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TRANSPORT OF LITHIUM AND RECTIFICATION BY FROG SKIN*

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SUMMARY

The isolated frog skin, bathed with Li^+-Ringer (Na^+-free) on the outside and Na^+-Ringer on the inside, can maintain a normal potential difference (PD) and short-circuit current (s.c.c.) for more than 6 h. The s.c.c. corresponded to the Li^+ influx. The Na^+ efflux was 4% of the s.c.c. 10^{-5} M ouabain depressed Li^+ influx and s.c.c. 10^{-5} M amiloride abolished the Li^+ s.c.c., while 0.1 unit/ml oxytocin stimulated it. When the inside of the skin was bathed with Li^+-Ringer, PD and s.c.c. fell to zero within 2 h. The oxygen consumption of skin slices bathed in Li^+-Ringer was 29% lower than controls bathed in Na^+-Ringer.

When the isolated frog skin is bathed in Na_2SO_4-Ringer it shows electrical rectification which has been correlated with the active transport of Na^+. In skins transporting Li^+, rectification characteristics are similar to those of skins transporting Na^+. When the inner face of the skin is bathed with Li^+-Ringer, rectification, PD and s.c.c. decline in a parallel fashion.

It is concluded that: (1) Li^+ can be transported when Na^+ is present at the inner face. (2) Amiloride, ouabain and oxytocin affect Li^+ and Na^+ transport in a similar manner. (3) Li^+ transport, like Na^+ transport, is associated with rectification. (4) Active transport of Na^+ and Li^+ seems to depend on two different but associated processes: one taking place at the external barrier (where rectification occurs) as shown by the effect of amiloride; and the other at an inner site related to energy requirements and affected by ouabain and Li^+. (5) The cation being transported is not necessarily activating the (Na^+–K^+)-ATPase.

INTRODUCTION

To study the degree of specificity of the mechanism of Na^+ transport by frog skin, Li^+ has been used as a replacing ion. In experiments in which both faces of the skin were bathed by solutions containing various concentrations of Li^+ and Na^+, Zerahn[1] showed that although Li^+ had a depressant effect on the short-circuit current (s.c.c.); it competed with Na^+ for the generation of the s.c.c. He indicated

Abbreviations: PD, potential difference; s.c.c., short-circuit current.

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that Li^+ can be transported against an electrochemical gradient for a short period of time when present in the outside solution in small concentration.

Recently, Biber and Curran[2] studied Na^+ and Li^+ fluxes across the outer barrier of the frog skin. Their experiments, and previous ones[3–5], suggested that Na^+ entry into the skin is not by free diffusion alone. Biber and Curran[2] showed both competitive inhibition of Na^+ flux by Li^+, and competitive inhibition of Li^+ flux by Na^+ in the outer barrier entry step. They proposed an interaction of both cations, with a membrane component.

This evidence indicates that Li^+ and Na^+ can selectively permeate the outside barrier of the skin and thus be available for transport by the pump. However, the question remains as to why the s.c.c. is rapidly depressed when the skin is bathed with Li^+ on both surfaces. In preliminary experiments we have found that the s.c.c. was only depressed when Li^+ was present in the inside solution.

Among the various models for Na^+ transport in frog skin, Candia[6] proposed one in which electrical rectification was closely associated with active Na^+ transport. To be consistent with this model Li^+ transport must be associated with rectification. Therefore, Li^+ could provide an additional means to study the association between active ionic transport and rectification. Experiments reported in this paper were designed for the following purposes: (a) To determine whether Li^+ can be actively transported by the frog skin, for a prolonged period of time. (b) To study the relationship between rectification and the transport of Li^+. (c) To study the effects of oxytocin, ouabain and amiloride on the Li^+-originated s.c.c. (d) To study the influence of Li^+ on oxygen consumption by skin slices.

MATERIALS AND METHODS

Rana pipiens kept in a container with water at room temperature (21–23 °C) were used. After pithing the animal the abdominal skin was removed, rinsed with Ringer's solution and mounted between the Ussing-type Lucite half chambers. The chambers used had several compartments which made it possible to simultaneously perform two or more experiments on the same skin (see ref. 6). The voltage and current bridges were made of 3% agar dissolved in the appropriate saline. Voltage bridges were connected to the recording equipment (Electrometer Model 200B, Keithley Instruments, Cleveland, Ohio; and recorder Model EU 20, Heath Co., Benton Harbor, Michigan) by means of calomel cells. The s.c.c. was measured with an automatic voltage-clamp system[7].

Solutions

The basic Ringer's solution (NaCl-Ringer) had the following composition (mM): NaCl, 103; KCl, 2.5; calcium gluconate, 1.8 and Tris–HCl, 2. The pH of the solution was 7.4. This saline was modified according to the experiment to obtain three additional solutions. In one type of solution NaCl was completely substituted by LiCl (LiCl-Ringer). For the determination of the resistance curves Cl^- was replaced by SO_4^{2-} (sucrose was added to keep the osmolarity constant) to make a Na_2SO_4 or a Li_2SO_4 Ringer's solution. A Na^+-free Ringer's solution was made by substituting the NaCl in the Ringer's solution by choline chloride or by an osmotically equivalent amount of sucrose.

Measurement of the Na^+ and Li^+ concentrations

Both cations were determined with a flame photometer (Eppendorf Model 700, Brinkmann Instruments, Westbury, N.Y.). Since in most cases Na^+ was measured in samples of Li^+-Ringer, and Li^+ in samples of Na^+-Ringer, special care was taken to avoid error due to interference by the other cation. The Na^+ standards were prepared by adding known amounts of Na^+ to the Li^+-Ringer and similarly, the Li^+ standards were made by adding known amounts of Li^+ to the Na^+ solution.

Measurements of Na^+ efflux

$^{22}Na^+$ (about 2 μCi/ml) was added to the solution bathing the inside face of the skin. After allowing 1 h for equilibration 3-ml samples were taken from the outside solution every 30 min, over a period of 3 h. The volume of the sample was replaced with non-radioactive solution. Specific activity in the labeled side was constant throughout the experiment, and the activity in the unlabeled side was 1% or less of that in the labeled compartment. The radioactivity was measured with a well scintillator detector and spectrometer.

Determination of the resistance curves

The technique for obtaining the I/V plots has been described in detail by Candia[6]. The resistance curve was determined by passing a slow current ramp, usually at a rate of about $8\,\mu A \cdot cm^{-2} \cdot s^{-1}$ through the skin, and was recorded on a chart recorder. The y axis indicated the potential difference (PD) of the skin, and the x axis indicated both time and current so that the slope of the trace was proportional to the skin resistance.

In this paper, the sign convention and current nomenclature described in a previous publication[6] will be used. The outside of the skin is considered as the reference side. Positive current circulating across the skin from the outside to the inside and having the effect of reducing the spontaneous PD of the skin is called depolarizing current. Conversely, current circulating through the skin from the inside to the outside increases the PD and is called hyperpolarizing current.

Determination of oxygen consumption

The abdominal skin was divided into two similar portions weighing between 150 mg and 300 mg each, which were then sliced into small pieces and placed in the incubation cell containing 5 ml of the Ringer's solution kept at a constant temperature of 25 °C. The solution in the cell was saturated with air and the decline in oxygen concentration was measured with an oxygen electrode connected to a polarographic circuit (Model YS 53, Yellow Springs Instrument Co., Yellow Springs, Ohio). The voltage output measured in the air-saturated solution was considered as 100%, and the oxygen consumption was determined between 100% and 80%. The recorder calibration was adjusted so that this range in oxygen tension (100% to 80%) gave a full scale deflection. The oxygen consumption was linear during the runs, which lasted about 20 min. The results are expressed as μl of oxygen (at standard temperature and pressure)/mg of wet tissue per h.

RESULTS

Short-circuit current in Li^+-Ringer

In these experiments the effect of replacing Na^+ by Li^+, in either the outside or inside solution, on the s.c.c. and PD was investigated. The experiments were started with the skin bathed with NaCl-Ringer on both sides. After the PD and s.c.c. were stable for at least 45 min, the inside or outside solution was replaced by the LiCl-Ringer.

When the inside face of the skin was bathed with LiCl-Ringer, a gradual decline of the PD and s.c.c. was observed. The inhibition was complete in about 90–120 min.

To test the action of Li^+ on the outside of the skin, the NaCl solution on that side was washed out with several volumes of a Na^+-free Ringer until the PD and s.c.c. declined to values near zero (Fig. 1). This was taken as an indication that the Na^+ had been effectively removed.

On several occasions Na^+ was added to the Na^+-free Ringer's solution to bring its concentration to 1 mM. This solution slightly stimulated the s.c.c., but it was always less than 10% of the s.c.c. recorded when the ordinary NaCl-Ringer was present.

Following this procedure, and about 2 min later, the Na^+-free solution in the outside was replaced by the LiCl-Ringer. This produced an immediate rise in the PD and s.c.c. to values similar to (or above) those previously recorded when Na^+ was the main cation present on both sides of the skin. Thereafter, the outside solution was completely replaced every 20 min with fresh LiCl-Ringer, and the Na^+ concentration determined in the solution just removed from the chamber. The Na^+ concentration was about 0.8 mM in the first sample and declined to about 0.3 mM during the following 90 min.

The s.c.c. reached a stable value generally 1 h after the change to LiCl-Ringer outside, and remained fairly constant for several hours. In experiments in which the s.c.c. was recorded for 6 h or more there was no indication that the decline observed was larger than that of the s.c.c. measured in ordinary NaCl-Ringer.

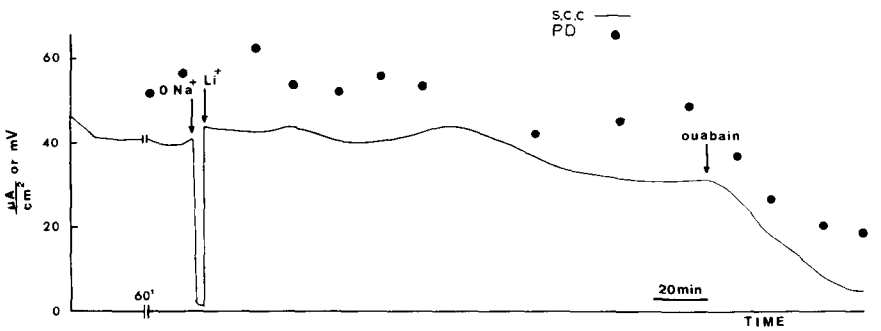

Fig. 1. Effect of the substitution of Na^+ by Li^+ in the outside solution on the s.c.c. The experiment was started with NaCl-Ringer on both sides. Once the s.c.c. was stable, Na^+-free Ringer was flushed (first arrow) to wash out the Na^+ in the outside and the s.c.c. fell to near zero. Then LiCl-Ringer was applied (second arrow). 10^{-5} M ouabain (third arrow) depressed the s.c.c. The LiCl-Ringer was renewed every 20 min to keep a very low (< 0.8 mM) Na^+ concentration in the outside Ringer.

In 15 skins the mean values of the stable s.c.c. were 29.2 ± 3.2 $\mu A \cdot cm^{-2}$ with NaCl-Ringer outside and 26.5 ± 3.3 $\mu A \cdot cm^{-2}$ with LiCl-Ringer outside. Paired comparison of individual values shows a statistically significant difference ($P < 0.005$).

Unidirectional fluxes of Li^+ and Na^+

The Li^+ influx, s.c.c. and Na^+ efflux were measured in the same skin according to the following protocol. The skins were short-circuited throughout the experiment and initially bathed in NaCl-Ringer. After the s.c.c. was stable for about 1 h, the outside was rinsed with a Na^+-free Ringer and then bathed with a LiCl-Ringer, as explained in the preceding section. This solution was renewed every 30 min for the rest of the experiment to ensure that the Na^+ concentration was negligible (maximum 0.8 mM). During 4–6 periods of 30 min, samples were taken from the inside to determine Li^+ influx by flame photometry. After taking the last sample for the Li^+ flux measurement, $^{22}Na^+$ was added to the inside solution and Na^+ efflux was determined by sampling from the outside during four periods of 30 min. The outside solution was then changed to NaCl-Ringer and the Na^+ efflux continued to be measured for four additional periods.

Table I shows the results of simultaneous measurements of s.c.c. and Li^+ influx in 10 skins bathed with LiCl-Ringer on the outside. The mean values of both parameters are similar. Moreover, the correlation between Li^+ influx and s.c.c. for the data in Table I is 0.78.

TABLE I

AVERAGE Li^+ INFLUX AND s.c.c. ACROSS THE FROG SKIN

Outside solution, LiCl-Ringer; inside solution, NaCl-Ringer. Mean values ±S.E. are given. Numbers in parentheses indicate number of experiments.

Li^+ influx ($\mu A \cdot cm^{-2}$)	*s.c.c. ($\mu A \cdot cm^{-2}$)*
27.4 ± 3.24 (10)	24.8 ± 3.57 (10)
Average Li^+ influx − s.c.c. (from paired data) =	2.57 ± 2.31 ($P > 0.1$)

TABLE II

AVERAGE Na^+ EFFLUX MEASURED IN FROG SKINS BATHED ON THE OUTSIDE WITH LiCl-RINGER FIRST AND SUBSEQUENTLY WITH NaCl-RINGER

The inside of the skin was bathed with NaCl-Ringer. Mean values ±S.E. are given. Numbers in parentheses indicate number of experiments.

Na^+ efflux ($\mu equiv \cdot cm^{-2} \cdot h^{-1}$)	
LiCl-Ringer outside	*NaCl-Ringer outside*
0.090 ± 0.023 (8)	0.109 ± 0.027 (8)
Mean of differences from paired data =	0.019 ± 0.008 ($P < 0.025$)

The values of the Na^+ efflux from 8 skins are shown in Table II. As can be seen, the passive permeability of the skin to Na^+ and presumably to Li^+, was not greatly modified by the presence of Li^+ in the outside solution. Nevertheless, the small difference observed was statistically significant.

Since the contribution of Na^+ to the s.c.c. was insignificant, the Na^+ efflux was only about 4% of the Li^+ influx, and the Li^+ influx was very similar to the s.c.c., it is most probable that the s.c.c. reflects the movement of Li^+ across the skin.

Effect of oxytocin, ouabain and amiloride on the s.c.c. and of ouabain on Li^+ influx

The experiments above demonstrate that the skin generates a s.c.c. with LiCl-Ringer as the outside bathing solution, providing Na^+ is present in the inside solution. However, the unlikely possibility remained that the s.c.c. was not the result of an active transport, but of different Na^+ and Li^+ mobilities in the skin. However, if this were so, drugs such as oxytocin, ouabain and amiloride would not be expected to affect the s.c.c. thus generated.

With LiCl-Ringer on the outside, the average s.c.c. from 6 experiments was increased from 16.5 $\mu A \cdot cm^{-2}$ to 32.9 $\mu A \cdot cm^{-2}$ by oxytocin in a concentration of 0.1 unit/ml. The same oxytocin concentration produced a comparable stimulation of the s.c.c. with NaCl-Ringer on both sides. Amiloride (10^{-5} M) in the outside solution, completely abolished the Li^+ s.c.c.

Ouabain (10^{-5} M) reduced the Li^+ s.c.c. (Fig. 1). In five experiments, s.c.c. and Li^+ influx, measured simultaneously, were reduced in every case. Average values in $\mu A \cdot cm^{-2}$ were: control s.c.c. 29.7 ± 4.0; ouabain s.c.c., 10.5 ± 1.2; control Li influx, 36.0 ± 3.8; ouabain Li^+ influx, 17.6 ± 3.0.

Resistance curves; effects of oxytocin, ouabain and amiloride

In a previous publication[6] current–voltage curves were studied in the skin of *Rana catesbeiana*. Rectification was found and shown to be associated with Na^+ transport. This non-linearity of the resistance is usually masked by the shunt pathway of Cl^- and it is necessary to replace Cl^- by SO_4^{2-} in the Ringer's solution to fully expose it. In this study, resistance curves were obtained from isolated skins of *Rana pipiens* bathed with Na_2SO_4- or Li_2SO_4-Ringer's solutions. The effects of oxytocin, ouabain and amiloride were also investigated.

A typical I/V plot is shown in Fig. 2a, obtained from a skin bathed on both sides with Na_2SO_4-Ringer. As can be seen, the slope resistance (dV/dI) increases as the skin is hyperpolarized, indicating rectification. To have a measure of the rectification, the slope resistance was determined at two points: where the PD across the skin is 0 mV ($R_{s.c.c.}$), and at about 160 mV, inside solution positive, (R_h). The rectification was estimated from the relation $(R_h - R_{s.c.c.})/R_{s.c.c.}$, which we shall refer to as rectification index. Its value for the curve shown in Fig. 2a is 0.95.

It has been previously postulated that active transport and rectification are closely related[6], the records of Figs 2a and 2b supporting this point of view. When the Na_2SO_4-Ringer bathing both faces of the skin (Fig. 2a) was replaced by Li_2SO_4-Ringer, $R_{s.c.c.}$ and R_h were reduced by about 25% during the first minutes, and the PD and rectification index remained almost unchanged (Fig. 2b). After extending the time of incubation of the skin in the Li_2SO_4-Ringer, the PD gradually fell to zero, $R_{s.c.c.}$ and R_h increased, and the rectification disappeared. This is illustrated in the

records c and d of Fig. 2 obtained 100 and 160 min after replacing the Na^+ in the Ringer's solution by Li^+. It should be noted that when no active transport of ions exists (Fig. 2d), the I/V plot shows that the skin behaves as a linear resistor. Similar results were obtained in another group of 6 skins. When the skins were bathed only on the inside with Li_2SO_4-Ringer, the results were the same.

If the rectifying property of the skin is associated with the active transport of ions it is expected that in skins bathed with Li^+ outside and Na^+ on the inside (a condition in which, as shown above, a s.c.c. can be maintained for several hours) the resistance curve will show rectification.

In a group of 7 skins control I/V plots were obtained with Na_2SO_4-Ringer bathing both sides of the skins. The outside solution was then removed by several quick washouts with Na^+-free Ringer until the PD fell to near zero. In some ex-

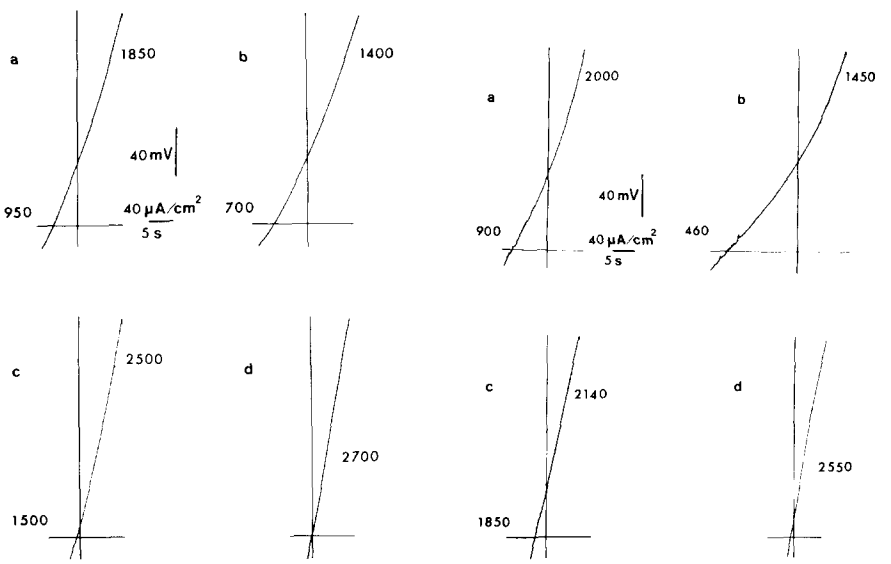

Fig. 2. I/V plots from the same frog skin showing the effect of substituting Na^+ by Li^+ in both sides. Ordinates: voltage across the skin. Abscissae: current and time. The numbers in the lower left of each curve indicate the value of the skin resistance ($\Omega \cdot cm^2$) when the PD was zero ($R_{s.c.c.}$). The numbers in the upper right corner indicate the value of R_h ($\Omega \cdot cm^2$), the resistance of the skin when it was hyperpolarized to about 160 mV. (a) Control resistance curve with the skin bathed on both sides with Na_2SO_4-Ringer. (b) Plot obtained 10 min after changing the bathing solution on both sides to Li_2SO_4-Ringer. Rectification still present. (c) 100 min after change of solution the PD, s.c.c. and rectification have decreased. (d) 160 min after change of solution the PD, s.c.c. and rectification have disappeared.

Fig. 3. Effect of drugs on rectification. I/V Plots are from the same skin. The numbers at the lower left and upper right on each curve correspond to the values of $R_{s.c.c.}$ and R_h, respectively. Skin bathed with Li_2SO_4-Ringer on the outside and Na_2SO_4-Ringer on the inside. (a) Control curve. The rectification index, $(R_h - R_{s.c.c.})/R_{s.c.c.}$, is 1.22. (b) 30 min after the addition of oxytocin (0.1 unit/ml). The PD and s.c.c. increased and the rectification index is 2.15. (c) 40 min after addition of ouabain (10^{-5} M). The PD and s.c.c. are reduced. Rectification index is 0.16. (d) 2 min after addition of amiloride (10^{-5} M). Rectification has disappeared; and PD and s.c.c. are very small.

periments resistance curves were then determined and no evidence of rectification was found. Following this preliminary step, intended to washout any remaining Na^+, Li_2SO_4-Ringer was applied to the outside of the skin and the PD recovered immediately. Resistance curves were then determined and compared to the previous controls. These results are summarized in Table III. There were no changes in the values of $R_{s.c.c.}$ and R_h, but the rectification index was significantly larger when Li^+ was the main cation in the outside solution ($P<0.01$). The effects of oxytocin, ouabain and amiloride were tested in 6 skins, and the results are illustrated in Fig. 3 and summarized in Table IV. Fig. 3a shows a resistance curve obtained in a skin bathed with Li_2SO_4-Ringer in the outside and Na_2SO_4-Ringer in the inside. Oxytocin (0.1 unit/ml) was added to the inside solution 30 min before the record shown in Fig. 3b was obtained. The PD increased from 72 mV to 82 mV, $R_{s.c.c.}$ and R_h were reduced by about 50% and 27%, respectively, and the rectification index increased from a control value of 1.22 to 2.15. Oxytocin was then washed out from the inside, and after the PD returned to normal, ouabain (10^{-5} M) was added to this solution. The PD began to decline steadily and about 45 min later $R_{s.c.c.}$ was almost doubled while R_h was practically unchanged. The rectification index was 0.16 (Fig. 3c). The addition of amiloride (10^{-5} M) to the outside solution immediately reduced the PD to 16 mV and the rectification index to zero.

The same type of experiments were carried out in 5 other skins bathed on both sides with Na_2SO_4-Ringer's solution, and similar results were obtained. They are shown in Table IV.

TABLE III

RESISTANCE (dV/dI) AND RECTIFICATION IN FROG SKINS BATHED ON THE OUTSIDE WITH Na_2SO_4-RINGER FIRST AND SUBSEQUENTLY WITH Li_2SO_4-RINGER

The inside of the skin was bathed with Na_2SO_4-Ringer. Rectification index $=(R_h - R_{s.c.c.})/R_{s.c.c.}$. Mean ± S.E. of differences from paired values of the rectification index: -0.11 ± 0.035 ($P<0.025$).

Na_2SO_4-Ringer outside			*Li_2SO_4-Ringer outside*		
$R_{s.c.c.}$ ($\Omega \cdot cm^2$)	R_h ($\Omega \cdot cm^2$)	*Rectification index*	$R_{s.c.c.}$ ($\Omega \cdot cm^2$)	R_h ($\Omega \cdot cm^2$)	*Rectification index*
1116	1542	0.38	1364	1820	0.33
656	1008	0.54	790	1358	0.72
792	1288	0.63	756	1392	0.84
904	1440	0.59	824	1344	0.63
560	1224	1.19	494	1142	1.31
752	1060	0.41	636	1012	0.59
586	1126	0.92	708	1428	1.02
$766 \pm 74^*$	$1241 \pm 74^*$	$0.67 \pm 0.11^*$	$796 \pm 103^*$	$1357 \pm 96^*$	$0.77 \pm 0.12^*$

* Mean ± S.E.

TABLE IV

EFFECTS OF OXYTOCIN, OUABAIN AND AMILORIDE ON RESISTANCE AND RECTIFICATION INDEX

(a) Differences between the paired values of the rectification index of: control and oxytocin, $-0.61 \pm 0.28^*$ ($P < 0.05$); control and ouabain, $0.67 \pm 0.13^*$ ($P < 0.005$); and control and amiloride, $0.78 \pm 0.17^*$ ($P < 0.005$). (b) Differences between paired values of the rectification index of: control and oxytocin. $-0.52 \pm 0.16^*$ ($P < 0.025$); control and ouabain, $0.71 \pm 0.19^*$ ($P < 0.025$); and control and amiloride, $0.86 \pm 0.21^*$ ($P < 0.025$).

	Control	*Oxytocin (0.1 unit/ml)*	*Ouabain (10^{-5} M)*	*Amiloride (10^{-5} M)*
(a) Skins bathed with Li_2SO_4-Ringer outside and Na_2SO_4-Ringer inside				
$R_{s.c.e.}^*$ ($\Omega \cdot cm^2$)	730 ± 70	400 ± 42	1466 ± 246	1610 ± 274
R_h^* ($\Omega \cdot cm^2$)	1324 ± 196	988 ± 182	1782 ± 484	1774 ± 280
Rectification index*	0.81 ± 0.16	1.41 ± 0.26	0.15 ± 0.13	0.04 ± 0.04
(b) Skins bathed on both sides with Na_2SO_4-Ringer				
$R_{s.c.e.}^*$ ($\Omega \cdot cm^2$)	446 ± 75	254 ± 40	988 ± 228	1128 ± 293
R_h^* ($\Omega \cdot cm^2$)	811 ± 138	616 ± 148	1140 ± 319	1128 ± 293
Rectification index*	0.86 ± 0.21	1.38 ± 0.24	0.15 ± 0.07	0

* Mean $\pm$S.E.

Oxygen consumption by skin slices

The rate of oxygen consumption by skin slices bathed in Li_2SO_4 and Na_2SO_4 solutions was measured. The effect of ouabain on the oxygen consumption in each of these conditions was also determined. The usual procedure was to divide a piece of abdominal skin into two symmetrical portions that were then sliced into small bits. One portion was placed in the Na_2SO_4-Ringer, the other in the Li_2SO_4-Ringer, and the rate of oxygen consumption determined. Solutions were then switched so that the portion of the skin originally in the Na_2SO_4 was placed in Li_2SO_4 and *vice versa*. In some experiments this procedure was repeated twice (three determinations of oxygen consumption were made every time the solution bathing the slices was changed or a drug was added). Thereafter, ouabain was added to both solutions to obtain a concentration $5 \cdot 10^{-4}$ M and the rate of oxygen consumption was again determined for as long as 180 min after the addition of ouabain.

In all cases, oxygen consumption by slices bathed in Li_2SO_4-Ringer was about 29% lower than when the same slices were bathed in Na_2SO_4-Ringer. The depression produced by Li^+ was reversible. Mean value $\pm$S.E. from 9 experiments of oxygen consumption in Na_2SO_4-Ringer was 0.154 ± 0.006 μl/h per mg of wet weight, while the oxygen consumption in Li_2SO_4-Ringer was 0.109 ± 0.004 μl/h per mg of wet weight. Ouabain depressed the oxygen consumption in Na_2SO_4-Ringer to 0.114 ± 0.004 μl/h per mg of wet weight, but did not significantly affect the oxygen consumption of the slices bathed in Li_2SO_4-Ringer. The degree of inhibition produced on the normal rate of oxygen consumption by ouabain and Li^+ was remarkably similar. Furthermore, ouabain did not modify the rate of oxygen consumption that was already depressed by Li^+-Ringer.

DISCUSSION

We have found that when Li^+ is present at the outer surface of the frog skin it can be actively transported for a prolonged period of time. However, the isolated frog skin[1] and the toad bladder[8,9] cannot maintain a normal s.c.c. when Li^+ is added to the Ringer bathing both sides of these membranes. No clear explanation of this inhibitory effect of Li^+ on s.c.c. has been reported. These earlier studies have provided some evidence indicating that Li^+ can be actively transported. For example, Zerahn[1] indicated that frog skin has the ability to move Li^+ against an electrochemical gradient, and Biber and Curran[2] showed that Na^+ and Li^+ interact with a membrane component at the entry step in the outer barrier of the epithelium. They further suggested that this process may represent an active step.

In the toad urinary bladder, Li^+ reduces the s.c.c. when present at either the mucosal or serosal side[8,9]. As in frog skin, the bladder can also transport Li^+ actively but only for short periods of time[8]. This transport of Li^+ in the toad urinary bladder is inhibited by ouabain[8] and blocked by amiloride[10].

Lindley and Hoshiko[11] have found that Li^+ can mimic Na^+ in the outside border better than Rb^+, K^+ or Cs^+ in its ability to maintain a PD. However, they also reported that "a skin with Li^+ in the outside sometimes deteriorates with time". They failed to see any "poisoning" with Li^+ inside, which may be due to the fact that they used a preparation in open circuit.

Opposite to that, we found that the replacement of Na^+ by Li^+ in the inside solution has a depressant effect on PD and s.c.c. However, a normal s.c.c. could be maintained for several hours when Li^+-Ringer was only placed on the outside of the skin, leaving Na^+-Ringer as the inside solution. Several interpretations of this result are possible. Li^+ from the outside solution may be replacing the Na^+ in the epithelial cells, while the latter is transported by the pump at the inner barrier. The Na^+ transport pool has been estimated between 0.07 and 0.17 $\mu equiv \cdot cm^{-2}$ of skin[5,12–14]; this amount of Na^+ could not maintain a s.c.c. of 26.5 $\mu A \cdot cm^{-2}$ for more than 11 min. Another alternative is that Li^+ is being accumulated while K^+ leaks out from the epithelial cells to the inside solution. The amount of K^+ in the skin is 1 $\mu equiv \cdot cm^{-2}$ (ref. 15). Again, this amount cannot maintain the observed s.c.c. for more than 1 h. Accumulation of Li^+ in the epithelial cells is also below the required level. Hansen and Zerahn[16] reported this to be in the order of 15 $\mu equiv/g$ or 0.075 $\mu equiv \cdot cm^2$. Therefore, it is logical to assume that a transepithelial movement of Li^+ from the outside to the inside solution is creating the PD and associated s.c.c. This was confirmed by the agreement between the Li^+ influx and the s.c.c. Furthermore, the Na^+ efflux was only 4% of the s.c.c. whether Li^+ or Na^+ was present in the outside solution, indicating that the passive permeability of the skin was not altered by Li^+. The movement of Li^+ was always "downhill", and it is possible (if the permeability of the skin to the Li^+ influx was 20–25 times larger than to the Na^+ efflux) that the s.c.c. could be produced by the difference between two entirely passive fluxes. Evidence from several experiments is against this possibility. With Li^+ in the outside, replacement of Na^+ by Li^+ in the inside solution did not immediately suppress the s.c.c. but only after 90–120 min. Na^+ in the outside and Li^+ in the inside did not reverse the s.c.c. The effects of ouabain on the Li^+ influx and of amiloride and oxytocin on the Li^+-originated s.c.c. are against a passive movement

of Li^+ and support the view that the Li^+ influx occurs through the same active channels utilized for the transport of Na^+.

The rectification curves with Li^+ outside are similar to those described by Candia[6] in the presence of Na^+. Furthermore, the effects of oxytocin, amiloride and ouabain confirmed the relation between rectification and active transport.

When Li^+ was used to replace Na^+ in the inside bathing solution, PD and s.c.c. were completely depressed in about 90–120 min. Simultaneously, and in a parallel fashion, the rectification index declined, whereas the resistance of the skin increased. This inhibitory effect of Li^+ resembles very much that of ouabain, and suggests a similar mode of action.

The normal rate of oxygen consumption in Na_2SO_4-Ringer was depressed when the skin slices were transferred to Li_2SO_4-Ringer. This inhibition was not further increased when ouabain was added to the Ringer. The inhibition by Li^+ was similar to that produced by ouabain when added to the control in Na_2SO_4-Ringer. These results suggest that both ouabain and Li^+ inhibit the same enzyme system, namely (Na^+–K^+)-ATPase, interfering with the supply of energy to the pump.

Results from this paper could be analyzed in the light of the model proposed in a previous publication[6] suggesting the existence of a two-step mechanism for active ion transport. The first step of this process is associated with electrical rectification. The second step requires an energy source, most likely the (Na^+–K^+)-ATPase system. It has been shown that Li^+ can permeate the outside barrier in a fashion similar to Na^+ (refs 2, 16) and would be, in principle, available for the translocation across the inner barrier. The second step would require the activation of the (Na^+–K^+)-ATPase system and we will assume that Li^+ cannot replace Na^+ in this function. This assumption is based on preliminary observations indicating that ATPase obtained from frog skin epithelium is activated by Na^+–K^+ but not by Li^+ or Li^+–K^+ (Siegel, G., personal communication). ATPase activity has been found in the inward facing membranes of frog skin[17]. Therefore, Na^+ may be needed at the inside barrier for the active translocation of an ion.

The energy derived from the (Na^+–K^+)-ATPase activity would be utilized by an undefined mechanism to translocate Li^+ or Na^+ across the inner barrier.

Li^+ present in the inside solution would prevent the activation of the enzyme, and neither Na^+ or Li^+ present in the outside solution could then be transported. When Li^+ is present only in the outside solution, and is being transported, the concentration in the epithelial cells is less than 10–20 mM[16]. This concentration apparently is either in a compartment unrelated to the (Na^+–K^+)-ATPase, or not enough to prevent the activation of the (Na^+–K^+)-ATPase by the Na^+ leaking in from the inside solution. The fact that in toad bladder Li^+ has an inhibitory effect from either serosal or mucosal solution[8,9] can be explained assuming that the (Na^+–K^+)-ATPase is this tissue is freely accessible from either side.

This interpretation of the mechanism of Na^+ and Li^+ transport is highly hypothetical, and requires further investigation for its confirmation.

We feel that we have clearly separated the dual behavior of Li^+ as an ion that can both inhibit the pump and be effectively transported. We have also further confirmed the close association between ionic transport and electrical rectification by frog skin.

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