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# Electrophysiologic Changes Associated with Potassium Depletion of Frog Skin

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**Summary.** Skins from the frog *Rana pipiens pipiens* were studied under short-circuited conditions during the course of removing and replacing potassium in the inner bathing media in 14 experiments. The intracellular potential ($V_{SC}$), fractional resistance ($FR$), short-circuit current ($I_{SC}$) and total tissue conductance ($g_T$) were constantly monitored during impalements of the epithelial cells. The mean value ($\pm$SE) for $V_{SC}$ was $-79$ ($\pm 3$) mV under baseline conditions. Removal of potassium from the inner bathing solution transiently stimulated the short-circuit current and hyperpolarized the basolateral membrane; with sufficiently long incubations, the basolateral membrane was eventually depolarized. Restoration of potassium to the inner solution within 43 min after initiating the perfusion with $K^+$-free solution depolarized the basolateral membrane. However, restoration of potassium after at least $1\frac{1}{2}$ hr of incubation hyperpolarized the membrane. Ouabain consistently depolarized the basolateral membrane, even after extended periods of potassium depletion as long as 320 min. In the presence of ouabain, restoration of potassium depolarized the basolateral membrane. The data provide further evidence for the concept that the Na – K exchange pump of frog skin is rheogenic. Furthermore, the results suggest that the pump continues to be active even during prolonged periods of potassium depletion, reaccumulating potassium which has leaked out of the epithelial cells.

Transcellular sodium movement is thought to proceed in two steps. Sodium enters from the mucosal or outer bathing solution into the epithelial cells down its electrochemical gradient. Sodium is thought to be subsequently extruded across the basolateral membranes of the transporting cells by the sodium-potassium exchange pump. In the classical formulation of Koefoed-Johnsen and Ussing (1958), the extrusion of sodium was considered to be stoichiometrically linked to potassium accumulation into the cell from the submucosal or inner bathing medium. Although a number of published data have supported this concept (Biber, Aceves & Mandel, 1972; Finn & Nellans, 1972; Nielsen, 1979), many other experimental results have suggested that coupling between potassium uptake and sodium extrusion is not fixed (Essig & Leaf, 1963; Curran & Cereijido, 1965; Essig, 1965; Biber et al., 1972; Candia & Zadunaisky, 1972; Giebisch, Sullivan & Whittembury, 1973; Nellans & Schultz, 1976; Robinson & Macknight, 1976*b*; Kimura & Fujimoto, 1977; Kimura, Urakabe, Yuasa, Miki, Takamitsu, Orita & Abe, 1977; Valenzeno & Hoshiko, 1977; DeLong & Civan, 1978; Nagel, 1980).

A particularly striking example of the apparent dissociation of cellular potassium accumulation from net sodium transport was provided by the different time courses of intracellular potassium activity and short-circuit current across toad urinary bladder following restoration of external potassium to potassium-depleted tissues (DeLong & Civan, 1978). The short-circuit current began to increase only 20–40 min after the intracellular potassium activity began to return to its baseline value. Taken together with previously published estimates of the changes in total ionic contents induced by potassium depletion (Robinson & Macknight, 1976*a*), these data led to the suggestion that potassium might be accumulated by a sodium-independent mechanism. Recent electron-microprobe analysis of toad bladder is in qualitative agreement with previous estimates based on chemical analysis of mucosal cell scrapings, but indicates that

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more sodium is actually gained by the transporting epithelial cells than had previously been estimated; the accumulation of sodium and loss of potassium are comparable in magnitude (Civan, Hall & Gupta, 1980). Thus, given the uncertainties of the experimental techniques, and the possibility that the stoichiometry of the pump might undergo modest changes even under physiologic conditions, the dissociation between the time courses of intracellular potassium activity and short-circuit current noted by DeLong and Civan (1978) might be consistent with the operation of a single Na – K rheogenic pump at the basolateral membrane.

In order to examine this possibility further, it would be helpful to monitor the difference in electrical potential across the basolateral membrane during the course of removing and subsequently restoring potassium to the serosal medium bathing toad urinary bladder. Unfortunately, this has not been found to be technically feasible in toad bladder (Higgins, Gebler & Frömter, 1977; DeLong & Civan, 1979, 1980), presumably because of the shallow depth of the mucosal epithelium and the activity of the submucosal bundles of smooth muscle. For this reason, we have turned to the frog skin, a preparation whose transport properties (Ussing, 1960) are similar to those of toad bladder (Leaf, 1965), but which is far more favorable for intracellular impalement (Nagel, 1976, 1980; Helman & Fisher, 1977; Helman, Nagel & Fisher, 1979).

## Materials and Methods

*Animals and Chamber*

Abdominal skins were excised from doubly-pithed frogs *Rana pipiens pipiens* obtained from West Jersey Biological Supply (Wenonah, NJ) and mounted horizontally in a chamber previously described (Nagel, 1976). The inner surface of the skin was supported by a copper grid. Silicone rubber grease (High Vacuum Grease, Dow Corning Corp., Midland MI) was applied, in order to reduce edge damage. A cross-sectional area of 0.4 $cm^2$ was exposed for study. A partial vacuum of some 60 cm water applied to the inner bathing medium immobilized the tissue. The volumes of inner and outer half-chambers were 0.2–0.3 ml. Perfusion of both surfaces of the preparation was driven by gravity without recirculation.

*Solutions and Chemicals*

The standard sodium Ringer's solution consisted of (mM): $Na^+$, 118.2; $K^+$, 3.4; $Ca^{++}$, 0.9; $Cl^-$, 117.2; $HCO_3^-$, 2.3; $HPO_4^{2-}$, 1.8; $H_2PO_4^-$, 0.3; the pH was 7.6–7.8 and osmolality 221–222 mosmol. The $K^+$-free Ringer's solution was similar, except for the equimolar replacement of $Na^+$ for $K^+$. Ringer's solutions containing higher concentrations of $K^+$ were prepared by equimolar replacements of $K^+$ for $Na^+$.

Amiloride was generously provided by Dr. George M. Fanelli, Jr. (Merck Institute for Therapeutic Research, West Point, PA).

*Electronics*

In most of the experiments, the skins were short-circuited by means of an automatic clamping circuit (W. Nagel, *in preparation*). At regular intervals, the transepithelial potential ($\Delta V$) was increased to 20 mV (inside positive to outside) for periods of 600 msec. The transepithelial current measured 200 msec after the onset of the pulse was compared with the current just prior to the pulse in order to calculate total tissue conductance. The fractional resistance (*FR*), defined as the voltage deflection observed between exploring micropipette and outer reference solution, divided by the voltage deflection across the entire tissue, was also determined automatically at this time. The transepithelial current ($I_{SC}$), transepithelial conductance ($g_T$), electrical potential of the exploring micropipette ($V_{SC}$) and fractional resistance were continuously displayed on two two-channel pen recorders.

In one experiment (Exp. XI, Table 1), skins were short-circuited (Yonath & Civan, 1971), and the transepithelial potential was increased to 10 mV for periods of 3–5 sec. In this case, $I_{SC}$, $\Delta V$ and $V_{SC}$ were the parameters recorded, while $g_T$ and *FR* were measured graphically from the tracings.

*Micropipettes*

Micropipettes were drawn from omega-dot borosilicate capillary glass tubing having an outer diameter of 1.5 mm and an inner diameter of 0.75 mm (Frederick Haer, Brunswick, ME). When filled with 3 M KCl solution, the resistances of the micropipettes were commonly 15–50 MΩ. In the presence of $K^+$-free solution, impalements were usually conducted with micropipettes having resistances in the higher range, as large as 120 MΩ. Use of micropipettes with higher resistance or micropipettes filled with 0.5 M KCl improved the success rate in obtaining stable prolonged, intracellular recordings. Micropipettes were mounted in a Lucite holder, and advanced with a step motor drive in increments of 1–3 μ. The resistance of each micropipette was monitored during the impalement by noting the voltage responses to passage of pulses of constant current, usually of 0.45 nA. Intracellular impalements were considered technically satisfactory only if: (1) abrupt changes in $V_{SC}$ were noted on entering and leaving a cell, (2) the $V_{SC}$ recorded within a cell was stable for at least 1 minute, and (3) upon withdrawing the micropipette from the cell, $V_{SC}$ returned to within 3 mV of the extracellular potential observed before impalement.

In acceptable impalements, the resistance of the micropipette was usually little changed upon entering the cell. When a substantial increase in resistance was noted, the micropipette was always retracted slightly, almost always resulting in a return of the resistance towards its pre-impalement value, with little change in the recorded intracellular potential.

## Results

The mean value of the intracellular potential obtained from acceptable impalements under baseline conditions in 14 skins from 14 animals was $-79 \pm 3$ mV; the uncertainties presented for this and subsequent averages are the standard errors of the mean. It was frequently possible to obtain intracellular recordings from single cells for prolonged periods of time, as long as an hour and a half in the present study.

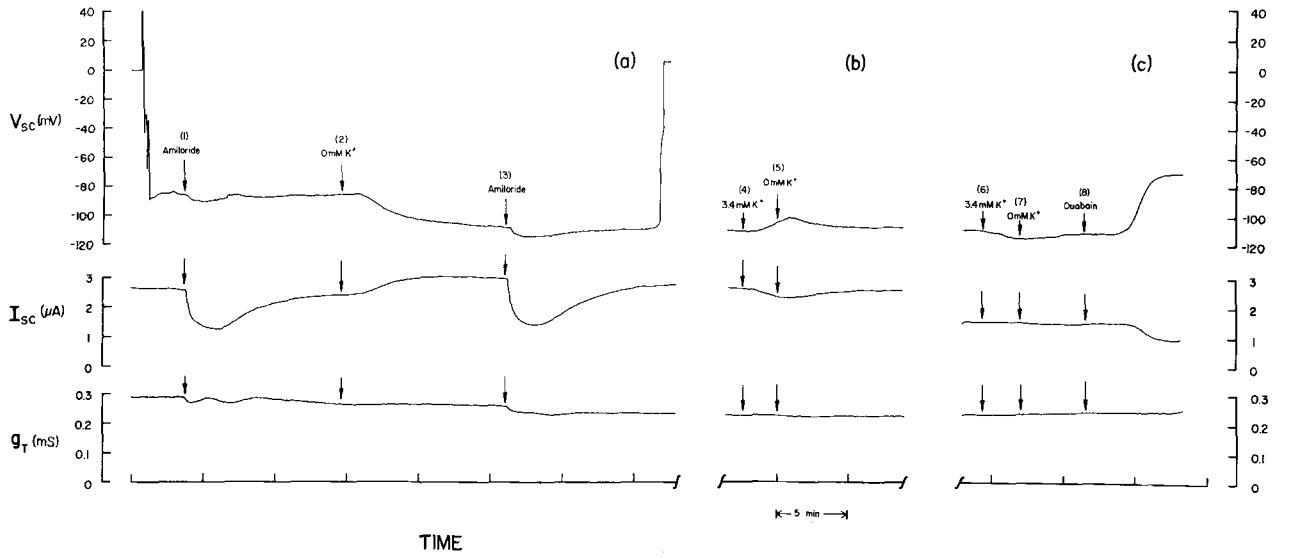


**Fig. 1.** Effects of withdrawing potassium and subsequently restoring potassium to the inner bathing medium. Panels (*a*), (*b*) and (*c*) were obtained during 3 separate intracellular penetrations from the same frog skin (Exp. VIII, Table 1). At the arrows marked (1) and (3), $10^{-5}$ M amiloride was added to the outer perfusing solution, hyperpolarizing the intracellular potential and increasing the apical fractional resistance from 0.92 to 0.96 and from 0.98 to 0.99, respectively. After each application of amiloride noted in Figs. 1 and 3, the diuretic was always washed off before initiating the next experimental manipulation. Removal of potassium from the inner perfusing solution at (2) led to a hyperpolarization of $V_{SC}$ of 23 mV and a transient stimulation of the short-circuit current. These effects were reversed [at arrow (4)] by restoring 3.4 mM potassium to the inner medium 39 min after potassium withdrawal; the removal of potassium at (5) once again hyperpolarized $V_{SC}$ and increased $I_{SC}$. When potassium was restored after 144 min of inner potassium depletion at (6), $V_{SC}$ was hyperpolarized by 4 mV, accompanied by very little change in $I_{SC}$. Addition of $2 \times 10^{-4}$ M ouabain to the inner perfusing solution substantially depolarized $V_{SC}$ even after extended periods of potassium deprivation, whether or not the potassium had been transiently restored to the inner medium, as here during the interval between (6) and (7). A biphasic response in tissue conductance to addition of amiloride, as noted after arrow (1), was occasionally observed, and is unexplained

Under these conditions, transient perfusion of the outer skin surfaces with Ringer's solution containing $10^{-5}$ M amiloride reduced the short-circuit current and tissue conductance, while hyperpolarizing the membrane potential and increasing the fractional resistance across the apical membrane (Figs. 1 and 3, Table 1).

The approximate degree of amiloride-induced membrane hyperpolarization expected could be calculated from the baseline value of the fractional resistance and the observed changes in short-circuit current and tissue conductance using the simple equivalent circuit of Fig. 2. The amiloride-sensitive transcellular pathway is indicated by the subscript *A*, while the paracellular pathway and amiloride-insensitive transcellular ionic pathway are lumped together within the loop symbolized by the subscript *p*. For example, at the arrow marked "(1) Amiloride" in Fig. 3, amiloride was noted to increase the fractional resistance from 0.94 to 0.98, while reducing $I_{SC}$ by 1.70 μA and $g_T$ by 0.015 mS. Using the circuit of Fig. 2, the membrane potential across the apical membrane would be expected to become more negative by about 6.8 mV, in reasonable agreement with the observed

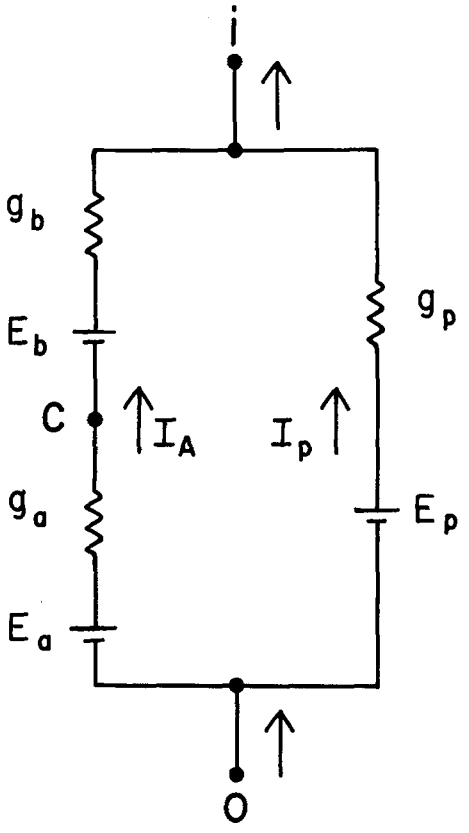


**Fig. 2.** Simple equivalent circuit of frog skin

**Table 1.** Absolute baseline values and changes noted following experimental manipulations

| Exp. | Baseline absolute values | | | | Changes following: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Amiloride | | $K^+$-Removal | | Restoration of $K^+$ | | Ouabain addition to $K^+$-depleted skin | Restoration of $K^+$ after ouabain addition to $K^+$-depleted skin |
| | | | | | | | Early | Late | Early | Late | | |
| | $V_{SC}$ (mv) | $FR$ | $I_{SC}$ (μA) | $g_T$ (mS) | $\Delta V_{SC}$ (mV) | $\Delta FR$ | $\Delta V_{SC}$ (mV) | $\Delta V_{SC}$ (mV) | $\Delta V_{SC}$ (mV) | $\Delta V_{SC}$ (mV) | $\Delta V_{SC}$ (mV) | $\Delta V_{SC}$ (mV) |
| I | −87 | 0.83 | 5.2 | 0.17 | — | — | − 3* | 5 | — | − 9 | — | — |
| II | −80 | 0.86 | 5.3 | 0.23 | −15 | 0.09 | −22 | 46 | — | — | — | — |
| III | −74 | 0.87 | 3.3 | 0.12 | — | — | −22 | 13# | — | − 8 | — | — |
| IV | −87 | 0.92 | 9.8 | 0.30 | − 8 | 0.03 | −20 | 9# | — | — | — | — |
| V | −84 | 0.95 | 5.2 | 0.24 | − 7 | 0.03 | −12 | − 7 | — | − 5 | 18 | 10 |
| VI | −89 | 0.96 | 4.3 | 0.20 | − 7 | 0.04 | −15 | — | — | — | — | — |
| VII | −77 | 0.91 | 3.1 | 0.22 | — | — | −23 | 17# | — | — | — | — |
| VIII | −86 | 0.93 | 2.5 | 0.28 | − 4 | 0.02 | −24 | −21 | 9 | − 4 | 41 | — |
| IX | −83 | 0.96 | 4.1 | 0.23 | − 3 | 0.01 | −30 | − 8 | — | −15 | 36 | 2 |
| X | −96 | 0.96 | 4.6 | 0.22 | − 6 | 0.02 | −21 | — | — | — | — | — |
| XI | −61 | 0.86 | 2.4 | 0.70 | − 5 | 0.03 | −12 | 6 | 12 | − 5 | — | — |
| XII | −70 | 0.89 | 9.7 | 0.31 | −10 | 0.07 | −21 | 10 | — | — | — | — |
| XIII | −60 | 0.82 | 5.0 | 0.12 | −26 | 0.17 | −46 | 21 | — | — | 5 | — |
| XIV | −73 | 0.79 | 10.8 | 0.29 | −20 | 0.12 | −22 | 9# | — | −25 | 17 | — |
| Mean | −79 | 0.89 | 5.4 | 0.26 | −10 | 0.06 | −21 | 8 | 10 | −10 | 23 | 6 |
| ±SE | ±2.8 | ±0.015 | ±0.73 | ±0.037 | ±2.2 | ±0.015 | ±2.6 | ± 4.8 | — | ± 2.8 | ± 6.6 | — |
| $N$ | 14 | 14 | 14 | 14 | 11 | 11 | 14 | 12 | 2 | 7 | 5 | 2 |

The term "early" refers to measurements made no later than 49 min after withdrawing potassium from the inner medium. "Late" refers to measurements made after potassium depletion for at least 1 hr. The symbol # indicates that the measurements were obtained after potassium depletions lasting at least 3 hr; after this time, the basolateral membrane was always found to be depolarized with respect to its baseline value. The asterisk entered for Exp. I indicates that the micropipette slipped out of the cell while the membrane potential was still changing following removal of potassium from the inner bath; the true hyperpolarization for this experiment was therefore in excess of the 3 mV observed. $N$ is the number of experiments in which each measurement of interest was made

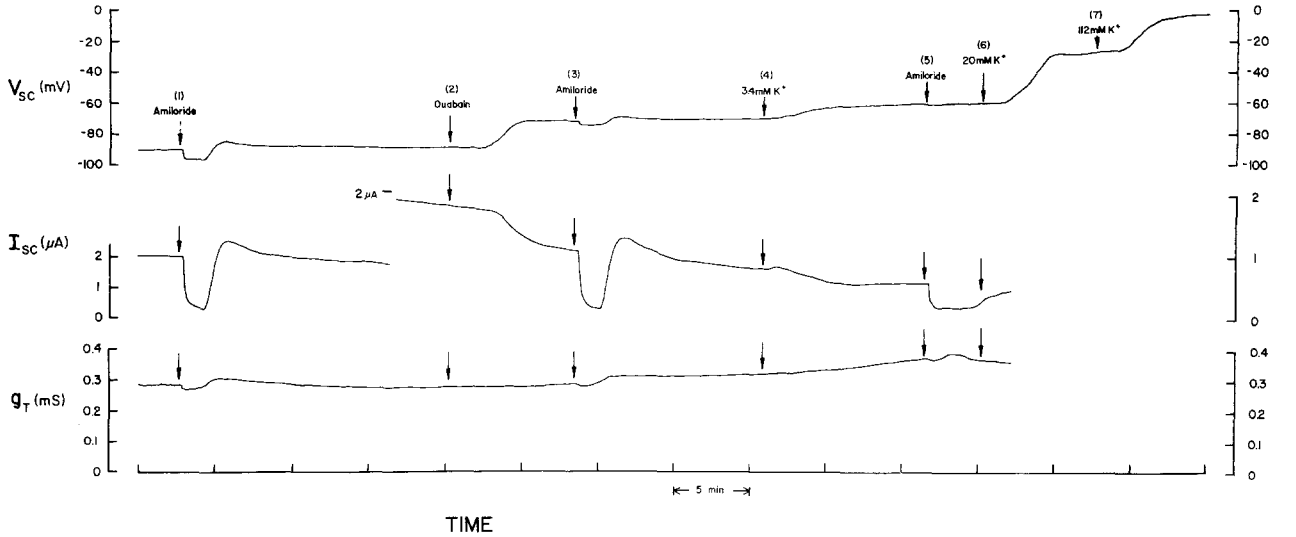


**Fig. 3.** Effects of restoring potassium to a potassium-depleted preparation following inhibition with ouabain. The entire record was obtained during the course of a single impalement (Exp. V, Table 1). At (1), (3) and (5), $10^{-5}$ M amiloride was applied to the outer surface of the skin. Ouabain was applied to the inner surface at a concentration of $2\times10^{-4}$ M at (2), 123 min following removal of potassium from the inner medium. Ouabain depolarized the basolateral membrane by 18 mV. In the presence of ouabain, addition of increasing amounts of potassium to the inner medium at (4), (6) and (7) resulted in increasingly large depolarizations of $V_{SC}$. Large transient increases in $I_{SC}$ and $g_T$ were noted following application of the very high potassium concentrations; these changes are not readily interpretable, and have been omitted from the end of the record. Note that the scale was changed for $I_{SC}$ shortly before arrow (2)

value of 6.3 mV.[1] This degree of agreement, which was commonly observed, is not considered to constitute a rigorous test of the validity of the equivalent circuit. Rather, the agreement is considered simply to provide additional confidence that the recordings were obtained from truly intracellular sites.

Perfusion of the inner surface of frog skin with $K^+$-free Ringer's solution promptly hyperpolarized the basolateral membrane and stimulated the short-circuit current across the entire tissue in all 14 experiments (Fig. 1). However, after the transient period of stimulation, the short-circuit current continuously declined with time. The basolateral membrane remained hyperpolarized for more prolonged periods; in Exp. VIII of Table 1, hyperpolarization was retained for 151 min following removal of potassium from the inner membrane. On the other hand, with very prolonged incubation in $K^+$-free inner media for 3 hr or longer, the basolateral membrane was inevitably depolarized below the baseline value.

It would be expected that restoration of potassium to the inner bathing solution soon after potassium removal should simply reverse the early effects noted. This was found to be the case in two experiments where potassium was returned soon after the initial withdrawal (Fig. 1; Table 1). However, the effects were very different following return of external potassium to tissues perfused with $K^+$-free media over prolonged periods. Fig. 1 presents the results of such an experiment. From consideration of the simple ionic diffusional movements across the basolateral membrane alone, addition of potassium to the inner medium would be expected to depolarize the basolateral membrane, as had been noted at earlier times during the incubation. In fact, $V_{SC}$ became more negative in this and in each of the other six experiments where this point was examined (Table 1).

The effects induced by restoring potassium to potassium-depleted tissues were also examined under conditions where the Na−K exchange pump was blocked. Fig. 3 presents the effects of including $2\times10^{-4}$ M ouabain in the inner perfusate 123 min after removing potassium from the bath. As noted in all five experiments where this point was examined, ouabain promptly depolarized the basolateral membrane and further inhibited the short-circuit current (Table 1). The total period required for ouabain's action, from the time of the initial decline in membrane potential to its completion was 1.5–3.6 min (mean ± SE = 2.8 ± 0.5 min). When potassium was then added to the inner solution, in the presence of ouabain in two experiments, both the membrane potential and short-circuit current were further reduced (Table 1), as expected from consideration of the simple diffusional contributions to the total difference in electrical potential across the basolateral membrane (Fig. 3).

## Discussion

The intracellular potential in skin from the frog *Rana pipiens pipiens* has been found to be −79 ± 3 mV under short-circuited conditions. Although in reasonable agreement with other recently published estimates (Nagel, 1976, 1980; Helman & Fisher, 1977; Helman et al., 1979) such measurements are subject to a number of artifacts. For example, impalement-induced membrane damage and diffusion of concentrated saline out of the micropipette tip can change the state of the cell studied (Lassen, Nielsen, Pape & Simonsen, 1971; Lindemann, 1975; Higgins, Gebler & Frömter, 1977; Sudou & Hoshi, 1977; Nelson, Ehrenfeld & Lindemann, 1978). Such effects are likely to have played only minor roles in the present study. Not only were impalements subject to strict criteria for acceptance, but it was often possible to obtain a continuous recording from a single cell for prolonged periods of time before, during, and after the experimental period. Furthermore, the rate of leakage out of micropipettes similar to those used here has been recently found to be very small (Helman et al., 1979). However, insofar as the precise value of the intracellular tip potential can never be determined (Civan, 1978), measurements of intracellular electrical potential must be considered estimates of the true membrane potential. It should be emphasized that the major concern of the present study was to determine the changes in, rather than absolute baseline value of, membrane potential during the course of the experimental manipulations. It frequently proved possible to observe the changes in membrane potential induced by these manipulations while the micropipette tip was maintained within a single cell.

[1] In this calculation, the magnitude of the amiloride-induced reduction in tissue conductance ($\Delta g_T$) is taken to be $[(1/g_a)+(1/g_b)]^{-1}$. From Fig. 2 and the definition of the fractional resistance,

$$(1/g_b)=(1-FR)\,[(1/g_a)+(1/g_b)]=(1-FR)(1/\Delta g_T). \quad (1)$$

The reduction in total transepithelial current ($\Delta I_{SC}$) produced by amiloride is taken to equal $I_A$. Therefore, the addition of amiloride should, by abolishing $I_A$, hyperpolarize the basolateral membrane by an amount ($\Delta V_{SC}$) given by:

$$\Delta V_{SC}=I_A(1/g_b)=(\Delta I_{SC})(1/g_b). \quad (2)$$

From Eqs. (1) and (2),

$$\Delta V_{SC}=\Delta I_{SC}(1-FR)(1/\Delta g_T). \quad (3)$$

From the data cited in the text,

$$\Delta V_{SC}=-(1.70)(1-0.94)(1/0.015)=-6.8\text{ mV}. \quad (4)$$

The results of the present study demonstrate clearly that the difference in electrical potential across the basolateral membrane of the transporting epithelial cells consists of contributions both from diffusive ionic movement across the bulk plasma membrane and from the rheogenic Na−K exchange pump, supporting several lines of evidence already published (Helman et al., 1979; Nagel, 1980). Following extended periods of potassium depletion, stimulation of the pump by addition of potassium to the inner medium hyperpolarized the basolateral membrane by some 10 mV, while inhibition of the pump by addition of ouabain further depolarized the basolateral membrane by about 23 mV (Table 1). Thus, charge transfer through the pump contributes to the total membrane potential. In the presence of ouabain, increasing the potassium concentration of the inner medium further depolarized the membrane potential, indicating that charge transfer through the transmembrane pathways in parallel with the pump also contributes to the membrane potential. The qualitative nature of these conclusions must be emphasized. In the absence of further information, the electrogenic contribution of the pump cannot be necessarily equated with the amount of membrane depolarization noted after ouabain.

The primary aim of the present study was to measure the basolateral membrane potential during the course of withdrawing and later restoring potassium to the inner bathing medium. Withdrawal of potassium has been found to hyperpolarize the basolateral membrane; prompt return of external potassium restores the membrane potential to its baseline value. In contrast to these expected effects, restoration of potassium to the inner bathing medium following an extended period of incubation in $K^+$-free inner solutions causes hyperpolarization of the basolateral membrane. This hyperpolarization can be abolished by prior treatment of the tissue with ouabain.

These observations complement the results of recent studies of the intracellular fluids of toad urinary bladder, using $K^+$-selective microelectrodes (DeLong & Civan, 1978, 1979, 1980) and using electron probe X-ray microanalysis (Civan et al., 1980). After incubation in $K^+$-free media for $1\frac{1}{2}$ to 2 hr, the transporting cells of toad bladder lose only some 20% of their intracellular potassium (Robinson & Macknight, 1976*a*; Civan et al., 1980). In part, the rate of net potassium loss is limited by the rate of apical sodium entry, leading to depolarization of the basolateral membrane in the short-circuited state. In addition, Civan et al. (1980) have suggested that this slow rate of net potassium loss reflects the continued operation of the Na−K exchange pump; even in the absence of potassium from the bulk media, potassium leaking from the epithelial cells is considered to be largely reaccumulated by the pump. This hypothesis now seems consistent with the observation of DeLong and Civan (1978, 1979) that restoration of potassium to the serosal medium bathing potassium-depleted tissues results in a prompt increase in the intracellular potassium activity, even before any increase in short-circuit current can be measured. Initial estimates of the relative gain in sodium and loss of potassium (Robinson & Macknight, 1976*a*) had suggested that insufficient intracellular sodium was available following potassium depletion to sustain potassium accumulation by the Na−K exchange pump. The more recent microprobe data of Civan et al. (1980) have indicated that the redistributions in sodium and potassium are comparable in magnitude, so that it is no longer necessary to invoke an additional transport mechanism responsible for cellular potassium accumulation.

Although the recycling hypothesis suggested by Civan et al. (1980) accommodates certain otherwise puzzling observations, it would be desirable to test the hypothesis directly. This has not yet been done, although the model has now been found to satisfy two necessary conditions. First, inhibition of the pump should increase the rate of net loss of potassium in $K^+$-free solutions; this has been found to be the case (Civan et al., 1980). Second, the Na−K pump should be operative, even in the prolonged absence of potassium from the inner or serosal bathing medium. The current study demonstrates that this is the case, at least in frog skin, since addition of ouabain depolarizes the basolateral membranes of potassium-depleted tissues. The rate of pump activity is clearly reduced below baseline values, as expected, since addition of potassium to the inner medium was observed to hyperpolarize the basolateral membrane. However, the rheogenic Na−K pump at the basolateral membrane appears to retain appreciable activity, even in the prolonged absence of potassium from the inner medium.

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## References

Biber, T.U.L., Aceves, J., Mandel, J.L. 1972. Potassium uptake across serosal surface of isolated frog skin epithelium. *Am. J. Physiol.* **222**:1366

Candia, O.A., Zadunaisky, J.A. 1972. Potassium flux and sodium transport in the isolated frog skin. *Biochim. Biophys. Acta* **255**:517

Civan, M.M. 1978. Intracellular activities of sodium and potassium. *Am. J. Physiol.* **234**:F261

Civan, M.M., Hall, T.A., Gupta, B.L. 1980. Microprobe study of toad urinary bladder in absence of serosal $K^+$. *J. Membrane Biol.* **55**:187

Curran, P.F., Cereijido, M. 1965. K Fluxes in frog skin. *J. Gen. Physiol.* **48**:1011

DeLong, J., Civan, M.M. 1978. Dissociation of cellular $K^+$ accumulation from net $Na^+$ transport by toad urinary bladder. *J. Membrane Biol.* **42**:19

DeLong, J., Civan, M.M. 1979. Intracellular potassium activity associated with potassium depletion from toad urinary bladder. *Colloq. Inst. Nat. Santé Rech. Med.* **85**:221

DeLong, J., Civan, M.M. 1980. Intracellular chemical activity of potassium in toad urinary bladder. *Curr. Top. Membr. Transp.* **13**:93

Essig, A. 1965. Active sodium transport in toad bladder despite removal of serosal potassium. *Am. J. Physiol.* **208**:401

Essig, A., Leaf, A. 1963. The role of potassium in active transport of sodium by the toad bladder. *J. Gen. Physiol.* **46**:505

Finn, A.L., Nellans, H. 1972. The kinetics and distribution of potassium in the toad bladder. *J. Membrane Biol.* **8**:189

Giebisch, G., Sullivan, L.P., Whittembury, G. 1973. Relationship between tubular net sodium reabsorption and peritubular potassium uptake in the perfused *Necturus* kidney. *J. Physiol. (London)* **230**:51

Helman, S.I., Fisher, R.S. 1977. Microelectrode studies of the active Na transport pathway of frog skin. *J. Gen. Physiol.* **69**:571

Helman, S.I., Nagel, W., Fisher, S.I. 1979. Ouabain on active transepithelial sodium transport in frog skin: Studies with microelectrodes. *J. Gen. Physiol.* **74**:105

Higgins, J.T., Jr., Gebler, B., Frömter, E. 1977. Electrical properties of amphibian urinary bladder epithelia. II. The cell potential profile in *Necturus maculosus*. *Pfluegers Arch.* **371**:87

Kimura, G., Fujimoto, M. 1977. Estimation of the physical state of potassium in frog bladder cells by ion exchanger microelectrode. *Jpn. J. Physiol.* **27**:291

Kimura, G., Urakabe, S., Yuasa, S., Miki, S., Takamitsu, Y., Orita, Y., Abe, H. 1977. Potassium activity and plasma membrane potentials in epithelial cells of toad bladder. *Am. J. Physiol.* **232**:F196

Koefoed-Johnsen, V., Ussing, H.H. 1958. The nature of the frog skin potential. *Acta Physiol. Scand.* **42**:298

Lassen, U.V., Nielsen, A.-M., Pape, L., Simonsen, O.O. 1971. The membrane potential of Ehrlich ascites tumor cells. Microelectrode measurements and their critical evaluation. *J. Membrane Biol.* **6**:269

Leaf, A. 1965. Transepithelial transport and its hormonal control in toad bladder. *Ergeb. Physiol. Biol. Chem. Exp. Pharmakol.* **56**:216

Lindemann, B. 1975. Impalement artifacts in microelectrode recordings of epithelial membrane potentials. *Biophys. J.* **15**:1161

Nagel, W. 1976. The intracellular electrical potential profile of the frog skin epithelium. *Pfluegers Arch.* **365**:135

Nagel, W. 1980. Rheogenic sodium transport in a tight epithelium, the amphibian skin. *J. Physiol. (London)* **302**:281

Nellans, H.N., Schultz, S.G. 1976. Relations among transepithelial sodium transport, potassium exchange, and cell volume in rabbit ileum. *J. Gen. Physiol.* **68**:441

Nelson, D.J., Ehrenfeld, J., Lindemann, B. 1978. Volume changes and potential artifacts of epithelial cells of frog skin following impalement with microelectrodes filled with 3 M KCl. *J. Membrane Biol.* **40**:91

Nielsen, R. 1979. Coupled transepithelial sodium and potassium transport across isolated frog skin: Effect of ouabain, amiloride and the polyene antibiotic filipin. *J. Membrane Biol.* **51**:161

Robinson, B.A., Macknight, A.D.C. 1976*a*. Relationships between serosal medium potassium concentration and sodium transport in toad urinary bladder. II. Effects of different medium potassium concentrations on epithelial cell composition. *J. Membrane Biol.* **26**:239

Robinson, B.A., Macknight, A.D.C. 1976*b*. Relationships between serosal medium potassium concentration and sodium transport in toad urinary bladder. III. Exchangeability of epithelial cellular potassium. *J. Membrane Biol.* **26**:269

Sudou, K., Hoshi, T. 1977. Mode of action of amiloride in toad urinary bladder: An electrophysiological study of the drug action on sodium permeability of the mucosal border. *J. Membrane Biol.* **32**:115

Ussing, H.H. 1960. The alkali metal ions in isolated systems and tissues. *Handb. Exp. Pharmakol.* **13**:1

Valenzeno, D.P., Hoshiko, T. 1977. Potassium reaccumulation by isolated frog epidermis. *Biochim. Biophys. Acta* **470**:273

Yonath, J., Civan, M.M. 1971. Determination of the driving force of the $Na^+$ pump in toad bladder by means of vasopressin. *J. Membrane Biol.* **5**:366

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