Intracellular Na\(^+\) and K\(^+\) Activities and Membrane Conductances in the Collecting Tubule of Amphiuma

JEAN-DANIEL HORISBERGER and GERHARD GIEBISCH

From the Yale University School of Medicine, Department of Physiology, New Haven, Connecticut 06510-8026

ABSTRACT Membrane potentials and conductances, and intracellular ionic activities were studied in isolated perfused collecting tubules of K\(^+\)-adapted Amphiuma. Intracellular Na\(^+\) (a\(_{\text{Na}}\)) and K\(^+\) (a\(_{\text{K}}\)) activities were measured, using liquid ion-exchanger double-barreled microelectrodes. Apical and basolateral membrane conductances were estimated by cable analysis. The effects of inhibition of the apical conductance by amiloride (10\(^{-5}\) M) and of inhibition of the basolateral Na-K pump by either a low K\(^+\) (0.1 mM) bath or by ouabain (10\(^{-4}\) M) were studied. Under control conditions, a\(_{\text{Na}}\) was 8.4 ± 1.9 mM and a\(_{\text{K}}\) 56 ± 3 mM. With luminal amiloride, a\(_{\text{Na}}\) decreased to 2.2 ± 0.4 mM and a\(_{\text{K}}\) increased to 66 ± 3 mM. Ouabain produced an increase of a\(_{\text{Na}}\) to 44 ± 4 mM, and a decrease of a\(_{\text{K}}\) to 22 ± 6, and similar changes were observed when the tubule was exposed to a low K\(^+\) bath solution. During pump inhibition, there was a progressive decrease of the K\(^+\)-selective basolateral membrane conductance and of the Na\(^+\) permeability of the apical membrane. A similar inhibition of both membrane conductances was observed after pump inhibition by low K\(^+\) solution. Upon reintroduction of K\(^+\), a basolateral membrane hyperpolarization of -23 ± 4 mV was observed, indicating an immediate reactivation of the electrogenic Na-K pump. However, the recovery of the membrane conductances occurred over a slower time course. These data imply that both membrane conductances are regulated according to the intracellular ionic composition, but that the basolateral K\(^+\) conductance is not directly linked to the pump activity.

INTRODUCTION

The cell model introduced by Koefoed-Johnsen and Ussing (1958) provides an explanation of the means by which epithelial cells bring about active transcellular Na\(^+\) transport, and at the same time, maintain low cell Na\(^+\) and high cell K\(^+\) levels. Recently, attention has been drawn to the fact that apical and basolateral transport processes must be coordinated to effect transcellular ion movement without jeopardizing...
dizing intracellular ion homeostasis (Schultz, 1981; Chase, 1984; Cohen and Giebisch, 1984; Schultz, 1985). This is borne out by the fact that very large changes in net transepithelial Na⁺ transport may be achieved, for instance, by hormonal treatment (aldosterone) without marked alteration in the cell Na⁺ level (Wills and Lewis, 1980).

In the present study we have used the isolated perfused collecting tubule of *Amphiuma* to investigate the relation between the active basolateral Na-K pump and the apical Na⁺ and basolateral K⁺ conductances. This preparation offers several advantages (Horisberger et al., 1987; Hunter et al., 1987): changes of net Na⁺ transport and pump activity can be easily achieved by pump inhibition or rapid and reversible abolishment of the Na⁺ entry across the apical membrane by amiloride. Previous studies have also shown that the basolateral membrane of the main cell type of this preparation was K⁺-selective so that large changes of the basolateral conductance could be safely attributed to modification of the K⁺-specific conductance.

We have shown previously (Horisberger et al., 1987) that collecting tubules taken from *Amphiuma* that have been exposed to a high K⁺ environment have a higher rate of transcellular Na⁺ transport and a higher apical membrane and transcellular conductance. The paracellular conductance was also smaller in these K⁺-adapted animals. The high ratio of the cellular to paracellular conductance makes the determination of the individual cell membrane conductance more precise. Furthermore, the apical and basolateral membrane conductances are of similar magnitude (resistance ratio near 1), which allows for precise determination of both membrane conductances simultaneously; in contrast, in animals not previously exposed to a high K⁺ environment, the high resistance of the apical membrane makes the measurements of the basolateral membrane conductance by transepithelial current injection less accurate. Finally, it can be predicted that the high transport rate in K⁺-adapted animals results in fast changes of the intracellular ionic composition when a transport process is altered at one membrane; this condition is favorable for the observation of such changes using intracellular electrodes. For these reasons, all the tubules were taken from *Amphiuma* previously exposed for at least 3 d to a high K⁺ environment.

In the present study, we observed that pump inhibition, either by ouabain or by a low K⁺ bath, produces an increase of the intracellular Na⁺ activity and a decrease of the K⁺ activity. Following a time course similar to that of the change of the intracellular ionic activities, there was a significant decrease of the apical Na⁺ conductance and an even larger decrease of the basolateral K⁺ conductance. The time course of the changes of the K⁺ conductance was slow and not immediately related to the activity of the Na-K pump.

**METHODS**

Preparation of Animals and Tubule Perfusion

*Amphiuma* of both sexes were obtained from C. Sullivan (Nashville, TE) and kept for at least 4 d in aquaria containing a 10 mM NaCl + 50 mM KCl solution.

The identification, preparation, and perfusion of single collecting tubules was carried out as described in another paper from this laboratory (Hunter et al., 1987). The perfusion appa-
ratus was modified to achieve fast luminal solution exchange and to allow for control of the intratubular hydrostatic pressure. As schematically shown in Fig. 1, a double-barreled fluid exchange pipette, extending to the tip of the conical part of the perfusion pipette, allowed for a fast exchange of the luminal perfusion solutions. As judged from the time course of the transepithelial repolarization after the removal of amiloride from the luminal fluid, the 90% exchange time was <1 s. To allow for control of the pressure on the collection side, the distal end of the isolated tubule was cannulated and connected to a manometer. A negative pressure (2–5 cm H₂O) was applied to the collecting pipette and was adjusted so that the tubule would tightly adhere around the collecting pipette. Thus, an electrically tight seal between the tubule and the perfusion pipette was achieved.

**Transepithelial Electrical Measurements**

The transepithelial potential at the proximal (Vₑp) and at the distal (Vₑd) end of the tubule was continuously recorded. The mean difference between these two measurements was $-0.3 \pm 1.4$ mV ($n = 24$) NS. The mean of $Vₑp$ and $Vₑd$ was used for the transepithelial potential ($Vₑ$). The transepithelial conductance ($Gₑ$) was calculated by cable analysis using the voltage deflections recorded at both ends of the tubule after current injection (10–55 nA) through the perfusion pipette (Hunter et al., 1987).

**Microelectrodes**

Conventional microelectrodes were pulled from Omega dot borosilicate glass tubing (Frederick Haer, Brunswick, Maine) (OD/ID, 1.2/0.6 mm) on a Narishige PD-5 puller, and were filled with 1 M KCl. Their resistance was 60–120 MΩ.

Double-barreled microelectrodes were constructed using a method suggested by R. Henderson (personal communication). Two pieces of glass tubing, stabilized by 2 O-rings cut from heat-shrink tubing, were heated and twisted by $-180°$, and then pulled on a Narishige PD-5 electrode puller.

For double-barreled Na⁺-sensitive electrodes, two pieces of aluminosilicate glass tubing (1.0 mm OD/0.76 mm ID) were used. Aluminosilicate glass has a resistivity 3 orders of magnitude higher than borosilicate, and wall shunting is minimal even when thin-walled glass tubing and high-resistance liquid ion exchanger (LIX) such as the Na⁺-LIX are used (Tripathi et al., 1985). The combined tip diameter of these double-barreled electrodes was <0.5 μm and each barrel had a resistance of 30–60 MΩ when filled with 1 M KCl.
Double-barreled K⁺-sensitive electrodes were pulled from pieces of either aluminosilicate or borosilicate (1.0 mm OD/0.5 mm ID) glass tubing, using the method described above. Each barrel had a resistance of 60–120 MΩ when filled with 1 M KCl.

Both barrels of the Na⁺- and K⁺-sensitive electrodes were silanized by exposure for 1 h to hexamethyldisilazane vapor (Fluka, Buchs, Switzerland). The ion-sensitive barrel was filled with either the Na⁺-LIX (catalogue number WPI IE-110, lot 9295/03F; purchased from World Precision Instrument, New Haven, CT) or the K⁺-LIX (catalogue number 477317, lot 121783; Corning Glass Works, Corning, NY). The reference barrel was filled with the reference liquid ion exchanger (RLIE), i.e., 2% K⁺-tetrakis-p-chlorophenyl borate in octanol (Thomas and Cohen, 1981).

The ion-sensitive electrodes were calibrated in mixed NaCl/KCl standard solutions, the sum of NaCl + KCl being constant (100 mM). The standard solutions contained 100, 30, 10, 3.0, and 1.0 mM Na⁺ for the Na⁺-sensitive electrodes and 100, 30, 10, 3.0, and 1.0 mM K⁺ for the K⁺-sensitive electrodes. The activity coefficients were assumed to be constant and equal to 0.77, a value obtained by interpolation of data by Robinson and Stokes (1959).

The values of the slope (S) and the selectivity coefficient for Na⁺ over K⁺ (1/K_{Na/K}) of the Na⁺-sensitive electrode were obtained by fitting the potential difference (ΔV) between the two barrels and the calculated activities (a_{Na}) of the standard solutions, using a least-square nonlinear fit method to the Nicolsky equation (Edelman et al., 1978):

\[
ΔV = S \log \left[ \frac{a_{Na} + K_{Na/K} \times a_K}{a_{Na}(100)} \right] - \log \left[ a_{Na}(100) \right],
\]

where \( a_{Na}(100) \) is the Na⁺ activity of the reference solution containing 100 mM Na⁺. Electrodes were calibrated before use to select those with a slope >50 mV/decade. They were calibrated again immediately after a successful impalement and the values obtained in this last calibration were used for intracellular activities calculation. For eight Na⁺-sensitive electrodes, the slope was 58.5 ± 0.7 mV/decade and the selectivity coefficient (1/K_{Na/K}) was 29 ± 4 for eight K⁺-sensitive electrodes the slope was 57.3 ± 0.8 mV/decade and the selectivity coefficient (1/K_{K/Na}) was 32 ± 4. The 90% response time were 11 ± 1 s and 10 ± 2 s for the Na⁺-sensitive and the K⁺-sensitive electrodes, respectively. The intracellular Na⁺ activities were then calculated using the following form of the Nicolsky equation, derived from Eq. 1, with the assumption that the sum of \( a_{Na} + a_{K} \) was constant and equal to the sum of these cations activities in the bath solution (77 mM):

\[
a_{Na} = 77 \left[ \frac{10 \left( \frac{V_{Na} - V_{RLIE}}{S} - K_{Na/K} \right)}{1 - K_{Na/K}} \right]
\]

where \( V_{Na} \) is the voltage read from the Na⁺-sensitive barrel and \( V_{RLIE} \) the voltage read from the reference barrel.

The intracellular K⁺ activities were calculated from the voltages recorded in the K⁺-selective electrodes, using the same method.

To test the assumption that the sum of the intracellular cation activities was constant and equal to the sum of the bath cation activities, we made double-barreled electrodes to compare the basolateral potentials recorded with conventional 1 M KCl-filled, and RLIE microelectrodes. These latter electrodes were made from borosilicate glass tubing and only one barrel of each electrode was silanized, using a method described by Henderson et al. (1986). A droplet of RLIE was deposited in the silanized barrel and after it had filled the tip, both barrels were back-filled with 1 M KCl. The 1 M KCl-filled barrel had a resistance ~120 MΩ and the RLIE-filled barrel of ~60 GΩ.
Estimates of Single-Barrier Conductances

As described in more detail in previous papers (Horisberger et al., 1987; Hunter et al., 1987), amiloride decreases the luminal membrane conductance to a negligible value and does not influence the paracellular conductance. Thus, the transcellular conductance was calculated as the difference between the transepithelial conductance in the presence and in the absence of amiloride

\[ G_{\text{cell}} = G_{\text{cell}}(\text{control}) - G_{\text{cell}}(\text{amiloride}). \]  

(3)

From the voltage divider ratio, the fractional apical resistance \((fR_a)\) was calculated using cable analysis as previously described (Hunter et al., 1987). The individual conductance of the apical \((G_a)\) and the basolateral \((G_b)\) membrane were then obtained from the following expressions:

\[ G_a = G_{\text{cell}}/fR_a \]  

(4)

\[ G_b = G_{\text{cell}}/(1 - fR_a) \]  

(5)

Individual membrane conductances were calculated only in tubules in which amiloride produced a decrease of the transepithelial conductance of >50%, i.e., in tubules in which the transcellular conductance was larger than the paracellular conductance. This decreased the errors in cell conductance measurement.

The results of membrane potential and conductance measurements obtained from cellular impalements by conventional 1 M KCl-filled electrodes \((n = 9)\), Na-LIX/RLIE double-barreled electrodes \((n = 8)\), and K-LIX/RLIE double-barreled electrodes \((n = 8)\) were pooled.

Apical and Basolateral Membrane Permeabilities

According to the electrodiffusion model of Goldman, a membrane conductance depends on the transmembrane voltage and on the concentration of the conductive ion species on both sides of this membrane (Schultz, 1980). Thus, changes of the experimentally measured conductance may be related either to changes of the membrane potential or to alterations of the intracellular ion concentrations as well as to a real change of the conductive properties of the membrane. Using the Goldman equation and taking the partial derivative of the current \((I)\) with respect to the potential \((V)\) provides an expression of the slope conductance \((G)\) for the ion pathway (Matsumura et al., 1984). This equation can also be solved for the permeability \((P)\), yielding

\[ P = \frac{G}{RT} \left[ \frac{C_1 - (1 + U)C_e e^U}{1 - e^U} + \frac{U e^U (C_1 - C_e e^U)}{(1 - e^U)^2} \right] \]  

(6)

with \(U = zVF/RT\)

where \(C_1\) and \(C_e\) are the intra- and extracellular ion activities, and \(R, T, z,\) and \(F\) have their usual meaning. We have calculated the apical Na\(^+\) permeability using values of the apical membrane potential and conductance, and of the intracellular Na\(^+\) activity from the experiments where these values could be measured simultaneously. Similarly, we have estimated the basolateral membrane K\(^+\) permeability from the simultaneously measured values of the basolateral membrane potential and conductance and the intracellular K\(^+\) activity. The values obtained for the K\(^+\) permeability may be overestimated by 20–30%, as the K\(^+\) conductance accounts for only 70–80% of the total basolateral membrane conductance, according to transference number measurements (Hunter et al., 1987; Horisberger et al., 1987).
Solutions and Drugs

The composition of the "control" solution was (expressed in millimolar) 97.0 Na⁺, 3.0 K⁺, 1.8 Ca²⁺, 1.0 Mg²⁺, 89.6 Cl⁻, 14.4 HCO₃⁻, and 0.8 H₂PO₄⁻/HPO₄²⁻. The "low K" solution was similar except for the 0.1 mM K⁺ and the 100.0 mM Na⁺ concentration. The same solution was used for the bath and the perfusate except that 2.0 mM glucose and 0.25 mM glycine were added to the bath solutions. All solutions were bubbled with a 2% CO₂/98% O₂ gas mixture. The pH of all solutions was 7.6. Amiloride (a gift of Merck, Sharp & Dohme, West Point, PA) was used at a concentration of 10⁻⁵ M, and ouabain (Sigma Chemical Co., St. Louis, MO) at 10⁻⁴ M.

Statistics

Results are expressed as mean ± SEM (n = number of observations). The statistical significance of the difference between mean values was determined by the Student's t test. When appropriate, the Student's t test for paired data was used (indicated in the text by "ptt"). P < 0.05 was chosen as the level of significance.

RESULTS

Baseline Transepithelial Measurements

The 24 isolated perfused tubules studied had a mean length of 599 ± 36 μm (range, 380–1,120 μm) and a mean internal diameter of 40 ± 4 μm. In the initial control condition, the mean value of Vₑ was -52 ± 4 mV (n = 24) and the transepithelial conductance 2.9 ± 0.2 mS · cm⁻² (n = 17) (in the remaining seven experiments, the values of the transepithelial and membrane conductances were not used because the transcellular conductance was <50% of the total transepithelial conductance). The transepithelial conductance with amiloride in the lumen provides an estimate of the paracellular conductance (see Methods section) and averaged 0.9 ± 0.15 mS · cm⁻² (n = 17). The transcellular conductance was 2.0 ± 0.16 mS · cm⁻² (n = 17). All these tubules were taken from *Amphiuma* previously exposed to a high K⁺ environment. The electrophysiological characteristics of these tubules are very similar to those previously reported for K⁺-adapted animals (Horisberger et al., 1987); the lower paracellular conductance in the present study is probably due to a better electrical seal achieved by cannulation of the tubule at both ends of the perfused tubule and the careful control of the intratubular pressure.

Intracellular Electrode Impalements

Impalements with conventional electrodes displayed the typical characteristics previously described (Hunter et al., 1987), consisting of an initial spike deflection, followed by a stabilization at a plateau potential level. The slower response time of the high-resistance LIX electrodes used in the present study prevented the observation of an initial spike deflection and led us to choose different criteria for accepting impalements with double-barreled ion-sensitive electrodes. Thus, impalements were considered acceptable if (a) after a fast initial deflection, the potential recorded from both barrels became stable (within ±2 mV) for at least 1 min in the control condition and (b) the potential recorded from the reference barrel (Vᵦᵝ) hyperpolarized and stabilized to a value of at least -65 mV after the addition of amiloride in
the luminal perfusion fluid. This potential value was chosen because it equals the mean + 1 SD of $V_b$ measured in previous experiments made in similar conditions with conventional electrodes (Horisberger et al., 1987).

A representative cell impalement with a Na⁺-sensitive electrode is shown in Fig. 2 A. When the electrode enters the cell, we recorded the fast appearance of a negative potential (upper tracing, $V_b$) and, simultaneously, a slightly slower potential shift of the differentially recorded potential (lower tracing), which indicates that the tip of the electrode had passed into a compartment of lower Na⁺ activity (~8 mM). After stabilization of both potential values, the addition of amiloride (10⁻⁵ M) to the luminal fluid produced a sharp hyperpolarization of $V_b$ and a decrease of $aNa$. The short upward deflection of the $V_b$ tracing is due to a luminal positive current injection, and its magnitude is used for the calculation of the resistance ratio as described in the Methods section. The downward deflection of the $V_b - V_{Na}$ tracing immediately after amiloride was due to the difference of the response time of the two barrels. The Na⁺-sensitive barrel had a slower response time and recorded the sharp voltage deflection due to amiloride with a delay, which produced this downward deflection in the differential recording. The reverse effect is seen when amiloride is removed (not shown).

(B) Recording of a cellular impalement with a double-barreled K⁺-sensitive microelectrode. The upper tracing is the recording of $V_b$ by the RLIE-filled reference barrel and the lower recording is the difference between the reference barrel and the K⁺-sensitive barrel. The comments of Fig 2 A apply similarly to this figure.

Fig. 2 B shows a cell impalement with a K⁺-sensitive microelectrode. The basolateral voltage trace and its response to amiloride was qualitatively similar to that of
the Na+-sensitive electrode. However, when the electrode entered the cell, the downward deflection of the differentially recorded potential indicated a large increase in the K+ activity. The observation that the potential recorded by the K+-sensitive barrel was +26 mV showed that K+ activity was above its electrochemical equilibrium. However, after the addition of amiloride to the luminal fluid the potentials recorded by the two barrels showed a similar value (Ek ~ Vl), a finding that demonstrated that, in the presence of luminal amiloride, K+ activity was now very near its equilibrium value expected from the basolateral potential difference. This means that if there are significant conductances other than to the K+ ion, the permeant ions must be passively distributed across the basolateral membrane.

**Baseline Values of Membrane Potentials and Conductances, and Intracellular Ionic Activities**

Table I summarizes transepithelial and transmembrane potentials as well as the intracellular Na+ and K+ activities in control and amiloride-treated tubules. Luminal amiloride had similar effects on Vte, Vtb, and Vb as previously described (Hunter et al., 1987). These effects consisted of the abolition of Vte and a marked hyperpolarization of Vtb and Vb. The effects on intracellular ion activities were a significant decrease of aNa and a modest but also significant increase of aK. The time course of the changes of aNa and aK were similar and could be roughly estimated to have a half-life of 30–60 s.

In the control condition the fractional resistance of the apical membrane (Rapp) was 0.49 ± 0.05 (n = 20), and the mean conductances of the apical and basolateral membrane were 5.7 ± 1.4 and 4.8 ± 0.6 mS · cm⁻² (n = 17), respectively.

**The Effects of Low K+ Concentration in the Bath**

The effects of lowering the bath K+ concentration (from 3.0 to 0.1 mM), a maneuver known to inhibit the Na-K-ATPase activity (Jorgensen, 1980), are summarized in Fig. 3. It is apparent that a biphasic response of Vmb and Vte occurred. First, lowering the K+ concentration in the bath induced a fast hyperpolarization of the basolateral membrane potential from −57 ± 3 to −83 ± 5 mV (n = 17) (P < 0.001, **t** test). After this initial hyperpolarization, Vmb decreased over a 2–3-min period. The time course

| TABLE I | Effect of amiloride on Vte, Vtb, and Vb, and on aNa and aK.
|----------|-----------------|-----------------|
| Control  | Amiloride*       |                 |
| n        | n               |                 |
| Vte (mV) | −52 ± 4         | 0 ± 1           |
| Vtb (mV) | −57 ± 3         | −82 ± 2         |
| Vb (mV)  | 5 ± 3           | 82 ± 2          |
| aNa (mM) | 8.4 ± 1.9       | 2.2 ± 0.5       |
| aK (mM)  | 56 ± 3          | 66 ± 3          |

Values are given as means ± SE.

*Values measured 3 min after the addition of 10⁻⁵ M amiloride to the luminal fluid.

**t** indicates a statistically significant difference between the "control" value and the "ouabain" value (**t** test).
FIGURE 3. Effects of a 4-min exposure to a low K⁺ concentration in the bath (from 3.0 to 0.1 mM) on the mean \( V_{\text{m}} \) and \( V_{\text{bl}} \), \( a_{\text{K,l}} \) and \( a_{\text{Na,l}} \), and \( G_{\text{b}} \) and \( G_{\text{a}} \). The number of observations and the statistical significance of relevant differences for the same data are given in Table II. The first point for each variable indicates the steady state value before the bath solution change was performed. The last point is the value measured after the return to a steady state (3–15 min after return to 3.0 K⁺ bath). The fast hyperpolarization of \( V_{\text{bl}} \) (and \( V_{\text{m}} \)) is explained by the sudden change of the basolateral equilibrium potential for K⁺ (\( E_{\text{K}} \)). The \( V_{\text{m}} \) change was not as large as when amiloride was present in the lumen (see Fig. 5) because (a) the apical resistance is lower without amiloride and a loop current is generated, and (b) the pump-generated potential is probably larger in the absence of amiloride as \( a_{\text{Na,l}} \) is higher. The following slow depolarization can be explained by the decrease of \( a_{\text{K,l}} \) and \( E_{\text{K}} \) and by the decrease of the ratio between the basolateral K⁺ conductance and the apical Na⁺ conductance. In contrast to what is observed when amiloride is present in the lumen (see Fig. 5), reintroduction of a normal (3.0 mM) K⁺ concentration in the bath produced a hyperpolarization of \( V_{\text{m}} \) and \( V_{\text{bl}} \). This is explained by the larger contribution of the electrogenic pump and the smaller contribution of \( E_{\text{K}} \) to the basolateral potential at this point (see the Discussion). The middle panel shows the large decrease of \( a_{\text{K,l}} \) and the large increase of \( a_{\text{Na,l}} \) after the inhibition of the pump. The reverse changes of Na⁺ and K⁺ activities occurred after raising the bath K⁺ concentration, which indicates immediate pump reactivation. The lower panel shows the \( G_{\text{b}} \) and the \( G_{\text{a}} \). There was an immediate increase of \( G_{\text{b}} \) after the change to the low K⁺ bath solution. This significant change is not in accordance with what is predicted from the constant field equation and is good evidence for an activation of a basolateral conductance by a hyperpolarization. Later there was a marked, 10-fold reduction of \( G_{\text{b}} \). Only the value of \( G_{\text{a}} \) after 4 min in the low K⁺ bath is significantly different from the initial control value, but taking into account the increase of \( a_{\text{Na,l}} \) and assuming a Na⁺ conductance that conforms to the prediction of the constant field equation, the Na⁺ permeability of the apical membrane is significantly decreased 3 min after the exchange for the low K⁺ solution. The vertical bars indicate ±1 SE of the mean.

of this voltage change was variable, in some tubules the depolarization was fast and followed by stabilization at the level of a new plateau value (example shown in Fig. 4). In other tubules, the depolarization was slower and \( V_{\text{m}} \) did not reach a new steady state value in the 4 min during which the tubule was exposed to the low K⁺ bath (example shown in Fig. 7). The transepithelial potential changes were qualitatively
FIGURE 4. Original recording of the basolateral membrane potential ($V_b$, upper tracing), the transepithelial potential at the proximal end of the tubule ($V_{te1}$, middle tracing) and at the distal end ($V_{te2}$, lower tracing) during exposure to the low $K^+$ bath. The short upward deflections of the potentials are due to luminal positive current injection. Their magnitudes were used for the calculation of the individual barrier resistances as described in the Methods section. The progressive increase in these deflections on the transepithelial potential recordings during the slow depolarization phase is an indication of the decrease of the transepithelial conductance. The more important increase of the voltage deflection of the $V_{te}$ tracing means that this decrease of the transepithelial conductance is mainly due to a decrease of the basolateral conductance. It must be noticed that when amiloride ($10^{-5}$ M) was briefly added to the lumen, the $V_{te}$ deflections almost completely disappeared, indicating the abolition of the apical conductance, while this blockage produced a large decrease of the transepithelial conductance (large deflections of the $V_u$ tracings). The square wave current injections (1 s) had the same amplitude during the whole experiment. In this example, the decrease of the basolateral conductance and the associated $V_u$ depolarization were fast and nearly complete after 2 min. In other tubules these changes were slower, as shown in Fig. 7. When $K^+$ concentration was returned to normal (3.0 mM), there was an immediate repolarization of $V_u$. We explain this repolarization by a high pump-generated potential when the pump is activated by the readmission of $K^+$ at a time when the intracellular $Na^+$ activity is very high and the basolateral conductance is low.
intracellular Na\(^+\) and K\(^+\) activities, and membrane conductances

The immediate inhibition of the basolateral Na-K pump by the low K\(^+\) concentration in the bath lead to a prompt increase of the intracellular Na\(^+\) activity and a decrease of that of K\(^+\). Simultaneously with the initial basolateral hyperpolarization, the basolateral conductance (G\(_b\)) presented a small, but statistically significant increase, from 3.9 ± 0.9 to 4.6 ± 0.8 mS · cm\(^{-2}\) (n = 14) (P < 0.05, pt). Later, however, G\(_b\) showed a marked, 10-fold reduction. In most tubules, the apical conductance (G\(_a\)) also decreased during this period, but the time course of this conductance change was slower than that of G\(_b\).

Upon restoring the control K\(^+\) concentration (3.0 mM) in the bath solution, V\(_{bl}\) immediately hyperpolarized by 23 ± 4 mV (n = 16) (from −24 ± 4 to −47 ± 4 mV, P < 0.001, pt). V\(_{a}\) hyperpolarized, with the same time course, by −13 ± 2 mV (n = 16). Shortly after the reintroduction of K\(^+\) (5–10 s), there was an initial significant increase of G\(_b\), from 0.5 ± 0.1 to 1.1 ± 0.2 mS · cm\(^{-2}\) (n = 12) (P < 0.05, pt), whereas G\(_a\) did not change significantly: 2.4 ± 0.5 to 2.7 ± 0.5 mS · cm\(^{-2}\) (n = 12) (NS, pt).

Table II provides a comprehensive summary of the values of membrane potentials, conductances, and intracellular Na\(^+\) and K\(^+\) activities before, during, and after exposure of single tubules to the low K\(^+\) bath solution. Of particular interest are the changes in cell ion activities and barrier conductances after restoring the bath K\(^+\) concentration to its initial value of 3.0 mM. This maneuver reactivated the Na-K pump as evidenced by the rapid decrease of the intracellular Na\(^+\) activity and the increase of the K\(^+\) activity. Note that after a 3-min period, a\(_{Na}\) and a\(_{K}\) had returned to the initial control values. However, as shown in Table II (RecI), both the basolateral membrane potential and the basolateral conductance were still depressed, and it was only after a much longer period (5–15 min) that they returned to their initial control value (Table II, RecII). During this time, the apical membrane conductance
did not recover after exposure to the low K⁺ bath, but remained depressed at a value of ~40% of the initial control value.

Fig. 5 presents the effects on the basolateral and the transepithelial potentials of the same maneuver, lowering the bath K⁺ concentration from 3.0 to 0.1 mM when amiloride was present in the lumen. This maneuver produced a larger hyperpolarization of the basolateral membrane potential, from \(-82 \pm 1\) to \(-142 \pm 2\) mV, \(\Delta V_{bl} 59 \pm 2\) mV, \(n = 11\) (\(P < 0.001\)) than in the absence of amiloride, where the \(\Delta V_{bl}\) was only \(-26 \pm 3\) mV \(n = 17\) (\(P < 0.001\)). During a 3-min period in low-K⁺ bath solution, \(V_{bl}\) slightly decreased to \(-138 \pm 3\) mV \(n = 10\), and upon restoring K⁺ to its initial concentration of 3.0 mM, \(V_{bl}\) returned to \(-89 \pm 1\) mV \(n = 10\), a value only slightly higher than before the low K⁺ exposure (difference \(-7 \pm 1\) mV, \(n = 10\) (\(P < 0.01\), ptt). During the 3-min period of the exposure to the low K bath in

![Figure 5](image)

**Figure 5.** \(V_{bl}\) and \(V_{te}\) during a 3-min exposition to a low K⁺ (0.1 mM) bath solution when amiloride \((10^{-5}\) M\) was present in the lumen. Eight measurements were obtained with conventional 1-M-KCl microelectrodes and three measurements were taken with the reference barrel of RLIE/Na-LIX double-barreled microelectrodes; as the values obtained by these two types of electrode were similar, the results were pooled (\(n = 11\)). The large hyperpolarization of \(V_{bl}\) obtained by the shift to the low K⁺ solution is an indication of the K⁺-selective properties of the basolateral membrane. At the time of the reintroduction of K⁺, a reverse change in potential was observed. This indicates that during the 3-min low K⁺ step the conductive properties of the membrane were not significantly modified, in contrast to what was observed when amiloride was not present in the lumen (see Fig. 3). The vertical bars indicate ±1 SE of the mean.

The presence of luminal amiloride, \(q_{Na}\) increased from \(2.3 \pm 0.6\) to \(13.4 \pm 2.6\) \((n = 3)\) (\(P < 0.05\), ptt).\(^1\) The effect on the transepithelial potential was a polarization of \(V_{te}\) from \(0 \pm 1\) to \(-5 \pm 1\) mV \(n = 12\) (\(P < 0.05\), ptt). The transepithelial conductance \(G_{se}\) decreased from \(0.87 \pm 0.14\) to \(0.80 \pm 0.14\) mS · cm⁻² \(n = 11\) (\(P < 0.05\), ptt). This confirms the presence of a significant K⁺ conductance of the

\(^1\)The Na⁺ entry pathway, in the presence of amiloride in the lumen and with a low K⁺ peritubular bath solution, is not known. A small residual apical membrane conductance (i.e., an amiloride-resistant part of the Na⁺ conductance) could explain part of this influx considering the very large driving force for Na⁺ entry under these circumstances \((V_{te} + E_{Na})\) in the range of \(-220\) mV. Electrically silent pathways, such as a Na⁺/H⁺ exchanger, in either membrane, could account for another part of the Na⁺ influx under these circumstances.
paracellular pathway as demonstrated in a previous study (Horisberger et al., 1987).

**The Effects of Ouabain**

Fig. 6 summarizes the effects of basolateral Na-K-ATPase inhibition by $10^{-4}$ M ouabain. The basolateral and transepithelial potential changes occurred in two distinct phases. First, there was a fast (<15 s) depolarization (change of $V_{te}$: $+7.6 \pm 0.9$ mV, $n = 13$, $P < 0.05$, ptt; change of $V_{bl}$: $+15 \pm 2$ mV, $n = 13$, $P < 0.05$, ptt). This was followed by a second phase of slower depolarization over the next 5 min. $V_{te}$ and $V_{bl}$ then stabilized (decreasing by $<2$ mV/min) at mean values of $-13 \pm 3$ mV ($n = 13$) and $-18 \pm 4$ mV ($n = 10$), respectively. The effects of ouabain were not readily reversible.

**Figure 6.** Mean $V_{te}$ and $V_{bl}$, $a_{Na,i}$ and $a_{K,i}$, and $G_a$ and $G_{bl}$, during the first 5 min after ouabain ($10^{-4}$ M) had been added to the bath solution. The $V_{bl}$ and $V_{te}$ measurements show the immediate fast depolarization after the addition of ouabain (values taken 10–15 s after the solution exchange). This depolarization is thought to be due to the immediate blocking of the pump and the abolition of the pump-generated potential. Using the mean measured values of $V_{bl}$, $R_{te}$, $R_{bl}$, and $R_a$ and Eq. 7, the pump current was estimated to be $58 \mu A/cm^2$. By using a paired t test to compare the experimental (after ouabain) with the control values, all experimental values of $V_{te}$ ($n = 11$) and $V_{bl}$ ($n = 11$) were found to be significantly different ($P < 0.05$) from the control values. The middle panel shows the increase of $a_{Na,i}$ and the decrease of $a_{K,i}$ during the same period. The immediate rise of $a_{Na,i}$ and the fall of $a_{K,i}$ indicate that the pump was rapidly and completely blocked by ouabain. By using a paired t test, all experimental values, after ouabain, of $a_{Na,i}$ ($n = 4$) and $a_{K,i}$ ($n = 4$) were found to be significantly different ($P < 0.05$) from the control values. The lower panel shows the marked decrease of the basolateral conductance ($G_{bl}$) and the smaller but significant decrease of the apical conductance ($G_a$); the values of both conductances, recorded at 3 and 5 min after ouabain addition, are significantly different from the control values (paired t test, $n = 8$, $P < 0.05$). The value recorded 10–15 s after ouabain addition is significantly lower (paired t test, $n = 8$, $P < 0.05$) for $G_{bl}$, but there was no change of $G_a$. This immediate decrease of $G_{bl}$ supports the hypothesis of a potential dependent conductance at the basolateral membrane. The vertical bars indicate $\pm 1$ SE of the mean.
The effects of ouabain on the intracellular Na⁺ and K⁺ activities were qualitatively similar to, but developed more slowly than those observed after pump inhibition by removing external K⁺. This difference may be explained by the fact that, in addition to blocking the Na-K pump, lowering the bath K⁺ concentration increases the driving force (larger K⁺ chemical gradient) for K⁺ exit across the basolateral membrane. In addition, this maneuver augments the driving force for Na⁺ entry across the apical membrane (apical membrane hyperpolarization). These increased driving forces then result in larger ion fluxes across both cell membranes, thus inducing a modification of the intracellular ionic content at an accelerated rate (compare Figs. 3 and 6).

Similar to the membrane potential changes, the changes of the basolateral conductance also occurred in two phases. First, we observed a fast initial decrease of $G_{bl}$, from $3.2 \pm 0.6$ to $2.6 \pm 0.4$ mS cm⁻² ($n = 10$) ($P < 0.02$, pt) $10-15$ s after ouabain. At this time changes in $a_{Na}$ and $a_{K}$ were still small. In the second phase, both the apical and the basolateral membrane conductances decreased with the same time course as $a_{Na}$ and $a_{K}$. The apical membrane permeability to Na⁺ (calculated according to Eq. 6) decreased significantly from $12.1 \pm 3.1$ to $5.4 \pm 1.2 \times 10^{-6}$ cm⁻¹ s⁻¹ ($n = 4$) ($P < 0.05$, pt) $5$ min after ouabain.²

The K⁺ permeability (calculated according to Eq. 6) showed a significant decrease from $76 \pm 13$, under the control conditions, to $34 \pm 14 \times 10^{-6}$ cm⁻¹ s⁻¹ ($n = 3$) ($P < 0.05$, pt) $5$ min after ouabain. This result indicates that the large decrease of the basolateral conductance cannot be fully explained by the change of intracellular K⁺ activity, but that the conductive properties of the membrane are also modified.

**Conventional vs. RLIE Electrodes for Intracellular Potential Measurements**

We tested the assumption that the sum of the intracellular cation activities is constant and similar to the sum of the extracellular fluid cation activities. This was done by measuring basolateral membrane potentials with double-barreled 1-M-KCl/RLIE microelectrodes. The RLIE provides a cation-selective membrane. (Thomas and Cohen, 1981) and thus gives a nernstian response to cation chemical gradient, without selectivity between cations. Accordingly, the voltage measured by an intracellular RLIE electrode is the sum of the membrane potential plus the chemical potential of the cations. When the sum of the activities of the cations in the bath and in the cell are identical, their chemical potential is equal inside and outside the cell and the voltage recorded from the RLIE microelectrode is the membrane potential. The mean potentials measured in the 1-M-KCl barrel and in the RLIE barrel were $-53.3 \pm 5.0$ and $-53.0 \pm 4.9$ mV ($n = 8$), respectively, in the control conditions. The mean difference between the two barrels was $0.5 \pm 0.8$ mV ($n = 8$), NS. No impalement acceptable according to our criteria (see the Methods section) presented a difference >4 mV between the two barrels. With two of these impalements, it was possible to carry out all the experimental maneuvers (amiloride, low K⁺ bath [shown in Fig. 7], amiloride and low K⁺ bath, ouabain, amiloride and ouabain) used with the other types of electrode. The difference of the voltage between the two

² The observed fractional decrease of the apical Na⁺ permeability is larger than the fractional change in apical Na⁺ conductance, a difference that is explained by the higher intracellular Na⁺ activity after ouabain treatment.
barrels never exceeded 4 mV, except for short periods (<10 s) immediately after a solution change that induced a large change of $V_{bl}$. This type of difference might have been due to the difference of the response time of the two barrels (RLIE barrels had a 95% response time of 1–2 s) and/or to transitory changes of the sum of the intracellular cation activities.

**DISCUSSION**

In the present paper we report the first measurements of the intracellular Na⁺ and K⁺ activities in amphibian collecting tubule cells, and provide evidence that the transcellular transport of Na⁺ in this epithelium is explained well by the model of Koefoed-Johnsen and Ussing (1958). First, the large decrease of $a_{Na,i}$ after the addition of amiloride to the luminal fluid shows that the main entry pathway of Na⁺ into the cell is an apical conductance. Second, the increase of $a_{Na,i}$ and the decrease of $a_{K,i}$ produced by ouabain or by lowering K⁺ in the bath solution demonstrate that the active step of the transcellular transport is a K⁺-dependent, ouabain-sensitive basolateral Na-K exchange pump. The immediate voltage changes after pump inhibition or reactivation also indicate that this pump is electrogenic. Finally, measurements of

![Figure 7](image-url)
the apical and basolateral membrane conductances show that both conductances are reduced when the pump is inhibited. All the experiments were performed on tubules taken from *Amphiuma* previously exposed to a high K⁺ environment. This treatment has been shown to increase the blood level of aldosterone in these animals (Oberleithner et al., 1983a) and to increase the transcellular Na⁺ transport and the apical membrane Na⁺ conductance (Horisberger et al., 1987). Thus the present results apply to a tight epithelium in which the transport rate is stimulated by a high level of mineralocorticoid. The rate and the magnitude of the changes of intracellular ionic activities and membrane conductances are probably larger under these conditions than in an unstimulated epithelium.

**Intracellular Na⁺ and K⁺ Activities**

The values of intracellular Na⁺ activity in the collecting tubule cells of *Amphiuma* are identical to those reported for another "tight" epithelium, the frog skin epithelium (Garcia-Diaz et al., 1986); they are also similar to those of the cells of the diluting segment of *Amphiuma* (Oberleithner et al., 1983c), the proximal tubule of *Ambystoma* (Tripathi et al., 1985), and of *Necturus* (Cemerikic and Giebisch, 1980). The intracellular K⁺ activities are slightly lower than those in the diluting segment of *Amphiuma* (Oberleithner et al., 1983b), but lower total bath osmolarity in our conditions may account for this difference. In the proximal tubule of *Necturus* (with a bath of similar osmolarity), the K⁺ activities were similar (Kubota et al., 1983).

The rapid decrease of aNai after luminal amiloride is consistent with a two-membrane cell model of the *Amphiuma* collecting tubule, in which amiloride blocks the major influx pathway for Na⁺ and the basolateral Na-K pump continues to extrude Na⁺ ions until a new steady state value is reached. The increase of the intracellular K⁺ activity is a consequence of the hyperpolarization of the basolateral membrane: amiloride induces an immediate increase of Vm to a value near the equilibrium potential for K⁺ (Eₓ) and the driving force for K⁺ exit becomes small (ΔμK/F = Eₓ - Vm) (it decreased from 29 ± 7 to 6 ± 2 mV, n = 6, P < 0.05), so that the K⁺ efflux across the basolateral membrane conductance becomes negligible. Thus, as long as the pump is still transporting K⁺ into the cell in exchange for Na⁺, aNai rises. When amiloride is removed, the reverse sequence of events takes place: Na⁺ enters the cell passively along an increased chemical gradient across the apical membrane and the induced transcellular current depolarizes the basolateral membrane. As a consequence, the driving force for K⁺ exit increases, producing a large K⁺ outflow. Thus aNai increases and aK increases until the pump rate, which depends on aNai (Turnheim et al., 1983), reaches a new steady state value.

**The Apical Membrane**

Inhibition of the Na-K pump and the subsequent changes of the intracellular Na⁺ and K⁺ activities were uniformly followed by a reduction of the apical membrane Na⁺ conductance. The apical membrane Na⁺ conductance of several tight epithelia has been shown to conform to the prediction of the Goldman-Hodgkin-Katz "constant field" equation (Fuchs et al., 1977; Thompson et al., 1982; Palmer, 1985). Thus we assume that the conductance of the apical membrane of the *Amphiuma* collecting tubule also conforms to the "constant field" model of membrane ionic...
conductance. Considering the increase of the intracellular Na\(^+\) activity, the measured decrease of the apical conductance corresponds to an even larger decrease of the apical permeability to Na\(^+\). These findings are consistent with the hypothesis that the apical Na\(^+\) permeability is down-regulated by the intracellular Na\(^+\) activity; this is also well documented in amphibian tight epithelia; and the mammalian bladder and colon (Larsen, 1973; Helman et al., 1979; Chase and Al-Awqati, 1981; Turnheim et al., 1983; Lewis and Hanrahan, 1985). Our data show that a similar phenomenon is present in renal tubular cells. Furthermore, in accordance with other investigators (Garty and Lindemann, 1984) we observed a decrease of the apical conductance only after Na\(^+\) had reached a high intracellular level (>20 mM).

The decrease of the Na\(^+\) conductance of the apical membrane observed after inhibition of the pump was not reversible within the time course of our experiments. Thus, the apical Na\(^+\) conductance remained reduced over the time period of our measurements (5–15 min). It is possible that recovery from the feedback inhibition might have occurred over a longer time period. Two points are relevant. First, we have in general observed a slow, spontaneous, and moderate decline of the apical membrane conductance in perfused collecting tubule, an event that could make it difficult to detect a slow recovery process. Second, Garty and Lindemann (1984) have also observed a very protracted time course of the recovery of the apical membrane Na\(^+\) conductance after it had been reduced by pump inhibition. They invoked slow Ca\(^{2+}\) extrusion as a possible cause. Whether a similar situation may explain our observations is presently not known.

**The Basolateral Na-K Pump is Electrogenic**

When the bath K\(^+\) concentration is decreased from 3.0 to 0.1 mM, \(V_m\) hyperpolarizes, an effect consistent with a K\(^+\) selective basolateral membrane. When, 4 min later, K\(^+\) is suddenly reintroduced, we do not observe the reverse effect, i.e., a membrane depolarization, but a significant hyperpolarization of \(-23\) mV. This change of \(V_m\) is in the opposite direction to that predicted for a passive conductance, and indicates that K\(^+\) activates an electrogenic mechanism in the basolateral membrane. The ensuing increase of \(a_{K_i}\) and decrease of \(a_{N,a}\) indicate that this electrogenic process is due to the activity of the Na-K pump (Graf and Petersen, 1974; Biagi et al., 1981). Furthermore, ouabain also produced a fast (10 s) depolarization of \(V_m\), an effect also consistent with inhibition by this drug of an electrogenic transport mechanism (Helman et al., 1979). The rheogenicity of the Na-K pump has already been demonstrated in the similar tight epithelium of the toad urinary bladder (Narvarte and Finn, 1985).

**The Na-K Pump Activity and the Basolateral K\(^+\) Conductance**

Earlier studies (Hunter et al., 1987; Horisberger et al., 1987) have demonstrated by transference number measurement that the K\(^+\)-specific conductance represents 70–80% of the total basolateral membrane conductance of the collecting tubule of *Amphiuma*. Thus, the large decrease (up to 90%) of the basolateral conductance measured after inhibition of the Na-K pump must be due, at least for the main part, to changes of a K\(^+\)-conductive pathway, even if the relative contribution of K\(^+\) to the total basolateral membrane conductance may change.
Several recent studies have shown that after alterations in Na-K pump activity, there are rapid and proportional changes in ionic permeability of the basolateral membrane (Davis and Finn, 1982; Chase, 1984; Cohen and Giebisch, 1984). The available evidence is consistent with the idea that these changes are the result of modifications in the basolateral K⁺ conductive pathway. Several mechanisms have been proposed by which the conductance of the basolateral membrane could be regulated.

One hypothesis proposes a direct and immediate link between the basolateral Na-K pump and the K⁺ conductance (Anner, 1981; Schultz, 1985); the "pump" and "leak" functions may be performed by a single membrane protein. Thus, an increase in the pump activity would be inherently linked to an increase of the K⁺ conductance. This view is supported by the fact that the basolateral membrane conductance decreases when the pump rate is reduced in conditions where intracellular Na⁺ either is low (luminal amiloride) (Davis and Finn, 1982) or high (pump inhibition) (Helman et al., 1979; Matsumura et al., 1984).

We can investigate the relations between the pump rate and the basolateral conductance using simple equivalent circuit analysis as shown in Fig. 8 (Boulpaep and Sackin, 1979). We can define two main components of the basolateral potential change when K⁺ is removed or added to the bath solution: (a) ΔVₚₚ, the change of Vₙₐ due to the modification of the basolateral electromotive force (EMF) produced by the K⁺ gradient across this membrane, and (b) ΔVₚₑₚ, the change of Vₙₐ generated by the electrogenic Na-K pump (assuming a constant Na⁺/K⁺ coupling ratio):

$$ΔV_{pump} = Δi_{pump}R_\text{ap}(R_a + R_s)/(R_a + R_\text{ap} + R_s)$$

and

$$ΔV_K = ΔE_{Kb}(R_a + R_s)/(R_a + R_\text{bl} + R_s),$$

where Δiₚₑₚ is the change of the pump current, ΔEₖ is the change of the basolateral EMF due to K⁺, and Rₐ, Rₚₚₚ, Rₛ, Rₖₗ are the apical, basolateral, and shunt resistance, respectively. If we neglect the smaller effect on Vₙₐ of the change of the basolateral internal resistance. The experiments and the analysis were all done in open circuit condition (zero net transepithelial current). The change of the bath K⁺ concentration directly affected two elements (a) the pump, whose activity is dependent on external K⁺ and (b) Eₖ. The effects of changes in the bath K⁺ concentration on Rₙₐ were neglected.
conductance, the change of $V_m$ when $K^+$ is added or removed from the bath solution is equal to the sum of the two components defined above:

$$\Delta V_m = \Delta V_{\text{pump}} + \Delta V_K.$$  \hfill (9)

It is interesting to compare the change of $V_m$ induced by the low $K^+$ bath in the presence and the absence of luminal amiloride. When amiloride is present in the lumen, little $Na^+$ enters the cell and the pump rate is reduced. Accordingly, the $\Delta V_{\text{pump}}$ term of Eq. 9 can be neglected, then $\Delta V_m$ equals approximately $\Delta V_K$, and the removal and reintroduction of $K^+$ in the bath produced changes of $V_m$ of opposite direction and very similar magnitude (see Fig. 5). Although we could not measure the basolateral conductance directly when the apical membrane conductance had been blocked by amiloride, we can deduce from the pattern of $V_m$ changes that there were no large modifications of $G_{bl}K^+$ during the low $K^+$ period and/or no large increases of the pump current when $K^+$ was reintroduced.

In contrast, when no amiloride was present in the lumen (Fig. 3), the change of $V_m$ after the introduction of the low $K^+$ bath was $-26$ mV, but when $K^+$ was suddenly reintroduced there was a hyperpolarization of $-23$ mV. As the $E_K$ change is of similar value but opposite sign, there must have been large changes in the basolateral resistance ($R_{bl}$) and/or in the pump current ($i_{\text{pump}}$) (see Eqs. 7–9). We have indeed measured a 10-fold increase of $R_{bl}$, and the high $a_{\text{eq}}K^+$ observed at this point might explain a larger $i_{\text{pump}}$. Thus, immediately after reduction of the bath $K^+$ concentration the basolateral $K^+$ conductance is large, while the pump current is minimal (as demonstrated by the rising $a_{\text{eq}}K^+$ and the decreasing $a_{\text{eq}}iK^+$). In contrast, immediately after reintroduction of a 3.0 mM $K^+$ concentration to the bath solution, the pump current was large and the basolateral conductance was considerably reduced. The observation that $G_{bl}$ uncouples from $i_{\text{pump}}$ argues against the hypothesis of a direct tight link between the size of the $K^+$ conductance and the activity of the Na-K pump.

If the basolateral membrane conductance is not directly linked to the pump activity, can the decrease of this conductance after inhibition of the pump then simply be explained by the reduction of the intracellular $K^+$ activity?

The dependence of the conductance on the $a_{\text{eq}}iK^+$ is often assumed to obey the Goldman “constant field” model. With this assumption, we have calculated what the predicted reduction of $G_{bl}$ would be if it were due to the change of $a_{\text{eq}}iK^+$. This calculation was made by using the mean values of $V_m$, $G_{bl}$, and $a_{\text{eq}}i$ under control conditions (values given in Table II) and Eq. 6 to estimate the $K^+$ permeability coefficient. This yielded a value of $P_K$ equal to $94 \times 10^{-6}$ cm $\cdot$ s$^{-1}$. Using this value of the $K^+$ permeability and the mean values of $V_m$ and $a_{\text{eq}}i$ after a 4-min low $K^+$ bath exposure (values given in Table II), the slope conductance was calculated by means of the derivative of the Goldman equation (Matsumura et al., 1984). The measured values of $G_{bl}$ under control conditions and after low $K^+$ were $4.4 \pm 0.8$ and $0.5 \pm 0.1$ mS $\cdot$ cm$^{-2}$, and the calculated value in the low $K^+$ bath was $1.4$ mS $\cdot$ cm$^{-2}$. Thus, the decrease of $a_{\text{eq}}i$ accounts only for 38% of the observed reduction in basolateral $K^+$ conductance after low $K^+$ exposure. A similar calculation was performed with the potential, conductance, and $a_{\text{eq}}i$ values before and after ouabain. The $P_K$ under control conditions was $58 \times 10^{-6}$ cm $\cdot$ s$^{-1}$, and, using this value, a $G_{bl}$ of 1.8 mS $\cdot$ cm$^{-2}$ was
calculated for the \( a_{\text{Ki}} \) and the basolateral membrane potential measured 5 min after ouabain, at which time the measured value of \( G_b \) was 0.5 \( \pm \) 0.1 mS \( \cdot \) cm\(^{-2}\). Therefore, the change of \( a_{\text{Ki}} \) accounts only for 28% of the observed reduction of \( G_b \).

Furthermore, after reactivation of the Na-K pump, normal \( a_{\text{Ki}} \) levels were rapidly attained, whereas \( G_b \) took a longer time to fully recover (Table II, Fig. 3). Thus, the "constant field" model with a constant permeability does not account for the reduction of the basolateral conductance observed after inhibition of the pump. Then, we can examine the two following hypotheses to explain the observed results: First, the basolateral \( K^+ \) conductance does obey the constant field model, but the permeability of the membrane is altered by a modification of the intracellular composition secondary to the blockage of the pump. Evidence for a blocking effect of the intracellular Na\(^+\) ions on \( K^+ \) conductances has been presented by Kawahara et al. (1987) and by Gitter et al. (1987). Changes in cytosolic calcium activity have also been proposed as a possible mechanism of the regulation of basolateral membrane conductance (Chase, 1984; Schultz, 1981). Finally, the intracellular pH could also control the basolateral \( K^+ \) conductance (Matsumura et al., 1984).

The second hypothesis postulates that the basolateral \( K^+ \) conductance is different from that predicted by the constant field model either because of an "anomalous" voltage dependency or because of a dependency on an \( a_{\text{Ki}} \) that is "steeper" than that predicted by the model. Indeed, two important observations indicate that the effect of the basolateral membrane voltage on the conductance of this membrane does not obey the constant field model. First, we observed a brief, statistically significant, increase of the basolateral membrane conductance that coincided with \( V_b \) hyperpolarization immediately after the change from normal to low \( K^+ \) concentration in the bath. A conductance conforming to the constant field model would be expected to undergo a large decrease, due to both the lower mean \( K^+ \) concentration and the hyperpolarization. Second, we also observed a statistically significant decrease of the basolateral conductance, 10–15 s after the addition of ouabain to the bath, coinciding with the fast decrease of the basolateral membrane potential, but before major changes in the intracellular activities of Na\(^+\) and \( K^+ \) had occurred. These observations strongly suggest that the basolateral \( K^+ \) conductance is larger at higher negative potentials or, in other words, is inward-rectifying and differs from that predicted by the constant field model of membrane conductance. Several investigators have indeed observed inward-rectifying properties of the basolateral membrane of Na\(^+\)-transporting epithelia (Boulpaep, 1966; Nagel, 1985), and others have reported a linear basolateral membrane conductance in conditions where the \( K^+ \) gradient and the constant field model predicted outward rectification (Schultz et al., 1984). However, results consistent with the constant field model have also been reported (Wills et al., 1979; Thompson et al., 1982; Schoen and Erlij, 1985). In studies on the isolated collecting tubule of \textit{Amphiuma}, in conditions similar to those of the present study, we have indeed recently observed inward-rectifying properties of the basolateral membrane conductance, which confirms the expectation that the basolateral \( K^+ \) conductance increases with hyperpolarization of the membrane (Horisberger and Giebisch, 1987).

If the basolateral membrane conductance is indeed inward rectifying, the effect of the intracellular \( K^+ \) activity on the basolateral membrane conductance is com-
plex: a lower \( a_{K} \) will reduce the basolateral conductance by simply decreasing the concentration of conductive ions (effect present in the constant field model), but a lower \( a_{K} \) will also decrease the basolateral conductance because of its effect on the membrane potential (a lower \( a_{K} \) means a lower \( E_{K} \), and, because \( E_{K} \) is the main determinant of the basolateral membrane potential, a lower \( V_{m} \)). Thus the reduction of the basolateral conductance, due to a decreased \( a_{K} \), will be more pronounced than that which is predicted by the constant field model.

In summary, the large reduction of the basolateral membrane \( K^{+} \) conductance that we have observed after inhibition of the pump can be explained by the combined effects of a decrease of the intracellular \( K^{+} \) activity and an inward-rectifying basolateral membrane conductance. An additional effect of the intracellular \( Na^{+} \), \( H^{+} \), or \( Ca^{2+} \) activities is also possible.

J.-D. Horisberger was supported by the Fogarty International Center. This work was supported by National Institutes of Health grant AM-17435.

Original version received 7 July 1987 and accepted version received 19 May 1988.

REFERENCES

Anner, B. M. 1981. A K-selective cation channel formed by Na-K-ATPase in liposomes. Biochemistry International. 2:265–371.

Biagi, B. A., M. Sotthell, and G. Giebisch. 1981. Intracellular potassium activity in the rabbit proximal straight tubule. American Journal of Physiology. 241:677–686.

Boulpaep, E. L. 1986. Potassium and chloride conductances of the peritubular membrane of proximal tubular cells of Necturus kidney. Biophysical Journal. 6:133. (Abstr.)

Boulpaep, E. L., and H. Sackin. 1979. Equivalent electrical circuit analysis and rheogenic pump in epithelia. Federation Proceedings. 38:2030–2036.

Cemerikic, D., and G. Giebisch. 1980. Intracellular sodium activity in Necturus kidney proximal tubule. Federation Proceedings. 39:1080. (Abstr.)

Chase, H. S., Jr., and Q. Al-Awqati. 1981. Regulation of the sodium permeability of the luminal border of toad bladder by intracellular sodium and calcium. Journal of General Physiology. 77:693–712.

Chase, H. S., Jr. 1984. Does calcium couple the apical and basolateral membrane permeabilities in epithelia. American Journal of Physiology. 247:869–876.

Cohen, B., and G. Giebisch. 1984. Relationship between potassium conductance and transport in renal tubular epithelium. In Information and Energy Transduction in Biological Membrane. C. L. Bolis, E. J. M. Helnreicher, and H. Passow, editors. A. R. Liss, New York, 391–401.

Davis, C. W., and A. L. Finn. 1982. Sodium transport inhibition by amiloride reduces basolateral membrane potassium conductance in tight epithelia. Science. 216:525–527.

Edelman, A., S. Curci, I. Samarzija, and E. Frömter. 1978. Determination of intracellular \( K^{+} \) activity in rat kidney proximal tubular cells. Pflügers Archiv. 378:37–45.

Fuchs, W., E. H. Larsen, and B. Lindemann. 1977. Current-voltage curve of sodium channels and concentration dependence of sodium permeability in frog skin. Journal of Physiology. 267:137–166.

Garcia-Diaz, J. F., G. Klemperer, L. M. Baxendale, and A. Essig. 1986. Cell sodium activity and sodium pump function in frog skin. Journal of Membrane Biology. 92:37–46.

Garty, H., and B. Lindemann. 1984. Feedback inhibition of sodium uptake in \( K^{+} \)-depolarized toad urinary bladders. Biochimica et Biophysica Acta. 771:89–98.
Gitter, A. H., K. W. Beyenbach, C. W. Christine, P. Gross, W. W. Minuth, and E. Fromter. 1987. High-conductance K⁺ channel in the apical membranes of principal cells cultured from rabbit renal cortical collecting duct anlagen. *Pflügers Archiv*. 408:282–290.

Graf, J., and O. H. Petersen. 1974. Electrogenic sodium pump in liver parenchymal cells. *Proceedings of the Royal Society of London, Series B*. 187:363–367.

Helman, S. I., W. Nagel, and R. S. Fischer. 1979. Ouabain on active transepithelial sodium transport in frog skin. Studies with microelectrodes. *Journal of General Physiology*. 74:105–127.

Henderson, R. M., P. B. Bell, R. D. Cohen, C. Browning, and R. A. Iles. 1986. Measurement of intracellular pH with microelectrodes in rat kidney in vivo. *American Journal of Physiology*. 250:209–209.

Horisberger, J.-D., and G. Giebisch. 1987. Current-voltage (I/V) relation of the basolateral membrane of the collecting tubule (CT) of *Amphiuma*. *Abstracts of the Xth International Congress of Nephrology (London)*. 571. (Abstr.)

Horisberger, J.-D., M. Hunter, B. A. Stanton, and G. Giebisch. 1987. The collecting tubule of *Amphiuma*. II. Effects of potassium adaptation. *American Journal of Physiology*. 253:1273–1282.

Hunter, M., J.-D. Horisberger, B. A. Stanton, and G. Giebisch. 1987. The collecting tubule of *Amphiuma*. I. Electrophysiological characterization. *American Journal of Physiology*. 253:1263–1272.

Jorgensen, P. L. 1980. Sodium and potassium ion pump in kidney tubules. *Physiological Reviews*. 60:864–917.

Kawahara, K., M. Hunter, and G. Giebisch. 1987. Potassium channels in *Necturus* proximal tubule. *American Journal of Physiology*. 253:488–494.

Koefoed-Johnsen, V., and H. H. Ussing. 1958. The nature of the frog skin potential. *Acta Physiologica Scandinavica*. 42:298–308.

Kubota, T., B. A. Biagi, and G. Giebisch. 1983. Effects of acid base disturbances on basolateral membrane potential and intracellular potassium activity in the proximal tubule of *Necturus*. *Journal of Membrane Biology*. 73:61–68.

Larsen, E. H. 1973. Effect of amiloride, cyanide and ouabain on the active transport pathway in toad skin. In *Transport Mechanism in Epithelia*. H. H. Ussing, editor. Munskgaard, Copenhagen, 131–143.

Lewis, S. A., and J. W. Hanrahan. 1985. Apical and basolateral membrane ionic channels in rabbit urinary bladder epithelium. *Pflügers Archiv*. 405(Suppl. 1):S83–S88.

Matsumura, Y., B. Cohen, W. B. Guggino, and G. Giebisch. 1984. Regulation of the basolateral potassium conductance of the *Necturus* proximal tubule. *Journal of Membrane Biology*. 79:153–161.

Nagel, W. 1985. Basolateral membrane ionic conductance in frog skin. *Pflügers Archiv*. 405:39–45.

Narvarte, J., and A. L. Finn. 1985. Effects of intracellular sodium and potassium iontophoresis on membrane potentials and resistances in toad urinary bladder. *Journal of Membrane Biology*. 84:1–7.

Oberleithner, H., F. Lang, W. Wang, G. Messner, and P. Deetjen. 1983a. Evidence for an amiloride-sensitive Na pathway in the amphibian diluting segment induced by K adaptation. *Pflügers Archiv*. 399:166–172.

Oberleithner, H., W. B. Guggino, and G. Giebisch. 1983b. Potassium transport in the early distal tubule of *Amphiuma* kidney. Effects of potassium adaptation. *Pflügers Archiv*. 396:185–191.

Oberleithner, H., F. Lang, R. Greger, W. Wang, and G. Giebisch. 1983c. Effect of luminal potassium on cellular sodium activity in the early distal tubule of *Amphiuma* kidney. *Pflügers Archiv*. 396:34–40.
Horisberger and Giebisch  Intracellular Na⁺ and K⁺ Activities, and Membrane Conductances  665

Palmer, L. G. 1985. Modulation of apical Na permeability of the toad urinary bladder by intracellular Na, Ca, and H. Journal of Membrane Biology. 83:57–69.
Robinson, R. A., and R. H. Stokes. 1959. Electrolyte Solutions. Butterworths, London. 571 pp.
Schoen, H. F., and D. Ertl. 1985. Current-voltage relations of the apical and basolateral membranes of the frog skin. Journal of General Physiology. 86:257–287.
Schultz, S. G. 1980. Basic principles of membrane transport. Cambridge University Press, Cambridge. 1–144.
Schultz, S. G. 1981. Homocellular regulatory mechanisms in sodium-transporting epithelia: avoidance of extinction by “flush-through.” American Journal of Physiology. 241:579–590.
Schultz, S. G., S. M. Thompson, R. L. Hudson, S. R. Thomas, and Y. Suzuki. 1984. Electrophysiology of Necturus urinary bladder. II. Time-dependent current-voltage relations of the basolateral membranes. Journal of Membrane Biology. 79:257–269.
Schultz, S. G. 1985. Regulatory mechanisms in sodium-absorbing epithelia. In The Kidney: Physiology and Pathophysiology. D. W. Seldin and G. Giebisch, editors. Raven Press, New York. 189–198.
Thomas, R. C., and C. J. Cohen. 1981. A liquid ion-exchanger alternative to KCl for filling intracellular reference microelectrodes. Pflügers Archiv. 390:96–98.
Thompson, S. M., Y. Suzuki, and S. G. Schultz. 1982. The electrophysiology of the rabbit descending colon. I. instantaneous transepithelial current-voltage relations and the current-voltage of the Na-entry mechanism. Journal of Membrane Biology. 66:41–54.
Tripathi, S., N. Morgunov, and E. L. Boulpaep. 1985. Submicron tip breakage and silanization control improve ion-selective microelectrodes. American Journal of Physiology. 249:514–521.
Turnheim, K., S. M. Thompson, and S. G. Schultz. 1983. Relation between intracellular sodium and active sodium transport in rabbit colon: current-voltage relations of the apical sodium entry mechanism in the presence of varying luminal sodium concentrations. Journal of Membrane Biology. 76:299–309.
Wills, N. K., D. C. Eaton, S. A. Lewis, and M. S. Ifshin. 1979. Current-voltage relationship of the basolateral membrane of a tight epithelium. Biochimica et Biophysica Acta. 555:519–523.
Wills, N. K., and S. A. Lewis. 1980. Intracellular Na⁺ activity as a function of Na⁺ transport rate across a tight epithelium. Biophysical Journal. 30:181–186.