Fig. 9). The resting potential rapidly depolarized by about 10 mV in low-chloride saline. The depolarization was sustained for the 6-min exposure to low-chloride saline, and the resting potential hyperpolarized slightly upon return to control saline (Figs 8, 9). However, the recovery of action potential duration and overshoot to control values was not associated with significant changes in resting potential (Fig. 9, t = 14–30 min).

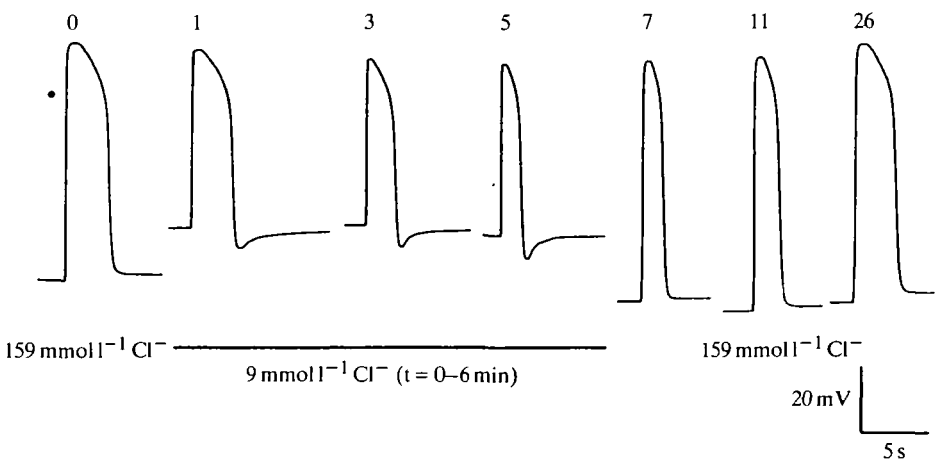

Fig. 8. Effects of a reduction in chloride concentration on resting and action potentials. Between 0 and 6 min the bathing saline was changed from control saline (159 mmol l^{-1} Cl^-) to a low-chloride (9 mmol l^{-1} Cl^{-1}) saline. Time (in min) after the start of the experiment is indicated above each recording and the zero potential (●) is indicated to the left of the first recording.

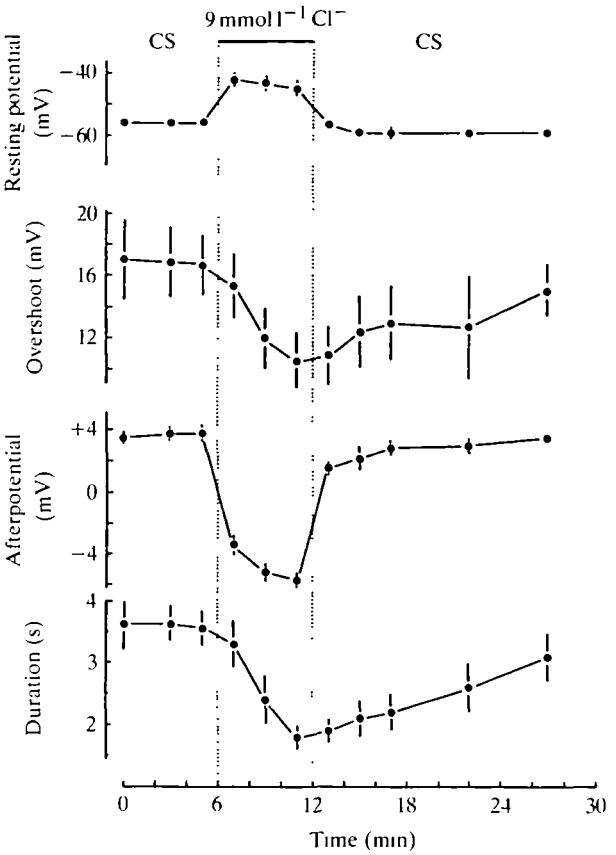

Fig. 9. Summary of the effects of low-chloride saline on resting and action potentials. The afterpotentials were measured as the difference between the resting potential prior to initiation of the action potential, and the most negative or plateau phase value which occurred 150–200 ms after the most negative slope of the falling phase of the action potential. Mean ± S.E., $N = 6$. CS, control saline. All salines were bicarbonate-free.

The prolonged nature of these effects relative to the time required to exchange the volume of the perfusion chamber (15 s) indicated that chloride reduction affected some aspect of membrane conductance that reversed slowly upon restoration of control Cl^- levels. A significant resting Cl^- conductance was suggested by the depolarization produced by low-chloride salines. Exposure to low-chloride solutions probably results, therefore, in a gradual decline in intracellular chloride activity. Because chloride/bicarbonate exchange mechanisms are involved in maintenance of intracellular pH in many invertebrate cells (Roos & Boron, 1981), it is possible that a drop in intracellular Cl^- activity during perfusion with low-chloride saline could inhibit such exchange, and result in gradual acidification of the oocyte. The interrelationships of intracellular pH regulation and the excitable properties of oocytes are currently under study.

DISCUSSION

The results of this study demonstrate three novel aspects of electrical excitability in *Rhodnius* oocytes. First, injection of hyperpolarizing current revealed inward rectification, and injection of depolarizing current revealed outward rectification. Second, excitability is increased, and repolarization after an AP is retarded, by agents which block voltage-dependent K^+ channels. Third, repolarization does not appear to involve a chloride influx, because replacement of chloride with impermeant anions did not increase AP duration.

Rectification

Inward rectification has not previously been demonstrated in insect oocytes, but is common in the oocytes of a number of marine invertebrates (Hagiwara & Jaffe, 1979), and is also found in muscles of vertebrates (Hagiwara, 1983) and insects (Ashcroft & Stanfield, 1982). Inward rectification, also referred to as anomalous rectification, is generally believed to be due to a voltage-dependent potassium conductance (Hagiwara, 1983). Some anion channels also show rectification (e.g. Hanrahan, Alles & Lewis, 1985). However, because repolarization does not appear to involve a chloride influx, it is unlikely that anion channels contribute significantly to rectification in *Rhodnius* oocytes.

Inward rectification will alter the potential jump produced by an inward current carried by ions such as calcium. Because membrane resistance increases at potentials less negative than the resting potential, less inward current is required to depolarize the membrane further. This process will continue until the potential reaches the more positive values, -10 to $0\,mV$, required for outward rectification. Calcium channels in the oocyte cell membrane are also voltage-dependent (O'Donnell, 1985), and the effects of inward rectification may contribute to the rate of rise of Ca^{2+} action potentials. The speed with which oocytes depolarize can have important consequences. Long-lasting depolarizations are known to occur at the time of fertilization in many marine invertebrates (Hagiwara & Jaffe, 1979), for example, and the high rate of rise of the depolarization is important in the production of a rapid electrical block to polyspermy. Although membrane potential has not been recorded during fertilization of insect eggs, inward rectification could alter the amount of inward current required to initiate and/or maintain a depolarization at that time.

Outward current rectification has been observed in *Drosophila* eggs when calcium concentration is increased from 1·8 to $20\,mmol\,l^{-1}$ (Miyazaki & Hagiwara, 1976), and in the developing oocytes of *Locusta* (Wollberg, Cohen & Kalina, 1976). TEA-sensitive rectification is also the first non-linear electrical property of embryonic grasshopper neurones (Goodman & Spitzer, 1981) and cultured cockroach neurones (Lees, Beadle, Botham & Kelly, 1985). Outward rectification is considered to be due to a voltage-dependent potassium conductance.

In *Rhodnius* oocytes, outward current rectification limits the overshoot of the calcium action potential, and hence the degree of excitability. Alterations of rectification, or regional differences in it, may be important in the development of

excitability in the embryo. Ionic channels present in molluscan egg cells, for example, can segregate into identifiable cells during cleavage, producing differences in excitability of localized regions as embryonic development progresses (Jaffe & Guerrier, 1981).

Blockage of K^+ channels increases excitability

The increase in AP duration in *Rhodnius* oocytes during exposure to agents such as TEA and 4-AP is further evidence for a voltage-sensitive potassium conductance. The increase in E_p produced by exposure to these agents is also to be expected, because the overshoot of Ca^{2+} action potentials in many systems is determined by the ratio of inward Ca^{2+} current to outward K^+ current (Hagiwara, Chichibu & Naka, 1964). It is important to note that although TEA also blocks Ca^{2+}-dependent K^+ currents in some tissues, 4-AP does not block the latter type of current. Further experiments, using voltage-clamp techniques, are necessary to determine the relative contribution of voltage-dependent and calcium-dependent potassium currents to total outward current flow during action potential repolarization.

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REFERENCES

ASHCROFT, F. M. & STANFIELD, P. R. (1982). Calcium and potassium currents in muscle fibres of an insect (*Carausius morosus*). *J. Physiol., Lond.* **323**, 93–115.

BLAGBURN, J. M., BEADLE, D. J. & SATTELLE, D. B. (1985). Development of chemosensitivity of an identified insect interneurone. *J. Neurosci.* **5**, 1167–1175.

GILLETTE, R., GILLETTE, M. U. & DAVIS, W. J. (1982). Substrates of command ability in a buccal neuron of *Pleurobranchaea*. II. Potential role of cyclic AMP. *J. comp. Physiol.* **146**, 461–470.

GOODMAN, C. S. & SPITZER, N. C. (1981). The development of electrical properties of identified neurons in grasshopper embryos. *J. Physiol., Lond.* **313**, 385–403.

HAGIWARA, S. (1983). *Membrane Potential-Dependent Ion Channels in Cell Membranes*. New York: Raven Press.

HAGIWARA, S., CHICHIBU, S. & NAKA, K. (1964). The effects of various ions on resting and spike potentials of barnacle muscle fibers. *J. gen. Physiol.* **48**, 163–179.

HAGIWARA, S. & JAFFE, L. A. (1979). Electrical properties of egg cell membranes. *A. Rev. Biophys. Bioeng.* **8**, 385–416.

HAGIWARA, S. & MIYAZAKI, S. (1977). Changes in excitability of the cell membrane during "differentiation without cleavage" in the egg of the annelid, *Chaetopterus pergamentaceous*. *J. Physiol., Lond.* **272**, 197–216.

HAGIWARA, S. & NAKA, K. (1964). The initiation of spike potential in barnacle muscle fibers under low intracellular Ca^{2+}. *J. gen. Physiol.* **48**, 141–162.

HANRAHAN, J. W., ALLES, W. P. & LEWIS, S. A. (1985). Single anion-selective channels in basolateral membrane of a mammalian tight epithelium. *Proc. natn. Acad. Sci. U.S.A.* **82**, 7791–7795.

HERMANN, A. & GORMAN, A. L. F. (1981*a*). Effects of tetraethylammonium on potassium currents in a molluscan neuron. *J. gen. Physiol.* **78**, 87–110.

HERMANN, A. & GORMAN, A. L. F. (1981*b*). Effects of 4-aminopyridine on potassium currents in a molluscan neuron. *J. gen. Physiol.* **78**, 63–86.

JAFFE, L. A. & GUERRIER, P. (1981). Localization of electrical excitability in the early embryo of *Dentalium*. *Devl Biol.* **83**, 370–373.

LEES, G., BEADLE, D. J., BOTHAM, R. P. & KELLY, J. S. (1985). Excitable properties of insect neurones in culture: A developmental study. *J. Insect Physiol.* **31**, 135–143.

MIYAZAKI, S. & HAGIWARA, S. (1976). Electrical properties of the *Drosophila* egg membrane. *Devl Biol.* **53**, 91–100.

O'DONNELL, M. J. (1985). Calcium action potentials in the developing oocytes of an insect *Rhodnius prolixus*. *J. exp. Biol.* **119**, 287–300.

PELHATE, M. & SATTELLE, D. B. (1982). Pharmacological properties of insect axons: a review. *J. Insect Physiol.* **28**, 889–903.

REUTER, H. (1973). Divalent cations as charge carriers in excitable membranes. *Prog. Biophys. molec. Biol.* **26**, 1–43.

ROOS, A. & BORON, W. F. (1981). Intracellular pH. *Physiol. Rev.* **61**, 296–434.

SCHLICTER, L. C. (1983). Spontaneous action potentials produced by Na and Cl channels in maturing *Rana pipiens* oocytes. *Devl Biol.* **98**, 47–59.

STANFIELD, P. R. (1983). Tetraethylammonium ions and the potassium permeability of excitable cells. *Rev. Physiol. Biochem. Pharmacol.* **97**, 1–67.

THOMPSON, S. H. (1977). Three pharmacologically distinct potassium channels in molluscan neurones. *J. Physiol., Lond.* **265**, 465–488.

WOLLBERG, Z., COHEN, E. & KALINA, M. (1976). Electrical properties of developing oocytes of the migratory locust, *Locusta migratoria*. *J. Cell Physiol.* **88**, 145–158.