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MEASUREMENTS OF THE INTRACELLULAR POTASSIUM ACTIVITY OF RETZIUS CELLS IN THE LEECH CENTRAL NERVOUS SYSTEM

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SUMMARY

The intracellular K activity of leech Retzius cells was measured using double-barrelled, liquid ion exchanger, microelectrodes. At the normal external K^+ concentration of 4 mM (equivalent to 3 mM-K activity, assuming an activity coefficient of 0·75) the mean K activity was $101{\cdot}3 \pm 7{\cdot}6$ mM (S.D., $n = 14$) in the cell bodies, and $4{\cdot}35 \pm 0{\cdot}4$ mV ($n = 27$) in the extracellular spaces surrounding them, indicating a K^+ equilibrium potential of -80 mV. The mean membrane potential was $-43{\cdot}6 \pm 4{\cdot}9$ mV ($n = 14$). In a K-free external solution, or in the presence of 5×10^{-4} M-ouabain, the intracellular K activity decreased by up to 14 mM min^{-1}. This indicates an efflux of K^+ ions across the cell membrane of approximately 2×10^{-10} mol cm^{-2} s, and an apparent K^+ permeability coefficient of 8×10^{-6} cms^{-1}. The cell membrane depolarized upon removal of K^+ and upon addition of ouabain, and transiently hyperpolarized beyond its initial level on return to the normal external K^+ concentration. The recovery from this hyperpolarization paralleled the increase of the intracellular K activity following the re-addition of K^+. Our results suggest that, despite the high K^+ permeability of the Retzius cell membrane, the intracellular K activity is maintained at a high level by an electrogenic pump.

INTRODUCTION

Ion-sensitive microelectrodes have been used to continuously measure the steady-state intracellular ion activities, and also to monitor changes in the ion activity (cf. Walker & Brown, 1977). Extensive studies of intracellular ion activities have been performed, for example, in *Helix* (cf. Thomas, 1977, 1978) and *Aplysia* neurones (Kunze & Brown, 1971; Russell & Brown, 1972; Eaton, Russell & Brown, 1975). These neurones contain high concentrations of K^+, which are actively maintained against electrochemical gradients by the activities of electrogenic Na^+/K^+ pumps.

The technique of intracellular ion-sensitive microelectrodes has so far not been applied to the annelid central nervous system. The intracellular K^+ content of nerve and glial cells in the classical annelid preparation, the leech central nervous system, has so far only been determined *indirectly* by measuring their membrane potential

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at various external K^+ concentrations (Nicholls & Kuffler, 1964), or by flame photometry (Nicholls & Kuffler, 1965). It was concluded from these studies that the K^+ distribution across the membrane of glial cells is near its equilibrium, as has been reported of glial cells in the amphibian optic nerve trunk (Orkand, Nicholls & Kuffler, 1966), and recently found in pigment (glial) cells of the drone retina (Coles & Tsacopoulos, 1979). In contrast to glial cells, the membrane potential of leech neurones was about 40 mV more positive than the extrapolated K^+ equilibrium potential (Nicholls & Kuffler, 1964). Later studies on identified sensory nerve cells (Baylor & Nicholls, 1969b; Jansen & Nicholls, 1973) and on Retzius cells (Walker & Smith, 1973) suggested that the membrane of these neurones contains an electrogenic Na^+/K^+ pump, which would help to maintain an intracellular ionic homeostasis.

In the present study we have used double-barrelled liquid ion exchanger microelectrodes to *directly* measure the intracellular K activity of Retzius cell bodies in the leech central nervous system. We chose the Retzius cells for this investigation, because they have the largest cell bodies within the leech nervous system. Our results suggest that the intracellular K^+ content of Retzius cells is maintained at a high level, presumably by the activity of an electrogenic Na^+/K^+ pump. After inhibition of this Na^+/K^+ pump by the cardiac glycoside ouabain, the intracellular K activity decreased with a fast time course, indicating a high membrane permeability to K^+ ions.

METHODS

Leeches (*Hirudo medicinalis*) were obtained from various supply houses. The dissection of the ganglia has been described in detail in a previous paper (Schlue & Deitmer, 1980). The ganglia were pinned by the connectives into the silicone ground of a perspex chamber (volume *ca.* 0·2 ml).

The normal external solution (physiological saline; Baylor & Nicholls, 1969*a*) had the following ionic composition (in mM): NaCl, 115; KCl, 4; $CaCl_2$, 1·8; Tris-Maleate, 10 (brought to pH 7·4 with approximately 12 mM-NaOH); glucose, 11. When the potassium concentration of the external solution was reduced or increased, equivalent amounts of NaCl and KCl were exchanged to maintain osmolarity and ionic strength. The cardiac glycoside ouabain (G-strophanthin, Serva) was added to the bathing solution to give a final concentration of 5×10^{-4} mol/l. This amount of ouabain had no effect on the response or time course of either the K^+-sensitive barrel or the reference barrel of the double-barrelled microelectrodes used.

The preparation was continuously superfused with a flow rate of 15 to 20 bath volumes min^{-1}. The experiments were performed at room temperature (22–25 °C).

Microelectrodes and electrical recording. The construction and calibration of the double-barrelled K^+-sensitive microelectrodes have been described in detail previously (Schlue & Deitmer, 1980; after Zeuthen & Monge, 1975). The tip of the K^+-sensitive barrel was filled with a liquid ion exchanger resin (Corning 477317), and the shaft with 0·5 M-KCl. The reference barrel was filled with either 3 M-sodium-acetate or 1 M-magnesium-acetate. The bath reference electrode was a calomel electrode, communicating with the solution in the experimental chamber via a polyethylene tube filled with 3 M-KCl in agar.

The preparation and the electrical recording system are shown schematically i

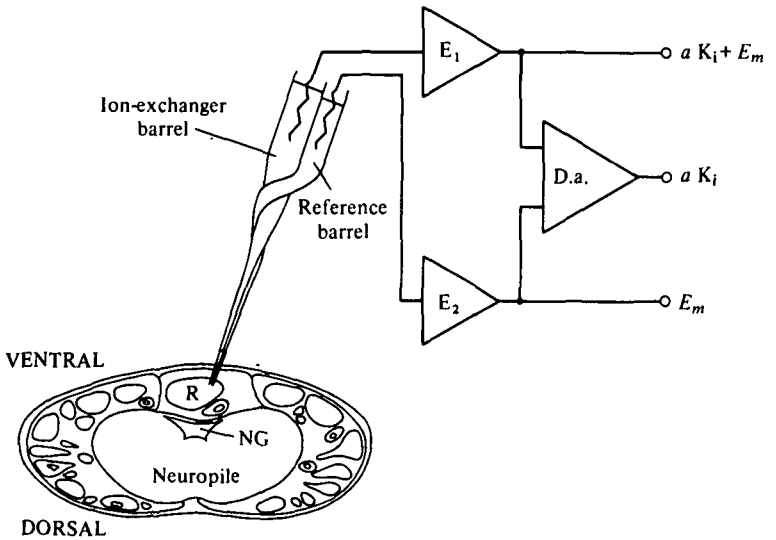

Fig. 1. Schematic drawing of the preparation and the electrical recording system. The lower part of the Figure shows a ganglion in cross section with the ventral side uppermost (R, Retzius cell; NG, neuropile glial cell). The ion-exchange barrel and the reference barrel of the double-barrelled microelectrode are each connected, via chlorided silver wires, to two independent electrometer probes (E_1 and E_2, input resistance to $10^{15}\ \Omega$, bias current less than 10^{-14} A). The probe outputs were fed into the inputs of a differential amplifier (D.a., WPI F-223A). The signal from the differential amplifier giving the pure intracellular K activity measurement, aK_i, and the signal from probe E_2, giving the cell membrane potential, E_m, were connected to two independent channels of a pen-recorder.

Fig. 1. The K^+-sensitive microelectrodes used in our experiments were calibrated in leech saline and responded with a potential of 45 to 52 mV (mean = $49 \pm 1{\cdot}8$ mV, $n = 34$), when the K^+ concentration was changed from 4 to 40 mM. Above 10 mM K^+, the mean response of the electrodes to a tenfold change in the K^+ concentration increased by up to 3 mV (see Fig. 1B in Schlue & Deitmer, 1980). The selectivity coefficient for Na^+ over K^+ determined by the best fit to the electrode responses at different K^+ concentrations according to the Nicolsky equation was 1/62.

Selection of cells. Previous studies using conventional single-barrelled microelectrodes have reported membrane potentials of between -30 and -60 mV in Retzius cell bodies (Hagiwara & Morita, 1962; Eckert, 1963). In the present study Retzius cells were discarded if their membrane potentials were below -35 mV, and if their action potentials had amplitudes less than $+15$ mV as recorded with the double-barrelled microelectrodes. When the membrane resting potential, and the amplitude and frequency of the spontaneously fired action potentials remained constant for at least 3 min after microelectrode impalement, the experiment was continued.

Potassium fluxes and permeability coefficient of the cell membrane. The changes in the intracellular K activity, aK_i, were assumed to result from K^+ fluxes across the membrane of the Retzius cells. The net flux, M_K, was calculated from these changes in aK_i by the equation

$$M_K = \frac{aK_i}{\gamma . t} \Big/ \frac{S}{V}, \qquad (1)$$

where γ is the K activity coefficient, which is 0·75 for the external solution (based on Robinson & Stokes, 1959) and assummed to be approximately the same for the cytoplasm, t the time in s, and S/V the surface to volume ratio of the cells. The mean diameter of the Retzius cells were approximately 80 μm, which gives a cell body surface of 2×10^{-4} cm^2 (disregarding possible invaginations of the cell membrane which would considerably enlarge the cell surface), and a cell body volume of $2{\cdot}7 \times 10^{-7}$ cm^3. With this assumption the S/V ratio of Retzius cells is approximately $7{\cdot}5 \times 10^2$ cm^{-1}. The 'apparent' permeability coefficient of the cell membrane, P'_K, was calculated from the K^+ fluxes across the membrane down the electrochemical gradient according to the constant field theory (Goldman, 1943; Hodgkin & Katz, 1949) when the *unidirectional* K^+ efflux is taken as the *net* K^+ flux across the membrane (M_K). This approximation assumes that unidirectional K^+ *efflux* is much larger than the unidirectional K^+ *influx*. Thus the 'apparent' permeability coefficient, P'_K, is given by the equation (Hodgkin & Horowicz, 1959)

$$P'_K = M_K \frac{RT}{E_m F} \frac{1 - \exp(-E_m F/RT)}{[K^+]_i}, \qquad (2)$$

where $[K^+]_i$ is the intracellular K^+ concentration, and E_m the resting membrane potential; R, T, and F have their usual thermodynamic meanings.

Unless otherwise stated, all results are expressed as the mean ± S.D.

RESULTS

The large Retzius cells were easily identified on the mid-ventral side of a leech ganglion. As shown in Fig. 1 the tip of the double-barrelled microelectrode was pushed through the endothelium and the outer ganglion capsule to enter the nerve cell body region. This produced small transient, asymmetric, potential shifts, recorded with both the K^+-sensitive and the reference barrel (Fig. 2). This was followed by abrupt potential shifts indicating the penetration of a Retzius cell by the double-barrelled microelectrode. As shown in Fig. 2, the recorded membrane potential was -52 mV and the intracellular K activity (aK_i) 105 mM. This intracellular K^+ level remained constant throughout the recording.

The mean membrane potential measured in physiological saline was $-43{\cdot}6 \pm 4{\cdot}9$ mV ($n = 14$). The mean intracellular potassium activity as measured with the double-barrelled K^+-sensitive microelectrodes was $101{\cdot}3 \pm 7{\cdot}6$ mM ($n = 14$). Assuming an intracellular activity coefficient for K^+ of 0·75 (as determined for pure KCl-NaCl solutions, see Robinson & Stokes, 1959), this would indicate an intracellular K^+ concentration of $135{\cdot}1 \pm 10$ mM. There was no difference in the measurement of the intracellular K activity when either Na-acetate or Mg-acetate was used as filling solution for the reference barrel. The K^+ concentration, measured in the extracellular spaces around the nerve cell bodies ('nerve cell body region') was significantly higher than the 4 mM in the external solution. We recently reported that the extracellular K^+ concentration in the nerve cell body region is $5{\cdot}8 \pm 0{\cdot}6$ mM (i.e. 45% higher than in the external solution, Schlue & Deitmer, 1980). This corresponds to an external K activity of 3 mM, and an extracellular activity of 4·35 mM, assuming

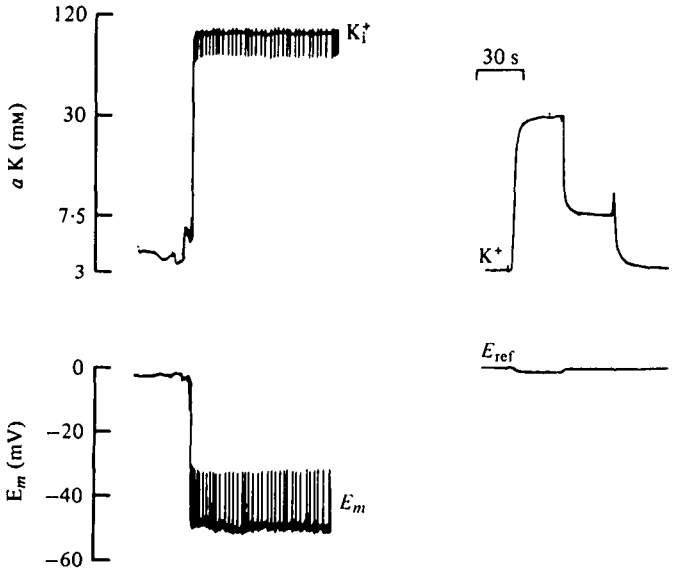

Fig. 2. Impalement of a Retzius cell with a double-barrelled K^+-sensitive microelectrode to record the intracellular K activity (upper trace), and the membrane potential (lower trace). The cell fires action potentials spontaneously (of about 20 mV in amplitude). These action potentials are partly recorded also in the K_i^+ trace, because the different time course of the ion-sensitive and the conventional electrode results in an imperfect electronic subtraction of these fast potential changes. On the right is shown a calibration of the K^+-sensitive microelectrode in the experimental chamber, when the K^+-concentration of the external solution was raised from 4 to 40 and 10 mM (equivalent to K *activity* change from 3 to 30 and 7·5 mM, respectively).

the same activity coefficient for the physiological saline and the blood in the intact ganglion.

From the mean intra- and extracellular K activities the K^+ equilibrium potential was calculated according to the Nernst equation to be −80 mV. This is 36 mV more negative than the mean membrane resting potential measured in these cells, indicating that the distribution of K^+ ions across the membrane of Retzius cells is far from being in equilibrium.

Changing the external potassium concentration

Raising the external K^+. Increasing the external K^+ concentration from 4 to 40 mM increased the average extracellular K^+ concentration from 5·8 mM to 41·9 mM (Schlue & Deitmer, 1980). As shown in Fig. 3 (upper trace) the K activity in the extracellular spaces rose from 4·5 to 33 mM (with a half-time of approximately 15 s) and subsequently fell to its initial level (with a half-time of 20 s) when the external K^+ concentration was raised from 4 to 40 mM (i.e. 3 mM to 30 mM-K activity). The intracellular K activity (aK_i) was 75 mM, which was by far the lowest value measured in any cell (Fig. 3, middle trace). When the external K^+ concentration was raised to 40 mM and subsequently lowered to 4 mM, the intracellular K activity remained unchanged. In some other experiments of this kind there appeared to be a small reversible increase in the intra-

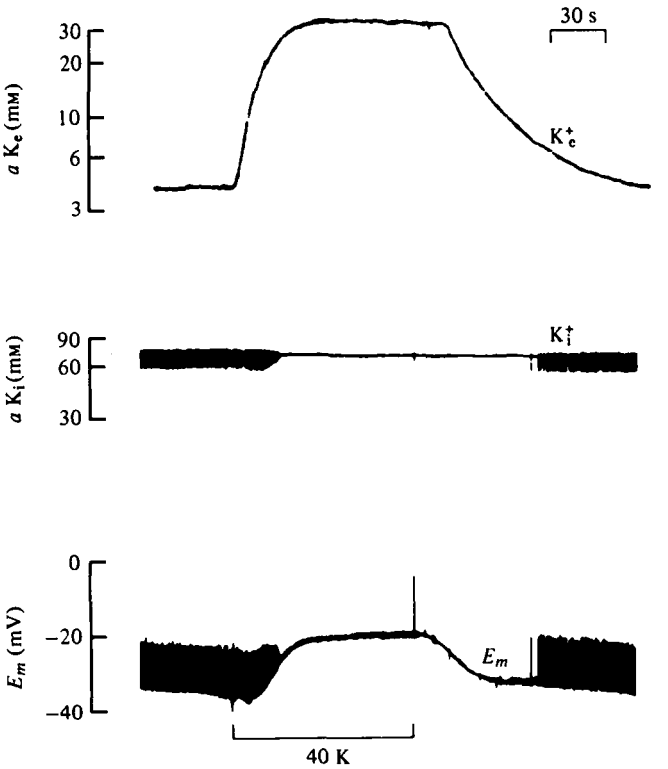

Fig. 3. The effect of raising the external K^+ concentration from 4 to 40 mM, on the K activity of the extracellular spaces in the cell body region (aK_e, upper trace), on the intracellular K activity of a Retzius cell (aK_i, middle trace), and on the membrane potential of the Retzius cell (E_m, lower trace). The recordings of aK_i and E_m are from the same cell, the recording of aK_e is from a different experiment. The black areas on the middle and lower trace are spontaneous action potentials.

cellular K activity of up to 5 mM during the exposure to 40 mM-K^+ in the external solution. As shown in Fig. 3 (lower trace), the initial membrane potential of the Retzius cell was −35 mV. The membrane depolarized, by about 15 mV, to −20 mV when the external K^+ concentration was raised to 40 mM. The measured intracellular and extracellular K activities indicate a K^+ equilibrium potential of −21 mV in this experiment. Thus, the membrane potential and the K^+ equilibrium potential appear to be very close, indicating that at high external K^+ concentrations the membrane behaves like a K^+ electrode, and that the distribution of K^+ ions across the cell membrane is in equilibrium under these conditions.

When the external K^+ concentration was raised to 40 mM the frequency of the spontaneous action potentials increased and then decreased to zero as the membrane depolarized. On return to normal external K^+ concentration, the membrane repolarized, and the cell fired action potentials again.

Lowering the external K^+. Previous measurements of the K^+ level in the extracellular spaces of the nerve cell body region, have shown that the K^+ level in intact leech ganglia remained considerably higher than in the external solution. In K-free sali[illegible]

example, the K^+ concentration in the extracellular spaces only decreased to 1·6 mM (Schlue & Deitmer, 1980). This maintained steady-state K^+ level does not appear to result from leakage of K^+ from damaged cells or from interference by other ions. In Fig. 4A is shown a typical recording of the K activity in the extracellular spaces of the nerve cell body region when the external K^+ concentration was reduced to zero for about 2 min. The extracellular K activity decreased slowly and had not reached a steady-state in the 2 min of exposure to zero potassium. On return to the normal external K^+ concentration (4 mM), the K^+ level in the extracellular spaces increased more rapidly and reached its initial value within 2 to 3 min. Fig. 4B shows the intracellular K activity and the membrane potential of a Retzius cell, when the external K^+ concentration was reduced to zero for about 100 s. The initial intracellular K activity, of 110 mM, fell slightly during this period. The cell membrane *depolarized* from -47 to -42 mV, despite the increased K^+ gradient across the membrane, while the action potential frequency increased by a factor of about 2. When the external K^+ concentration was again raised to 4 mM, the intracellular K activity increased and returned to its original level. The membrane potential did not return directly to its initial level upon restoring the external K^+ concentration, but underwent a marked undershoot. This transient hyperpolarization reached a peak membrane potential of -55 mV (i.e. 8 mV below the initial resting potential). During this period spontaneous action potentials were suppressed. Fig. 4C shows an experiment in which the preparation was exposed to zero K^+ for > 6 min. During this period the intracellular K activity decreased from 110 mM to about 45 mM, and the cell membrane depolarized from -52 to -44 mV. With extracellular K^+ concentrations of around 5·8 mM (at an external K^+ concentration of 4 mM) and 1·6 mM (in the absence of K^+ from the external solution) the K^+ equilibrium potentials are -82 and -92 mV, respectively. A simultaneous membrane depolarization from -52 to -45 mV indicated that membrane potential and K^+ equilibrium potential were shifted in opposite directions, thus *increasing* the electrochemical gradient for K^+ ions from 30 to 47 mV during this period.

When the K^+ concentration of the external solution was restored to 4 mM, the intracellular K activity increased rapidly. After 6 min the intracellular K activity had risen to approximately 80 mM, and then continued to increase more slowly. The membrane hyperpolarized upon re-addition of K^+ by 13 mV, to -62 mV, and then slowly depolarized to its initial level. This depolarization (or recovery from the maximum hyperpolarization) occurred with a much slower time course than that after the shorter exposure to zero K^+ (Fig. 4B). The durations of these depolarizations (approximately 2·5 min for the short and 5 min for the longer exposure to zero K^+) were independent of the time course of the rise in extracellular K^+ following return to normal external K^+ (between 1·5 and 2 min after exposures to zero K^+ for 2–15 min). There was also no depletion of K^+ ions observed in the extracellular spaces of the nerve cell body region when the K^+ concentration of the external solution was restored.

As shown in Fig. 5 the onset of the increase in the intracellular K activity occurred during the maximum hyperpolarization of the membrane. The steep rise in the intracellular K activity was followed by a slower phase after about 6–9 min, when the

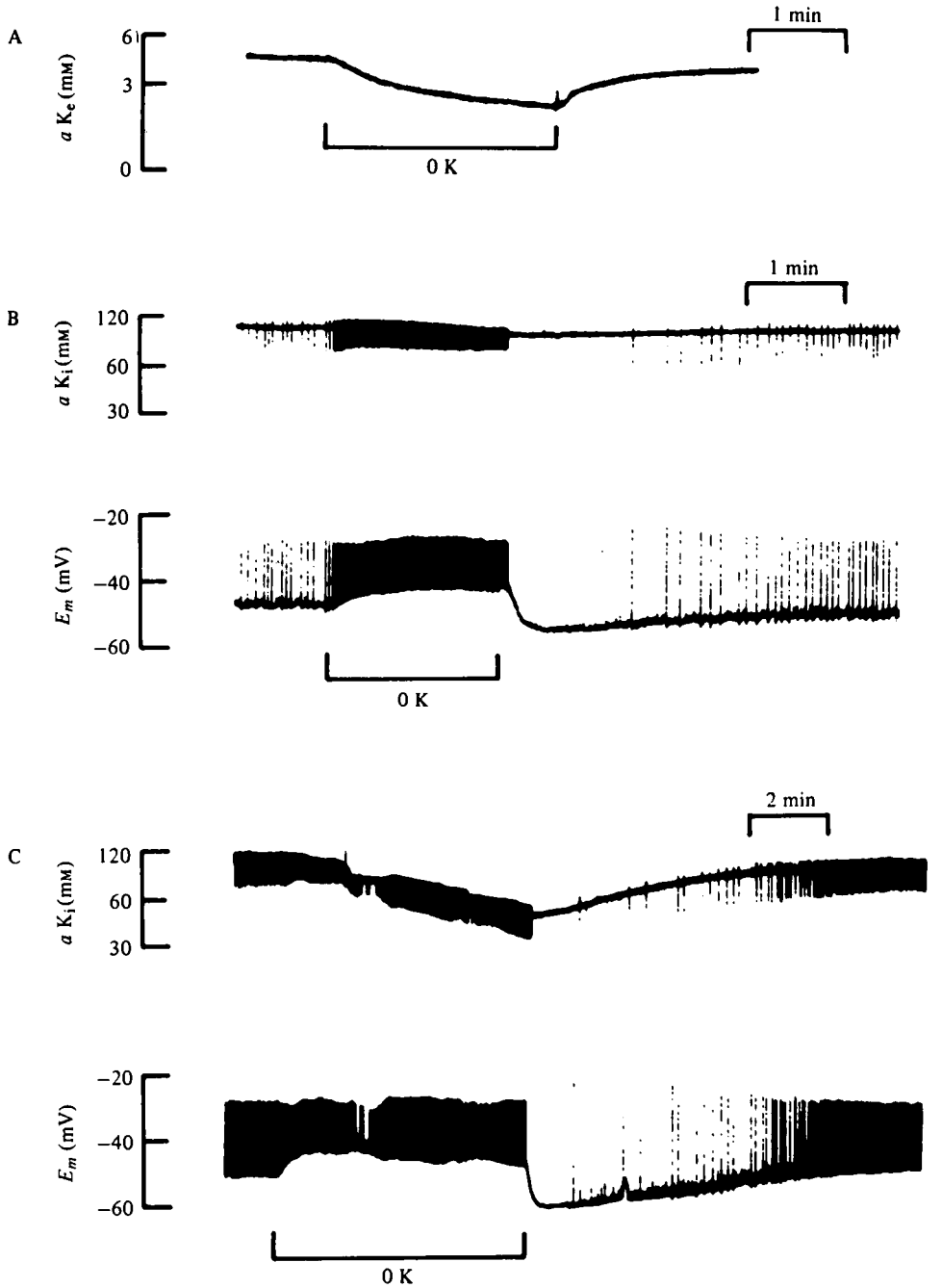

Fig. 4. The effect of zero external K^+-concentration on the K activity in the extracellular spaces of the nerve cell body region (A), and on the intracellular K activity and the membrane potential of a Retzius cell (B, C). The exposure to zero K^+ was less than 2 min in (B), and more than 6 min in (C) (note the different time scale in (B) and (C)). The recordings in (B) and (C) are from the same cell, that of (A) is from a different experiment.

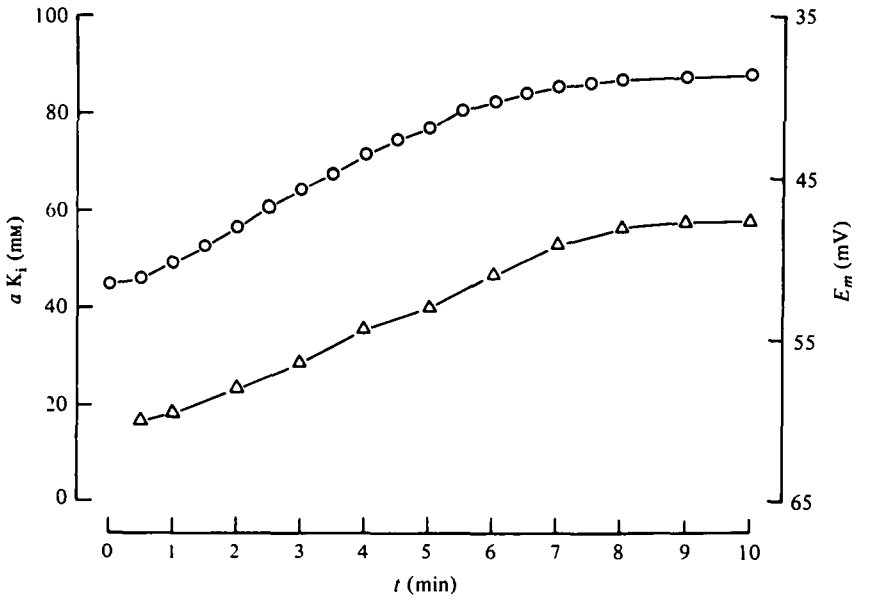

Fig. 5. The rise in the intracellular K activity, plotted on a linear scale (circles, left ordinate), and the recovery of the membrane potential (triangles, right ordinate) from maximum hyperpolarization after re-addition of 4 mM-K^+ to the external solution (following zero K). Both traces are drawn from the experiment shown in Fig. 4C.

membrane potential had reached its initial level. This suggests that the transient membrane hyperpolarization on return of external K^+ might be correlated with a simultaneous, rapid, increase in intracellular K activity.

Effects of ouabain

The influence of the cardiac glycoside ouabain on intracellular K activity and membrane potential was investigated. A high ouabain concentration (5×10^{-4} M) was added to the normal physiological saline to block the Na^+-K^+ pump. Addition of ouabain caused the intracellular K activity to fall, from 90 mM to about 9 mM, within 10 min (Fig. 6). The cell membrane depolarized rapidly from -43 to -35 mV, and further to about -10 mV in the next 10 min. The first ouabain-induced depolarization by 8 mV was usually too early and rapid to be produced by decreased intracellular K^+, although the second , slower, depolarization was probably caused by the loss of K^+ ions from the cell.

Exposure to zero external K^+ in the continuous presence of ouabain caused a further decrease in intracellular K activity to 6 mM. The membrane potential changed biphasically, a rapid brief hyperpolarization was followed by a slow depolarization (Fig. 6). Restoration of the external K^+ concentration to 4 mM caused the intracellular K activity to increase again to about 10 mM, and the cell repolarized after a brief depolarization. Removal of external K^+ a few minutes after the addition of ouabain induced a membrane hyperpolarization, in contrast to the depolarization

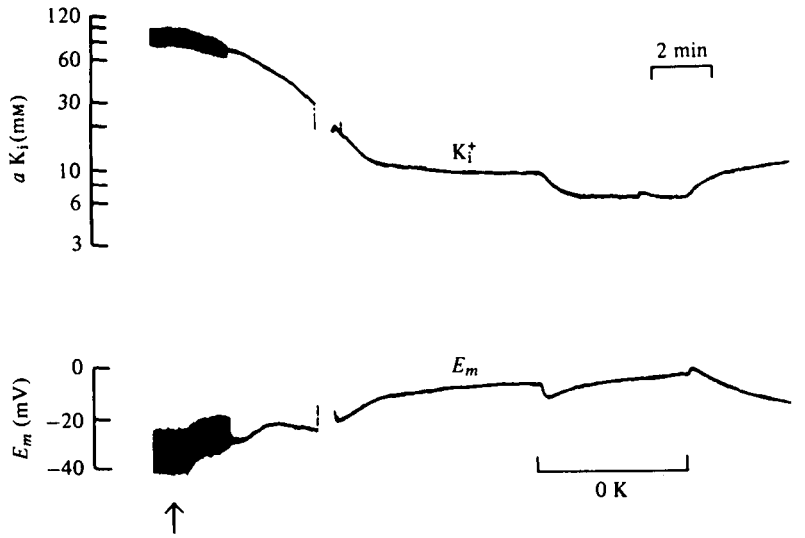

Fig. 6. The effects of the addition of 5×10^{-4} M-ouabain to the external solution (arrow) on the intracellular K activity (upper trace), and on the membrane potential (lower trace). At the end of the experiment is shown an exposure to zero K^+ in the continuous presence of ouabain. The interruption in the traces was due to an offset on both recordings lasting for about 40 s.

observed in the absence of ouabain. The change in membrane potential produced by the removal of K^+ was, thus, ouabain-sensitive.

Removal of ouabain was associated with an increase in intracellular K activity and membrane potential, after a delay of 5–20 min. The recovery of intracellular K activity and membrane potential lasted from 30 to 120 min, and was not always complete; its time course and duration depended upon the duration of the ouabain application.

Estimates of the K^+ permeability of the cell membrane

The loss of intracellular K^+ after the removal of the external K^+ occurred with a maximum rate of 12 mM min^{-1} (Fig. 7). A similar maximal rate of decrease in intracellular K activity (up to 14 mM min^{-1}) occurred 3–5 min after the addition of ouabain. The similarity of the rates of K^+ decrease in the absence of external K^+, and in the presence of ouabain is probably coincidental since these two conditions differ as e.g. the remaining K^+ in the extracellular spaces in nominal absence of external K^+ would maintain some Na^+-K^+ pumping across the cell membrane while 5×10^{-4} ouabain probably inhibits all Na^+-K^+ pumping. The decrease in the intracellular K activity in the presence of 10^{-5} M-ouabain probably reflects a *net* passive K^+ efflux from the cells. The maximal decrease in intracellular K^+ would then indicate a net K^+ efflux across the membrane of about 0·23 nmol cm^{-2} s. Using the constant field equation (Goldman, 1943; Hodgkin & Katz, 1949; see Methods), the net K^+ efflux under these conditions would indicate an 'apparent' membrane permeability co-

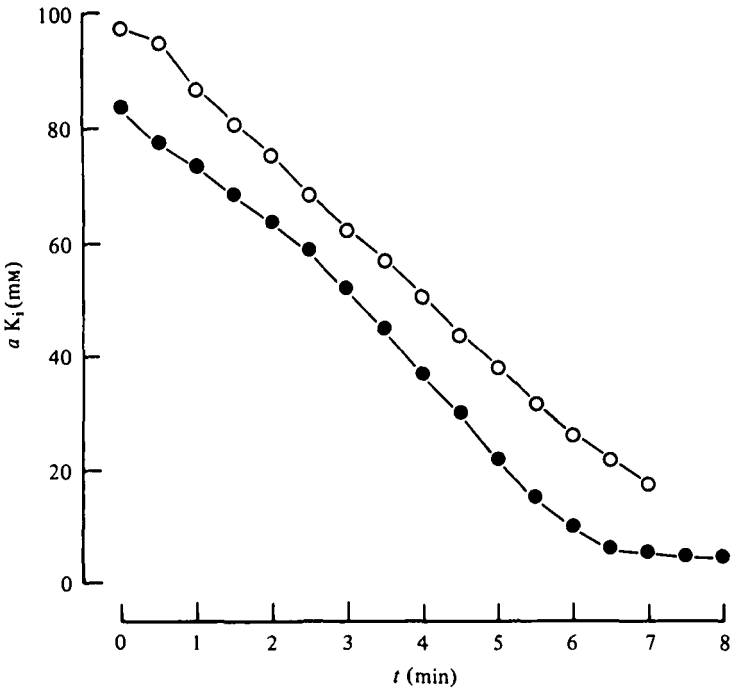

Fig. 7. The decrease of the intracellular K^+ activity from a Retzius cell plotted linearly versus time, after the K^+ concentration of the external solution had been reduced from 4 to zero mM (open circles), and after the addition of 5×10^{-4} M ouabain to the external solution (filled circles).

efficient for K^+ ions, P'_K, of approximately 8×10^{-6} cm s^{-1} (at a K^+ concentration of 5·8 mM in the extracellular spaces).

The *increase* in intracellular K activity after return to normal external K^+ (Fig. 4C) occurs against the electrochemical gradient for K^+ ions, and must therefore be linked to an active K^+ uptake mechanism. The time course of the intracellular K^+ rise thus indicates the rate, at which this active pump system works. The maximum increase in the intracellular K activity under these conditions was 8 mM min^{-1}. Assuming a *maximal* net loss of K^+ from the cells, as measured in the presence of ouabain, this increase in the intracellular K activity would indicate an uptake of K^+ ions across the cell membrane of 0·36 nmol cm^{-2} s.

DISCUSSION

The present study reports the first direct measurements of the intracellular K activity of leech nerve cells using ion-sensitive microelectrodes. The results suggest that a high intracellular K activity in the Retzius cell somata is maintained, against a gradient of 20 to 40 mV, by an electrogenic Na^+-K^+ pump. The passive K^+ efflux from these cells indicates an 'apparent' K^+ permeability coefficient of about 8×10^{-6} cm s^{-1}.

Intracellular K^+ and membrane potential

The intracellular K activity of the Retzius cells varied from 75 to 122 mM. The membrane potential in these cells ranged between −35 mV (lowest resting potential accepted, see Methods) and −55 mV. The membrane potential tended to be more negative with higher intracellular K activity, and *vice versa*. A direct proportionality of membrane potential and intracellular K level at constant extracellular K^+ concentration is consistent with the Goldman–Hodgkin–Katz equation. However, some degree of damage would also tend to decrease both the membrane potential and the intracellular K activity. The size of the action potentials and the frequency of their spontaneous generation might therefore be a better indication of possible membrane damage. We therefore carried out experiments only with cells firing action potentials with amplitudes usually greater than +15 mV, and with a frequency of lower than 0·5 impulses s^{-1}. Similar values for the action potential amplitude and the spike frequency have been reported in the literature (for references see Lent, 1977). Control experiments in the present study using conventional, single-barrelled, microelectrodes agreed well with our measurements with the double-barrelled microelectrodes, although there was a higher success rate of good impalements with single-barrelled microelectrodes.

The K^+ equilibrium potential across the Retzius cell membrane was calculated to be around −80 mV from our direct measurements of the extracellular and intracellular K^+ level. This value is somewhat lower than that reported by Nicholls & Kuffler (1964), who found a K^+ equilibrium potential of unidentified neurones (presumably not Retzius cells) of −89 and −83 mV, depending upon the preparation of the ganglia. Their extrapolated value of the intracellular K^+ concentration, (determined from membrane potential measurements of neurones *in situ*) was 138 mM. The intracellular K^+ concentration of the neurones, determined by flame photometry was about 130 mM (Nicholls & Kuffler, 1965). These values agree well with our measurements using intracellular ion-sensitive microelectrodes in Retzius cells. The deviation concerning the calculation of the K^+ equilibrium potential probably results from taking different values for the *extracellular* K^+ concentration: the external K^+ concentration (4 mM, Nicholls & Kuffer, 1964), and the K^+ concentration measured with ion-sensitive microelectrodes in intact ganglia (5·8 mM, Schlue & Deitmer, 1980).

The membrane resting potential of leech neurones were reported to be −30 to −60 mV for Retzius cells (Hagiwara & Morita, 1962; Eckert, 1963; and present study), around −40 mV for unidentified neurones (Nicholls & Kuffler, 1964), and −40 to −45 mV in identified sensory nerve cells (Baylor & Nicholls, 1969*a*, *b*; Schlue, 1976). Thus, our results agree with the conclusion of previous studies (Nicholls & Kuffler, 1964; Baylor & Nicholls, 1969*a*) that K^+ ions are not passively distributed across the cell membrane of leech neurones. The difference between the mean resting potential and the K^+ equilibrium potential as determined in the present study ranges between 20 and 40 mV.

Effects of extracellular K^+ and ouabain

Nicholls & Kuffler (1964) concluded from their experiments that the intracellular K^+ concentration does not change significantly, when the external K^+ concentration is raised. Our measurements using intracellular K^+-sensitive microelectrodes confirm this conclusion for leech Retzius cells. The magnitude of change in membrane potential upon raising the external K^+ concentration suggests that the membrane of Retzius cells is a poor K^+ electrode (15 to 25 mV/tenfold change of $[K^+]_o$).

Lowering the external K^+ concentration to zero, or adding 5×10^{-4} M-ouabain, produced a depolarization of the Retzius cell membrane of 5 to 8 mV, as has previously been reported by Walker & Smith (1973). Measurements of the *extracellular* K^+ concentration in the nerve cell body region of intact leech ganglia had shown that even after complete removal of K^+ from the external solution, there remains a small but significant amount of K^+ (1·6 mM on average) in the extracellular spaces (Schlue & Deitmer, 1980). Addition of ouabain produced a *transient* increase in the K^+ concentration in these extracellular spaces. Upon removal or addition of K^+ we had never observed a depletion or accumulation of K^+ in the extracellular spaces around the nerve cell bodies, which could explain the membrane depolarization by an ionic diffusion potential.

It was shown that the membrane potential of leech sensory neurones has a component which is due to electrogenic Na^+-K^+ pumping (Baylor & Nicholls, 1969*b*; Jansen & Nicholls, 1973). In the present study the following additional evidence are consistent with, or strongly suggest the presence of an electrogenic pump in the Retzius cell membrane: (1) the membrane depolarization on removal of external K^+ was converted to a hyperpolarization in the presence of ouabain; (2) return of external K^+ caused a transient hyperpolarization, the extent and the time course of which depended on the duration of the exposure to zero K^+; (3) the time course of this hyperpolarization was *not* related to a change in the K^+ concentration in the extracellular spaces.

The potassium permeability of the cell membrane

The ouabain-induced decrease in the intracellular K activity presumably reflects the net passive efflux of K^+ ions across the cell membrane. From the maximum K^+ efflux a K^+ permeability of 8×10^{-6} cms^{-1} was calculated according to the constant field theory (Goldman, 1943; Hodgkin & Katz, 1949). Compared with the reported K^+ permeability coefficients, for example, for the membrane of *Aplysia* neurones (Eaton *et al.* 1975), the K^+ permeability of the Retzius cell membrane is 50 to 150 times larger. As pointed out by Eaton *et al.* (1975) *Aplysia* neuronal cell membranes have an unusually low K^+ permeability, which is in accordance with their very high specific resistance of about 10^5 Ωcm^2. In contrast the membrane input resistance of Retzius cells of between 5 and 18 MΩ (cf. Lent, 1977), gives a specific membrane resistance of only 1 to 4×10^3 Ωcm^2. Despite the electrotonic coupling between the two Retzius cells in the leech ganglia (Hagiwara & Morita, 1962; Eckert, 1963), which would lead to an underestimation of the specific membrane resistance, the resistance of Retzius cell membranes appears to be significantly lower than that of *Aplysia* neuronal mem-

branes. In spite of this comparatively high K^+ permeability, the Retzius cell membrane potential is 20–40 mV more positive than the K^+ equilibrium potential. This indicates that the membrane permeability to ions other than K^+ ions probably greatly contributes to the membrane potential, especially, since the activity of an electrogenic Na^+/K^+ pump would also tend to increase the membrane potential.

From the action of 5-hydroxytryptamine on the Retzius cell membrane, the Cl^- equilibrium potential was estimated to be around -70 mV (Walker & Smith, 1973). This is 10–40 mV more negative than the normal membrane resting potential. It is, therefore, concluded that the Retzius cell membrane might also have a relatively high resting permeability to Na^+ ions, which holds the resting potential at a considerably more positive level than the K^+ and Cl^- equilibrium potentials.

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