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THE EFFECTS OF LITHIUM AND SODIUM ON THE POTASSIUM CONDUCTANCE OF SNAIL NEURONES

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SUMMARY

1. The iontophoretic injection of lithium into snail neurones reversibly increased the resting relative potassium permeability (P_K).

2. Long exposures to snail Ringer containing 25 mM-Li and correspondingly reduced Na also caused an increase in P_K. This did not occur with Ringer in which the same reduction of Na was made by replacing it with Tris.

3. Replacement of part of the Ringer Na by either Li or Tris led to proportional decreases in internal Na.

4. Injecting large quantities of Na into ouabain-treated cells caused effects similar to those of Li injection. Without ouabain, Na injection stimulated the electrogenic Na pump.

5. A number of tests failed to produce any clear evidence that intracellular Ca was involved in the response to Li.

INTRODUCTION

Chemically, lithium is very similar to sodium. Physiologically Li can sometimes, but not always, replace Na for short periods. In many nerves the normal inward current carrier is Na, but it can be completely replaced by Li (Hille, 1970). The Na pump, however, will not significantly transport Li (Keynes & Swan, 1959). Experimentally, Li has frequently been substituted for Na to produce Na-free solutions. Clinically, Li has been used in the treatment of manic-depressive illness (Maletzky & Blachly, 1971). This suggests it may have some specific neural effects as well as simply acting as a Na substitute.

As part of a study on the electrogenic Na pump (Thomas, 1968), it was noticed that the injection of large doses of Li or Na into snail neurones appeared to increase the resting potassium permeability (P_K).

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We have now investigated this effect in more detail, and have found that even relatively low intracellular levels of Li cause small increases in P_K. This occurs whether Li is applied by intracellular injection or in the external solution. High levels of intracellular Na have a similar effect. A preliminary account of some of these experiments has already appeared (Partridge & Thomas, 1974).

METHODS

Detailed descriptions of most of the methods used here have already been published (Thomas, 1972). Experiments were done on the large cell 1 (Neild & Thomas, 1974) at the rear of the right pallial ganglion of the snail *Helix aspersa*. The brain was removed from dormant snails and mounted in a small bath which allowed the ganglion to be superfused with snail Ringer flowing at the rate of about five bath-volumes per minute. The connective tissue was dissected from the ganglion and the cell was then penetrated with various micro-electrodes. Cell diameters were measured with a calibrated eyepiece on the dissecting microscope and the cell volume calculated assuming that the cells were spherical. Experiments were done at room temperature (14–22° C).

Micro-electrodes. Conventional glass micro-electrodes were used to (*a*) record membrane potential, (*b*) pass current pulses across the cell membrane, and (*c*) inject various ions into the cell by means of interbarrel electrophoresis (a technique in which current is passed between two intracellular micro-electrodes, carrying anions out of one and cations out of the other). Injection electrodes were filled with the following solutions as necessary: 2 M-KCl; 2 M-LiCl; 2 M-NaCl; 0·5 M EGTA, (ethylene glycol bis-(2-amino ethyl) tetra-acetic acid, K salt), or 1 mM-$LaCl_3$ in 1 M-KCl. The amplitude and duration of the injection current was measured and thus the amount of injected ion could be calculated, assuming that all the injection current was carried by ions moving out of the electrodes. Recessed-tip Na^+-sensitive micro-electrodes were constructed as previously described (Thomas, 1972) and were used in several experiments where a continuous record of intracellular Na activity was required.

Solutions. The normal snail Ringer used had the following composition: KCl, 4 mM; NaCl, 80 mM; $CaCl_2$, 7 mM; $MgCl_2$, 5 mM; Tris maleate buffer, 20 mM; pH 7·5. Test solutions were made by: replacing some of the NaCl with an equal amount of LiCl, $MnCl_2$, or Tris Cl; by altering the $CaCl_2$ or KCl concentration with appropriate adjustment of the NaCl; or by adding $LaCl_3$, $Pr(NO_3)_3$ or ouabain to the Ringer.

Electrical arrangements. The membrane potential micro-electrode was connected via a high-input impedance unity gain amplifier to an oscilloscope and pen recorder. Na^+-sensitive micro-electrodes were connected to a varactor diode amplifier (Analog Devices 311 J) the output of which was led to the pen recorder after having subtracted from it a low pass filtered membrane potential signal. In most experiments the cell's input (or, more exactly, chord) resistance was measured once a minute by passing a 3 nA current pulse lasting 6 sec from an intracellular micro-electrode to earth.

RESULTS

Effects of LiCl injection

Fig. 1 shows the effects of an injection of LiCl (calculated to raise the intracellular concentration of Li to 22 mM) on the membrane potential of a snail neurone. During the injection the membrane potential gradually increased and the cell ceased firing. After the injection, the cell began to recover slowly but, as was frequently the case with large injections, following the initial recovery phase it remained slightly hyperpolarized. The rate at which the initial hyperpolarization developed depended

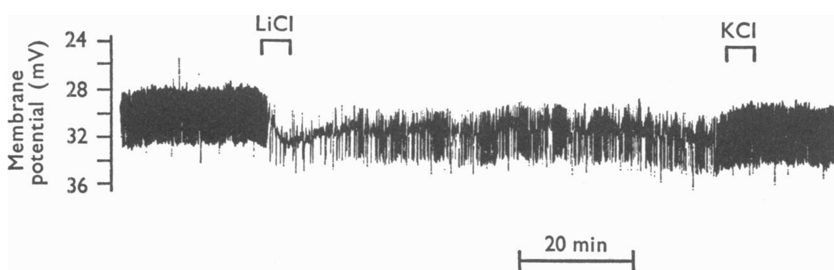

Fig. 1. Pen-recording showing the effect on the membrane potential of the injection of LiCl and KCl into the same cell. Left-hand bar indicates the 5 min period during which LiCl was injected iontophoretically by a 20 nA current. This was calculated to give a maximum concentration of injected ions of about 22 mM. Right-hand bar represents a similar injection of KCl produced by reversing the direction of the injection current.

upon the size of the injection. The amplitude of the hyperpolarization increased with increasing amounts of Li injected and recovery did not occur if the amount was too great. To show that this was a specific effect of Li injection, comparable amounts of KCl were injected. Fig. 1 shows that such an injection of KCl produced only a slight depolarization.

If constant current pulses were applied from an intracellular electrode to earth the resulting changes in membrane potential could be measured and the cell input resistance (R_i) determined. During an injection of LiCl we found that R_i fell rapidly by up to one third of its initial value and slowly recovered. The fall in R_i appeared to be the result of an increase in the relative K permeability (P_K). P_K increased with increasing intracellular Li over the range tested, 1–20 mM (see Partridge & Thomas, 1974). Fig. 2 shows that the recovery of R_i (if expressed as actual resistance less steady-state resistance) following a Li injection follows an approximately exponential time course, in this case with a time

constant of 36 min. In eight cells where the measurement was made the recovery of R_i following an injection of Li occurred with time constants between 8 and 36 min, with a mean of 20·5 min.

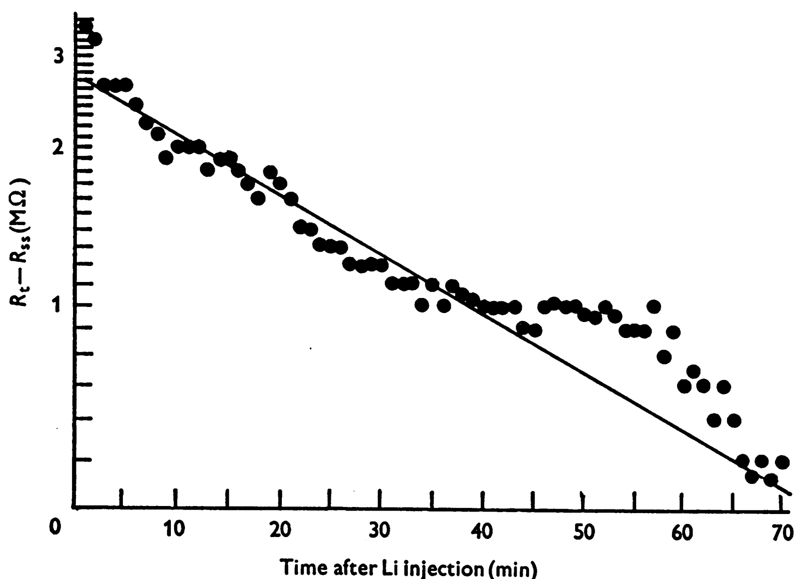

Fig. 2. Graph showing the recovery of the input resistance following the injection of enough LiCl into a cell to raise the intracellular concentration to 29 mM. Points represent the difference between the input resistance at that time (R_t) and the steady-state input resistance (R_{ss}). The line was drawn by eye as an approximate fit to the readings obtained over the first 30 min after the injection.

Effects of external Li

Similar effects to those observed when Li was injected into a cell were seen upon applying Li externally. As can be seen in Fig. 3, a detectable hyperpolarization and decrease in R_i was found after about 50 min in 25 mM-Li. The slight increase in R_i during the time in 10 mM-Li probably represents recovery from the trauma of electrode penetration. For low levels of external Li the effect on R_i was not always pronounced. The P_K was therefore estimated, as before, by changing the Ringer to ones containing 2 and 8 mM-K in the presence of each concentration of Li. By measuring the resultant membrane potentials and plotting K_0 *vs.* $e^{FV/RT}$ (Moreton, 1968) an estimate of P_K was obtained. Relative to that in normal Ringer, P_K increased to 1·3 after 95 min in 10 mM-Li and to 2·2 after 52 min in 25 mM-Li. On return to normal Ringer a recovery to 1·1 was observed.

To test that this was not a result of removing some of the external Na, control experiments were made using Tris-substituted Ringer. In the experiment shown in Fig. 4, 40 mM-Li applied for 43 min caused the

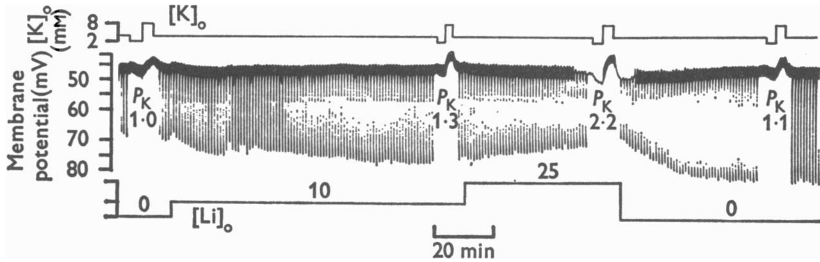

Fig. 3. The effect of external Li on the input resistance and on the response to changing external K. Hyperpolarizing current pulses of 3 nA are passed to earth for 6 sec once a minute in order to determine R_i, these are interrupted during the times when external K was changed so that a more accurate determination of the membrane potential could be obtained. Values of P_K are relative to the resting level before Li Ringer was applied.

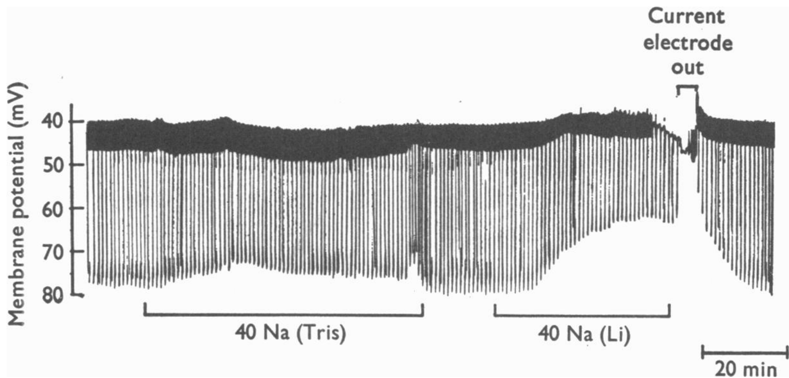

Fig. 4. Comparison of the effect of replacing half of the Na in the Ringer with Tris or Li. The electrode used to apply current pulses became dislodged at the end of the Li application but was re-inserted with only a minimal effect on the membrane potential.

expected decrease in R_i, a hyperpolarization, and cessation of firing. However, when Ringer with an equivalent amount of Na replaced by Tris was applied to the cell for 68 min only a slight decrease in R_i was noted and this was not accompanied by any hyperpolarization.

The recovery of R_i following application of Li in the Ringer showed less variability than the recovery after Li injection. In eight cells where 40 mM-Li had been applied until firing ceased, the recovery of R_i had time constants ranging from 8 to 15 min with a mean of 11·5 min.

It has been shown (Thomas, 1972) that decreasing external Na causes

a decrease in internal Na, presumably as a result of the Na pump's activity in the face of a reduced Na influx. Fig. 5 shows a continuous record of intracellular Na activity measured during the application of Ringer in which half the Na was replaced by Li. The Na activity decreased from about 5·2 to about 3·9 mM, and stayed at this new level until normal snail Ringer was returned. Similar decreases of intracellular Na activity by 1–2 mM were seen in six cells where this measurement was made. That this was an effect due to the lowered Na and not specifically due to the Li was shown by replacing a similar amount of Na with Tris. When this was done the intracellular Na activity fell by an equivalent amount and with a similar time course. Thus reduced external Na decreases Na influx, and the presence of Li does not alter this.

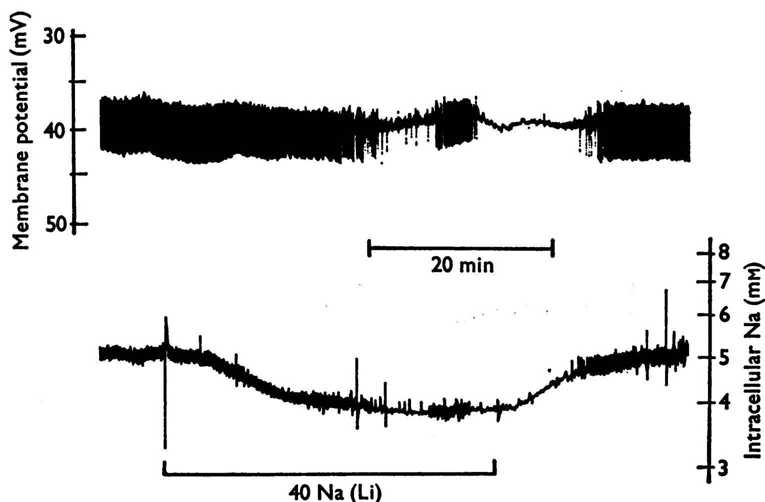

Fig. 5. The effect of external Li on membrane potential and internal Na. Upper trace shows membrane potential and lower trace is a continuous record of internal Na as measured with a recessed-tip Na^+-sensitive micro-electrode.

Effects of increasing intracellular Na

To investigate whether large increases in internal Na would have similar effects, experiments similar to those described above but using Na instead of Li, were undertaken. Fig. 6 shows that a small injection of Na, which raised the intracellular concentration by about 6 mM, caused a hyperpolarization but no change in input resistance. This hyperpolarization is the result of the action of the electrogenic Na pump in these cells (Thomas, 1969). When a large injection was made (raising the intracellular Na by a calculated 22 mM) a considerably larger hyperpolarization resulted, with a marked decrease in input resistance. Ouabain

was then applied and within 5 min the hyperpolarization following a small injection was completely blocked. However, a large injection still caused a hyperpolarization and decrease in R_i. Unfortunately this treatment was irreversible in the four cells where it was tried.

A convenient method of raising internal Na is to block the Na–K pump with ouabain. This method eliminates both the trauma of penetrating the cell with two additional injection electrodes and the complications of altered K fluxes due to the pump activities. A linear rise in internal Na at rates of 0·31–0·77 mM/min from an initial level of about 4 to levels above 20 mM at the termination of the experiments was noted in five cells treated with 10^{-4} M ouabain.

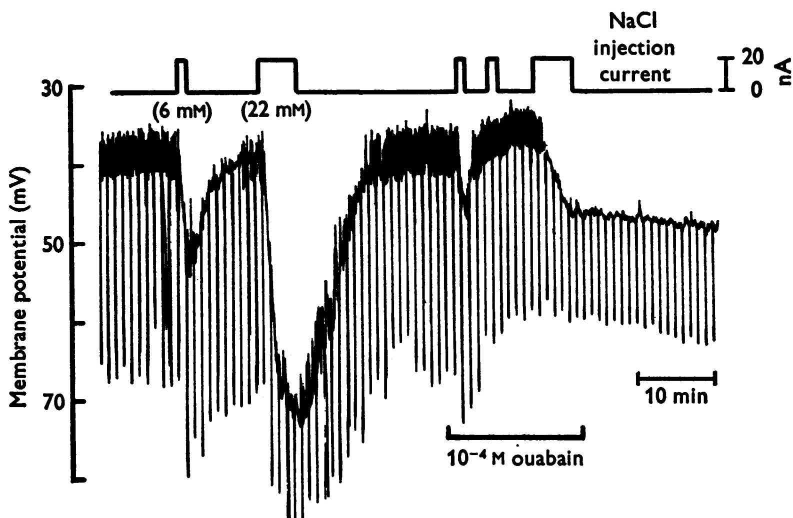

Fig. 6. The effects of the injection of Na on the membrane potential and input resistance, the latter being determined by the response to 3 nA hyperpolarizing pulses. The three short injections were calculated to raise $[Na]_i$ by 6 mM each while each of the two long injections raised $[Na]_i$ by a calculated 22 mM. 10^{-4} M ouabain was applied in the bath during the period marked by the bar.

In the experiment of Fig. 7 external K levels were periodically changed from 4 to 2 and 8 mM and the resultant changes in membrane potential measured. As the intracellular Na increased, the same changes in external K concentration produced considerably greater changes in membrane potential. After the ouabain was removed, intracellular Na stopped rising and the cell hyperpolarized, presumably as a result of a partial return of electrogenic Na pumping.

P_K was determined at intervals, as in the experiment of Fig. 3, and the results are shown in Fig. 8 (circles) for five cells. In all cases there

was an increase in P_K with increasing Na activity, although the experiment shown in Fig. 7 (open circles in Fig. 8) was the most dramatic example. Very similar effects of prolonged ouabain treatment on P_K have recently been reported by Gorman & Marmor (1974) with the *Anisodoris* giant neurone, but their effects took several hours to develop.

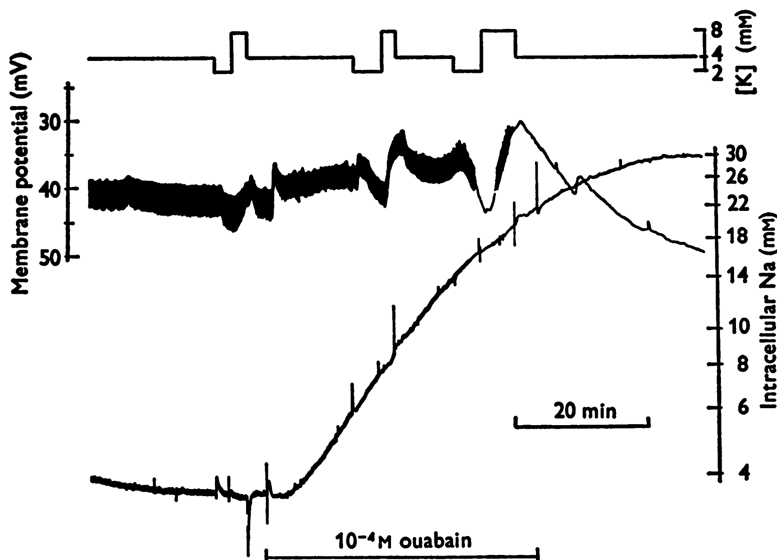

Fig. 7. Effect on the membrane potential response to changing external K of an increased internal Na resulting from inhibition of the Na pump. External K was changed at intervals to determine P_K, and intracellular Na was measured with a Na^+-sensitive micro-electrode.

Also shown in Fig. 8 (crosses) are data for the change in P_K following Li injection in seven different cells. Both Na and Li caused an increase in P_K as their intracellular concentrations rose, but generally Li had a more pronounced effect at any given estimated concentration.

Investigation of possible role of Ca

It is possible that Li has its hyperpolarizing effect through the following mechanism: Li, entering the cell either by injection or through passive influx, accumulates inside since it is pumped out poorly, if at all, by the Na pump (Keynes & Swan, 1959). However, if with respect to the Na–Ca exchange mechanism (Baker, 1972) intracellular Li resembles Na and thus decreases the Ca efflux, Ca ions will accumulate intracellularly. Since intracellular Ca ions are known to affect K permeability (Meech, 1972), this increased intracellular Ca would increase P_K, hyperpolarizing

the cell towards the K equilibrium potential. A similar mechanism for the long-term effect of ouabain on *Anisodoris* cells has been proposed by Gorman & Marmor (1974).

To examine the possible role of Ca in the Li effect, we have tested various procedures which are known to affect intracellular Ca. Table 1 gives a summary of the effects of these procedures.

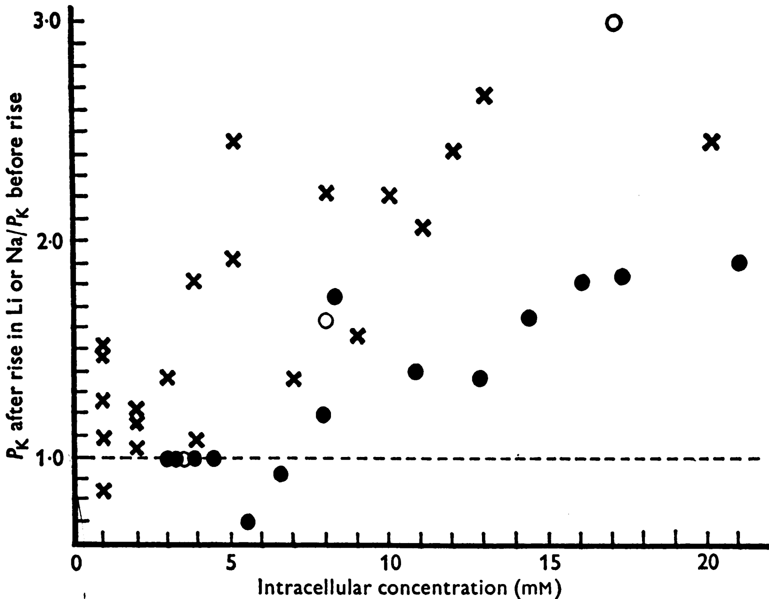

Fig. 8. Effect on P_K of intracellular Li (×) or Na (● and ○). Potassium permeability was determined relative to that before the injection (Li) or before the ouabain-induced rise in Na. Li concentrations were estimated from the injection charge used, and Na concentrations were measured with a Na^+-sensitive micro-electrode. Open circles are data from the experiment shown in Fig. 7.

EGTA, which would be expected to chelate free Ca (Meech, 1974), was injected throughout the period of Li application in three cells. With EGTA, these cells continued to fire a few spikes at the end of the hyperpolarizing pulse; during a similar period when Li was applied without EGTA injection they stopped firing. It is thus possible that EGTA gives some protection from the effect of Li. Lanthanum ions have been reported to block Ca uptake by mitochondria (Mela, 1968). Two cells were injected with La, calculated to bring the intracellular La concentration to about 30 μM, without causing any measurable change in the effect of externally applied Li.

External Mn and La have been shown to block Ca influx in several

preparations (Mayer, van Breeman & Casteels, 1972; Reuter, 1973). 15 mM-Mn in two preparations and 0·5 mM-La in another two were sufficient to increase R_i appreciably and to block spikes but did not prevent the hyperpolarization resulting from Li injection. Another rare earth element, praseodymium, Pr, which blocks influx and mitochondrial uptake of Ca (Alnaes & Rahamimoff, 1974), did not alter the effect of Li in one cell when it was applied at 100 μM in the Ringer.

Finally, Ringer with no $CaCl_2$ added in two cells and Ringer with twice the normal $CaCl_2$ (14 mM) in another two cells, caused no measurable alteration of the effect of externally applied Li.

TABLE 1. The results of various treatments designed to establish whether or not Ca is involved in the response to Li

Ion	Applied	Effects
EGTA	Inject	Some protection from Li effect
La^{3+}	Inject	Increases R_i, does not block Li hyperpolarization
Mn^{2+}	Bath	Increases R_i, stops spontaneous firing, does not block Li hyperpolarization
Pr^{3+}	Bath	Increases R_i, increases after hyperpolarization, does not block Li effect
La^{3+}	Bath	Increases R_i, stops spontaneous firing, does not block Li hyperpolarization
low Ca^{2+}	Bath	Decreases R_i, stops spontaneous firing, does not block Li hyperpolarization
$2 \times Ca^{2}$	Bath	Increases R_i, does not alter Li effect

DISCUSSION

We have shown that the injection of Li into snail neurones causes a decrease in R_i which recovers with an exponential time course. This decreased R_i results from an increase in the membrane permeability to K. This effect can also be produced by applying Li externally. Similarly, increased intracellular Na in amounts greater than those necessary to stimulate the electrogenic Na pump, causes a decrease in R_i and increases P_K. Although there is a possibility that internal Ca is involved in this effect we have failed to demonstrate this in any definitive manner.

It is not yet possible to monitor intracellular Li^+, thus its distribution and flux rates can only be estimated. We have made an estimate of the Li influx rate in the following manner. The time from application of 40 mM-Li until the cells ceased spontaneous firing was an average of 37 min (S.E. of mean, 3·1 min) in nineteen cells, including those of Figs. 4 and 5. To obtain an estimate of the corresponding intracellular Li concentration we have used the results of five experiments where sequential

injections, of increasing duration, of Li^+ were made. A mean value of 11·2 mM (S.E. of mean, 1·9 mM) was found for the minimal calculated intracellular concentration which caused the cells to stop firing spontaneously. This suggests that Li enters the cells at a rate which would cause the internal concentration to increase by about 0·3 mM/min. The mean Na^+ influx from Ringer containing 80 mM-Na would cause the internal Na^+ to increase by 0·54 mM/min (Thomas, 1972) but would be expected to be roughly halved (or 0·27 mM/min) in half the extracellular Na concentration. Li, then, appears to be entering at about the same rate as that for passive Na entry. Carmeliet (1964) has similarly found a prominent passive influx of Li ions in cardiac muscle cells.

Since Li is probably not pumped by the Na–K pump, we must assume that it leaves the cell passively. The recovery of R_i after its depression in the presence of Li has a time constant in the range 10–20 min. Thus either R_i recovers more rapidly than the actual Li efflux or Li efflux is much faster in snail neurones than has been reported in frog muscle. (Keynes & Swan (1959) found an efflux time constant of 10 hr and Yonemura & Sato (1967) found one of 2 hr.) The Na pump's ability to lower intracellular Na in the face of a decreased Na influx gradient does not appear to be affected by the presence of Li.

Raising internal Na by blocking the Na pump with ouabain introduces the complication that the internal K will fall at approximately the same rate. This would cause a decrease in the K equilibrium potential. For instance, if the internal K were to fall by 30 mM from about 100 mM then the equilibrium potential would decrease by about 15 mV. This could account for the gradual depolarization of the cell during ouabain treatment (Fig. 7).

The observation that the response to changing external K increases with high internal Na (Fig. 8) in an analogous manner to its increase with intracellular Li favours the hypothesis that Li is acting through a mechanism normally involving Na, perhaps one connected with that causing the increase in P_K during the action potential. The differences in the apparent effectiveness of the two ions has a number of possible explanations. It could be due to an error in the calculation of internal Li, although one would expect that such an error would be in the direction of overestimation of volume and thus underestimation of concentration. Another possible error is in the determination of P_K. Ouabain might cause a systematic change in P_{Na} or might directly affect P_K. Finally, and perhaps most likely, Li may actually be more effective in the mechanism responsible for the change in P_K.

The experiments concerning Ca, which we hoped would shed light on the mechanism of the Li effect, have proved to be ambiguous. The

fact that intracellular EGTA may have reduced the effect and that injected La^{3+} did not change it are both consistent with an intermediate role of Ca. Since zero external Ca was most certainly not attained without a chelating agent (cells did not tolerate 1 mM EGTA in the Ringer), the results with altered external Ca suggest that the Li effect simply is not responsive to external Ca levels. Mn, Pr and La in the Ringer would be expected to block Ca influx although their specific effect on Ca entering during the action potential could be less than that on other mechanisms of Ca influx. We thus have no evidence to support a specific role for Ca in the Li effect, but neither have we good evidence against Ca involvement.

Several mechanisms for the clinical effects of Li in the treatment of manic depressive illness have been proposed, although they are mostly speculative. The results presented here provide a possible basis for an alternative mechanism. If Li is passively distributed between the inside and outside of the neurones of patients one would expect an elevated intracellular concentration with respect to serum levels. Patients are maintained at serum Li levels of about 1 mM during prophylactic treatment so a passively distributed intracellular concentration (by the Nernst equation) could be over 10 mM (although there is no evidence that such levels actually occur). We have shown that at intracellular Li^{+} levels as low as 1 or 2 mM there is an increase in P_K. The effect of this would be to increase the membrane potential slightly and to stabilize the cell, reducing both depolarizing and hyperpolarizing swings of the membrane potential.

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REFERENCES

ALNAES, E. & RAHAMIMOFF, R. (1974). Dual action of praseodymium on transmitter release at the frog neuromuscular synapse. *Nature, Lond.* **247**, 478–479.

BAKER, P. F. (1972). Transport and metabolism of Ca ions in nerve. *Prog. Biophys. molec. Biol.* **24**, 177–223.

CARMELIET, E. E. (1964). Influence of Li ons on the transmembrane potential and cation content of cardiac cells. *J. gen. Physiol.* **47**, 501–530.

GORMAN, A. L. F. & MARMOR, M. F. (1974). Long-term effect of ouabain and sodium pump inhibition on a neuronal membrane. *J. Physiol.* **242**, 49–60.

HILLE, B. (1970). Ionic channels in nerve membranes. *Prog. Biophys. molec. Biol.* **21**, 1–32.

KEYNES, R. D. & SWAN, R. C. (1959). The permeability of frog muscle fibres to lithium ions. *J. Physiol.* **147**, 626–638.

MALETZKY, B. & BLACHLY, P. H. (1971). *The Use of Lithium in Psychiatry.* London: Chemical Rubber Company.

MAYER, C. J., VAN BREEMAN, C. & CASTEELS, R. (1972). The action of lanthanum and D600 on the calcium exchange in the smooth muscle cells of the guinea-pig *taenia coli*. *Pflügers Arch. ges. Physiol.* **337**, 333–350.

MEECH, R. W. (1972). Intracellular calcium injection causes increased potassium conductance in *Aplysia* nerve cells. *Comp. Biochem. Physiol.* **42A**, 493–499.

MEECH, R. W. (1974). The sensitivity of *Helix aspersa* neurones to injected calcium ions. *J. Physiol.* **237**, 259–277.

MELA, L. (1968). Interactions of La^{3+} and local anaesthetic drugs with mitochondrial Ca^{2+} and Mn^{2+} uptake. *Archs Biochem. Biophys.* **123**, 286.

MORETON, R. B. (1968). An application of the constant-field theory to the behaviour of giant neurones of the snail *Helix aspersa*. *J. exp. Biol.* **48**, 611–623.

NEILD, T. O. & THOMAS, R. C. (1974). Intracellular chloride activity and the effects of acetylcholine in snail neurones. *J. Physiol.* **242**, 453–470.

PARTRIDGE, L. D. & THOMAS, R. C. (1974). The effect of intracellular Lithium on snail neurones. *Nature, Lond.* **249**, 578–580.

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

THOMAS, R. C. (1968). Measurement of current produced by the sodium pump in a snail neurone. *J. Physiol.* **195**, 23–24*P*.

THOMAS, R. C. (1969). Membrane current and intracellular sodium changes in a snail neurone during extrusion of injected sodium. *J. Physiol.* **201**, 495–514.

THOMAS, R. C. (1972). Intracellular sodium activity and the sodium pump in snail neurones. *J. Physiol.* **220**, 55–71.

YONEMURA, K. & SATO, M. (1967). The resting membrane potential and cation movement in frog muscle fibres after exposure to lithium ions. *Jap. J. Physiol.* **17**, 678–697.