J. Membrane Biol. 79, 153–161 (1984)

The Journal of
Membrane Biology

Regulation of the Basolateral Potassium Conductance of the *Necturus* Proximal Tubule*

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Summary. Two methods, the measurement of the response of the basolateral membrane potential (V_{bl}) of proximal tubule cells of *Necturus* to step changes in basolateral K^+ concentration, and cellular cable analysis, were used to assess the changes in basolateral potassium conductance (G_K) caused by a variety of maneuvers. The effects of some of these maneuvers on intracellular K^+ activity (a^i_K) were also evaluated using double-barreled ion-selective electrodes. Perfusion with 0 mM K^+ basolateral solution for 15 min followed by 45 min of 1 mM K^+ solution resulted in a fall in basolateral potassium (apparent) transference number (t_K), V_{bl} and a^i_K. Results of cable analysis showed that total basolateral resistance, R_b, rose. The electrophysiological effects of additional manipulations, known to inhibit net sodium reabsorption across the proximal tubular epithelium of *Necturus*, were also investigated. Ouabain caused a fall in t_K accompanied by large decreases in a^i_K and V_{bl}. Lowering luminal sodium caused a fall in t_K and a small reduction in V_{bl}. Selective reduction of peritubular sodium, a maneuver that has been shown to block sodium transport from lumen to peritubular fluid, also resulted in a significant decrease in t_K. These results suggest that G_K varies directly with rate of transport of the sodium pump, irrespective of the mechanism of change in pump turnover.

Key Words potassium conductance · basolateral membrane · *Necturus* · proximal tubule · sodium-potassium pump

Introduction

A ouabain-sensitive sodium-potassium ATPase (pump) and a potassium conductance (leak) are integral parts of the basolateral membrane of a variety of epithelial transporters (Ussing & Leaf, 1978). Recent work in epithelia has shown that changes in sodium transport often do not result in changes in intracellular K^+ activity despite the expected increase in inward flux of K^+ through the pump (White, 1976). Further work has suggested that this is due to parallel changes in pump rate and potassium conductance G_K (Davis & Finn, 1982*a*; Grasset, Gunter-Smith & Schultz, 1983). Schultz (1981) has reviewed this recently and discussed some of the possible mechanisms that could account for the relationship between pump rate and potassium conductance. In light of what is known about the presence of calcium-sensitive K^+ pathways in several tissues (Schwarz & Passow, 1983), a change in intracellular calcium is a prime candidate. Alternatively, ATP or other metabolic factors, thought to regulate the calcium sensitivity of some of these pathways, could be involved (Lew & Ferreira, 1976; Romero, 1978; Fink, Hase, Lüttgau & Wettwer, 1983). Another type of explanation involves an inherent coupling of the pumps and leaks, either through simultaneous addition or removal of both units, or through a structural link between the two (Blum & Hoffman, 1971).

We have studied the changes in G_K in the basolateral membrane of the *Necturus* proximal tubule during several maneuvers known to alter the rate of active transport. These manipulations often had opposite effects on certain cell parameters (pH, Ca^{++}, a^i_{Na}, a^i_K) yet similar effects on G_K. This suggests that the changes in G_K were brought about by a change in pump turnover per se or, alternatively, that several factors are important in regulating the relationship between pump rate and potassium conductance.

Materials and Methods

Kidney Preparation and Solutions

Kidney preparation was similar to that reported in a companion paper (Matsumura, Cohen, Guggino & Giebisch, 1984). Peritu-

* Part of this material has been presented at the 10th International Conference on Biological Membranes (Cohen & Giebisch, 1984).

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bular flow-rate was 2.6 ml/min in transference number experiments and 1.0 ml/min in the cable experiments. Peritubular solution changes were made by changing portal-perfusion solution only and luminal changes by aortic perfusion change only.

Solutions

The composition of the control Ringer was 100.5 mM Na^+, 2.5 mM K^+, 1.8 mM Ca^{++}, 1.0 mM Mg^{++}, 98.1 mM Cl^-, 10.0 mM HCO_3^-, 0.5 mM $H_2PO_4^-$, 15 g/liter polyvinylpyrrolidone (PVP), 0.4 g/liter glucose and 2000 units/liter heparin. pH was maintained at 7.6 by bubbling with a 99% O_2/1% CO_2 mixture. The zero-K and 1.0-K solutions were made by deleting the appropriate amount of KCl and adding 2.5 and 1.5 mM mannitol, respectively, as partial osmotic compensation. The 10-K solution was made using an equimolar substitution of KCl for NaCl. In the 0.5 mM Na solution, 90 mM BIDAC (Bis(2-hydroxyethyl) dimethylammonium chloride) and 10 mM tetramethylammonium hydroxide (TMAOH) substituted for equimolar amounts of NaCl and $NaHCO_3$. In the 10.5 mM Na solutions the TMAOH was not used and in the 10 K-10.5 Na solution, equimolar substitution of KCl for BIDAC was made.

Changes in peritubular perfusion solution were made by way of a four-way stopcock placed near the site of caudal vein cannulation. All experiments were performed at room temperature (19–22°C). Ouabain (Sigma Chemical Co., St. Louis, MO) was added to the peritubular solution in some experiments.

Electrical Potential Measurements and Calculation of Transference Number

The measurement of membrane potentials and the calculation and interpretation of apparent transference numbers for potassium (t_K) have been described in detail in the previous paper (Matsumura et al., 1984). Double-barreled electrodes (*see* below) and electrodes used in cable analysis were beveled as described previously (Guggino, London, Boulpaep & Giebisch, 1983).

The problem of attenuation in electrical response due to current loops has been discussed elsewhere (Matsumura et al., 1984). It is unlikely that a change in this attenuation factor produced the alterations in the apparent transference numbers reported here. In these experiments decreases in t_K were found at a time when the only other expected significant resistance change would have been an increase in R_a (apical membrane resistance). This alone would have caused an increase in apparent potassium transference number and therefore could not account for the results.

Measurement of Intracellular Potassium Activity

Intracellular potassium activity (a_K^i) was measured with double-barreled potassium selective microelectrodes either according to the methods of Fujimoto and Kubota (1976) or by using a different silanizing agent as described below. Double-barreled microelectrodes were pulled using the glass described above. The reference barrel was filled with acetone initially. The tip was then dipped for 30 sec in a 1.5% solution (vol/vol) of silanizing agent A137 (Pharmacia Fine Chemicals, Piscataway, NJ) in 1-chloronaphthalene. The electrode was then heated for 1 hr on a heating plate set at 300°C. Corning 477317 exchanger was introduced into the tip by backfilling with a syringe with polyethylene tubing (pulled to a fine tip) attached. The K^+-sensitive electrode was then backfilled with 1.0 M KCl and the reference barrel with 1.0 M NaCl. The ion-sensitive barrel and the reference barrel were connected to the *A* and *B* inputs, respectively, of a high input impedance dual-differential electrometer (F223A, W-P Instrument, New Haven, CT). The *A*, *B* and *A-B* signals were recorded on the Gould 220 recorders.

The mean slope of the potassium electrodes used in this study was 58 ± 2 mV/tenfold change in potassium activity. Selectivity constant for sodium compared with potassium (K_{KNa}) was calculated from the equation $E = E_o + S \log(a_K + K_{KNa}a_{Na})$ where E is the voltage of the ion-sensitive electrode in a solution of K activity a_K and sodium activity a_{Na}. S is the slope of the electrode as determined from calibration in solutions of varying activities of pure KCl, and E_o is a constant for a given electrode. Solutions of 5, 10, 50 and 100 mM pure KCl or pure NaCl were used, and the mean K_{KNa} for the first eight electrodes used in this study was 0.036 ± 0.007. Thus interference by sodium on the measurement of a_K^i can be ignored as previous studies from this laboratory have demonstrated that normal a_{Na}^i is less than 10 mM (Cemerikic & Giebisch, 1980) and rises to 30 mM with 1 hr of ouabain perfusion. Even in the latter case the interference would be equivalent to less than 1.5 mM potassium. The problem is more significant in the external solution, so the intracellular activity was calculated from the equation:

$$a_K^i = 76\ (10^{[(V_K^i - V_K^{0.1M})/S]})$$

where V_K^i is the *A–B* value recorded inside the cell and $V_K^{0.1M}$ is the value recorded by the ion-sensitive barrel *in vitro* in a 0.1 M KCl solution (with K^+ activity of 76 mM). S is the slope of the electrode as discussed above.

Cable Analysis

In order to estimate the basolateral membrane resistance (R_b) under free flow conditions, two measurements were needed. These are α, the ratio of apical membrane resistance to basolateral membrane resistance (R_a/R_b), and R_z, the apical and basolateral membrane resistances in parallel $(R_aR_b)/(R_a + R_b)$. The theoretical and technical aspects of these measurements as well as their validity in this preparation have been explored in detail in recent work from this laboratory (Guggino, Windhager, Boulpaep & Giebisch, 1982).

To measure α, a current-passing electrode was introduced into the tubular lumen and 0.5-sec pulses of either 1×10^{-7} or 5×10^{-7} A were delivered. A second electrode at another site along the tubule was introduced first into the cell to measure the change in V_{bl} caused by the current pulses (ΔV_{bl}) and then advanced into the lumen at the same site to measure the transepithelial voltage deflections caused by the current (ΔV_{TE}) (*see* Fig. 1*a*). α was then calculated as $\frac{\Delta V_{TE}}{\Delta V_{bl}} - 1$. The validity of the use of α as an estimate for R_a/R_b in this preparation is discussed by Guggino et al. (1982). In most cases, two or three measurements of α were made in each tubule (minimum of one, maximum of four) at different distances from the current electrode. No systematic variation of α with distance was noted and variability was small so the measurements on a given tubule were averaged to provide a mean value for that tubule. This mean was then used to calculate the mean α and the mean $\frac{\alpha + 1}{\alpha}$ (for estimating R_b; *see* below) for the set of tubules under each condition.

R_z was measured from the decay pattern of the voltage response in the cell cable to current passed by an electrode within a cell. Depolarizing current pulses of either 2.5×10^{-8} or 1×10^{-7} A were injected through the intracellular current-passing electrode. The voltage deflections at two points along the tubule were measured by sequential impalements with a second electrode (Fig. 1*b*). The distance between current-passing and voltage-sensing electrodes was always greater than one tubule diameter and less than 700 μm. This distance was measured carefully with an ocular micrometer.

The voltage decay in this situation can be approximated by $\Delta V_{bl}^x = \Delta V_{bl}^o e^{-x/\lambda_c}$ where ΔV_{bl}^x is the voltage change recorded at distance x from the current electrode and ΔV_{bl}^o the calculated voltage deflection at the current source obtained by determining the intercept of a logarithmic plot of the ΔV_{bl} *vs.* distance obtained from the data points in a given tubule. λ_c, the length constant of the cellular cable, is determined from the inverse slope of this plot. R_{input} is then calculated from $\Delta V_{bl}^o/I$ (where I is the current used) and this is used to calculate the specific resistance of the cell cable, ρ_c, as:

$$\rho_c = \frac{4\pi a_o d\, R_{input}}{\lambda_c}$$

where a_o is the tubular radius and d, the thickness of the cell layer. R_z free-flow is then given by:

$$R_z = \frac{\rho_c \lambda_c^2}{d}$$

As

$$R_z = \frac{R_a R_b}{R_a + R_b}$$

and

$$\alpha = \frac{R_a}{R_b},$$

R_b can be estimated as

$$R_b = (R_z)\left(\frac{\alpha + 1}{\alpha}\right).$$

The α and R_z determinations were made in different sets of tubules to avoid impalement damage. Therefore the mean R_b was estimated in each experimental situation as $(\overline{R_z})\left(\overline{\frac{\alpha + 1}{\alpha}}\right)$ where $\overline{R_z}$ is the mean of R_z for all tubules in that group and $\left(\frac{\alpha + 1}{\alpha}\right)$ the mean of the value of $\frac{\alpha + 1}{\alpha}$ calculated for each tubule individually.

We have previously explored in detail the validity of cable analysis for measuring individual barrier resistance in this preparation (Guggino et al., 1982). One problem not discussed there is a statistical one arising from the technical problems that prevent doing all of the measurements in one tubule. In this study we needed only to measure the apical-to-basolateral resistance ratio (α) and resistance of the apical and basolateral membranes in parallel (R_z) to derive a measure of R_b. We felt though that measuring R_z in the same tubules as α would possibly lead to artifacts resulting from the multiple impalements required. We thus avoided a technical problem but added some statistical uncertainty. In a given tubule, $R_b = (R_z)\left(\frac{\alpha + 1}{\alpha}\right)$. Because the two measurements are done in separate tubules we can only use the product $(\overline{R_z})\left(\overline{\frac{\alpha + 1}{\alpha}}\right)$ to estimate $\overline{R_b}$. This is only correct if R_z and $\frac{\alpha + 1}{\alpha}$ are uncorrelated variables and therefore have a covariance equal to zero. (In general for variables X and Y, $(\bar{X})(\bar{Y}) + \mathrm{cov}(X,Y) = \overline{XY}$). In some tight epithelia, most resistance variation occurs in the apical membrane and hence α and R_z tend to be positively correlated (and therefore $\frac{\alpha + 1}{\alpha}$ and R_z negatively correlated). There is no evidence that a similar relation holds in the proximal tubule. We also have no reason to think that any such correlation changes significantly from the control to the experimental situation (low K^+ perfusion). We thus assume that $(\overline{R_z})\left(\overline{\frac{\alpha + 1}{\alpha}}\right)$ gives us a reasonable estimate for R_b, or at least that the calculated change from control to low K^+ perfusion cells is a reasonable approximation. The lack of data on the covariance prevents us from doing tests of significance on the change in R_b (Eisenberg & Gage, 1969).

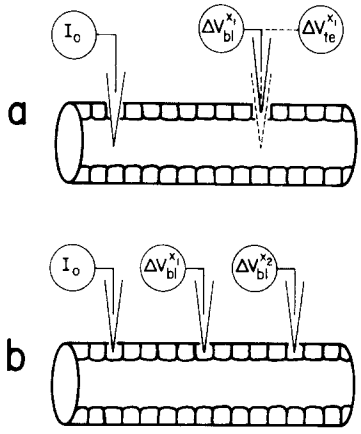

Fig. 1. Schematic of experimental set-up for cable analysis, showing (*a*) measurement of α and (*b*) measurement of R_z. *See* text for details

Statistics

Results are expressed as mean ± SEM. Significance was tested by paired or unpaired t test as noted below.

Results

Low K^+ Perfusion

In this set of experiments a kidney was initially perfused with control solutions and, at the start of the experimental period, the peritubular solution was switched to the zero-K solution for 15 min. Following exposure to the zero-K solution, a maintenance

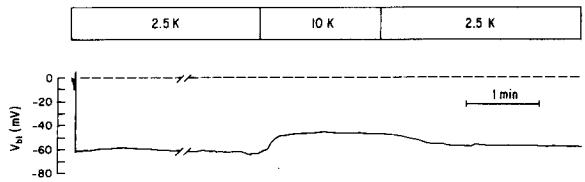

Fig. 2. Recording of the response of V_{bl} to a step increase in peritubular potassium concentration from 2.5 to 10 mM under control conditions. Some of the delay in the response from the time of the solution change is due to the transit time of the solution from stopcock to the animal. Impalement occurs at left

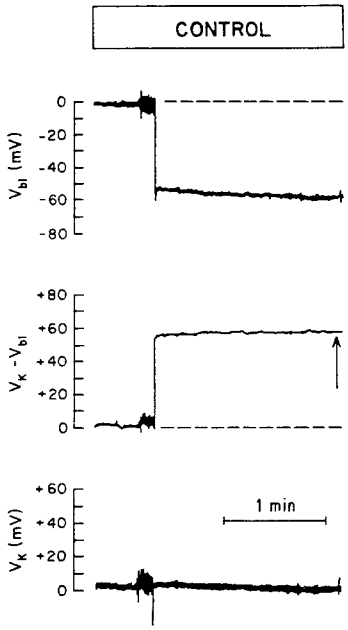

Fig. 3. Typical recording of intracellular potassium activity using a double-barreled microelectrode under control conditions. The top panel gives V_{bl} as measured by the reference barrel and the bottom panel the response of the ion-sensitive barrel. The middle panel gives the difference between the two. This signal is compared to the results of *in vitro* calibration to calculate the intracellular potassium activity which was 63 mM at the arrow. Potassium does not appear to be above equilibrium (bottom panel) because of interference by sodium when the electrode is still on the kidney surface

infusion containing 1.0 mM K was used for the remainder of the experimental period. Reduction in peritubular potassium has previously been found to reduce net fluid reabsorption in this preparation (Giebisch, Sullivan & Whittembury, 1973). The rise in intracellular sodium activity seen under these conditions (Cemerikic & Giebisch, 1980) also suggests that sodium-potassium pump rate has been reduced by this maneuver. All data were obtained after 30–45 min perfusion with the 1.0 mM K solution, a time when a new steady state had been reached. Control V_{bl}, t_K and a^i_K were determined in a separate group of animals. Typical recordings showing the determination of t_K and a^i_K under control conditions are shown in Fig. 2 and Fig. 3, respectively. Separate groups of control and experimental animals were also used for the cable analysis.

Table 1 shows V_{bl}, a^i_K and t_K values in the control and low K cells. Control t_K was 0.38 ± 0.03 using K steps from 2.5 to 10 mM and the experimental t_K was 0.09 ± 0.01 using steps from 1.0 to 10 mM ($P < 0.001$). In addition, using steps from 1.0 to 2.5 in both groups, the t_K was found to fall from 0.36 ± 0.06 ($n = 7$) in control to 0.17 ± 0.02 ($n = 8$) in the low K perfusion cells ($P < 0.01$). While there is no difference between the two control t_K's measured with different step changes, the two t_K's in low K^+ perfusion cells were significantly different ($P < 0.001$). This could be due to greater stimulation of the sodium-potassium pump by the larger step increase in potassium. Since the electrogenicity of the pump is particularly demonstrable after low K^+ perfusion (Biagi, Sohtell & Giebisch, 1981; Sackin & Boulpaep, 1981), the hyperpolarizing effect of pump stimulation may have reduced the depolarization caused by the effect of the K^+ increase on the conductive pathway. This would lead to an underestimate of the potassium transference number with the larger underestimate occurring when pump stimulation was greatest. That this effect is not responsible for all of the measured fall in t_K is shown by two findings. First, the mean driving force for K exit increased from 31 ± 2 mV ($n = 17$) in controls to 66 ± 3 mV ($n = 12$) in the experimental group ($P < 0.001$). Since apical potassium permeability is known to be low (Kimura & Spring, 1980), in the absence of a large change in pump coupling ratio (sodium to potassium), this implies that potassium conductance decreased as the decreased influx of potassium through the pump must be balanced by decreased exit through the leak. This is also borne out by the results of cable analysis, shown in Table 2. R_z increased significantly in the experimental group despite a lack of significant change in α, giving an increase in the estimated R_b. Thus, though a fall in intracellular potassium activity was found, this fall was minimized by a decrease in the leak pathway for potassium.

Pump Inhibition by Ouabain

Ouabain was applied in the peritubular solution at a concentration of 10^{-4} M, and measurements were made approximately 1 hr later. Control values were

Table 1. Effects of inhibition of transport on potassium transference number

	V_{bl} (mV)	a^i_K (mM)	t_K
Control	59 ± 2 (17)	67 ± 2 (17)	0.38 ± 0.03 (15)
Low K^+ perfusion	39 ± 2 (12)	49 ± 3 (12)	0.09 ± 0.01 (21)
	$P < 0.001$	$P < 0.001$	$P < 0.001$
Control	54 ± 1 (8)	77 ± 5 (8)	0.32 ± 0.03 (12)
10^{-4} M ouabain	14 ± 2 (8)	26 ± 3 (8)	0.19 ± 0.02 (9)
	$P < 0.001$	$P < 0.001$	$P < 0.01$
Control	61 ± 1 (8)	—	0.23 ± 0.03 (8)
Low luminal Na^+	53 ± 3 (6)	—	0.11 ± 0.03 (6)
	$P < 0.05$		$P < 0.02$
Control	68 ± 1 (28)	—	0.38 ± 0.02 (28)
Low basolateral Na^+	47 ± 1 (8)	—	0.20 ± 0.02 (8)
	$P < 0.01$		$P < 0.001$

Number of observations is given in parenthesis.

Table 2. Effects of low K perfusion on basolateral resistance

	Control	Low K Perfusion
R_a/R_{bl}	2.9 ± 0.6 (7)	2.1 ± 0.3 (7)[a]
R_t ($\Omega \cdot cm^2$)	1181 ± 88 (20)	1766 ± 189 (14)[b]
ρ_c ($\Omega \cdot cm$)	6411 ± 776 (20)	6879 ± 2956 (14)[a]
R_{bl} ($\Omega \cdot cm^2$)	1583	2611

Number of observations is given in parenthesis.
[a] NS.
[b] $P < 0.01$.

obtained in these same animals before the ouabain perfusion. This concentration of the drug has been shown to inhibit sodium reabsorption profoundly in this preparation (Giebisch et al., 1973) and increase cell sodium activity (Cemerikic & Giebisch, 1980). Thus the situation is similar to that in the low K^+ perfusion experiments except that the pump inhibition is presumably more complete in this group. As shown in Table 1, large decreases in V_{bl} and a^i_K were found and t_K fell from 0.32 ± 0.03 in control, to 0.19 ± 0.02 in the ouabain-treated tubules ($P < 0.01$). t_K's in this and all subsequent groups were measured using step changes of potassium from 2.5 to 10. A typical recording is shown in Fig. 4.

Low Luminal Na^+

In this set of experiments control V_{bl} and t_K were determined and then the aortic solution was changed to the 0.5-Na solution. After 30 min V_{bl} and t_k were again measured. V_{bl} appeared to have achieved a new steady-state value by this time. Results are shown in Table 1. Potassium apparent transference number fell from 0.23 ± 0.03 in control to 0.11 ± 0.03 in the low sodium group ($P < 0.02$). These experiments were done in the month of January and, given the known seasonal variation in these animals (Spring & Giebisch, 1977), this may explain the lower control t_K in this group compared to the other groups presented here.

This degree of lowering of luminal sodium will certainly reduce sodium-potassium pump rate. Sodium transport has been shown to vary inversely with ambient sodium concentration in this preparation (Spring & Giebisch, 1977). It has also been shown that little or no change in intracellular potassium activity occurs in these circumstances (Spring & Giebisch, 1977; Kubota, Biagi & Giebisch, 1983*a*).

Low Basolateral Na^+

In these experiments V_{bl} and t_K were measured within 15 min of switching the basolateral solution from control to the 10.5 solution. Control values were obtained from a different group of animals in concurrent experiments. As shown in Table 1, V_{bl} depolarized as reported previously (Guggino et al., 1983) and t_K fell significantly from 0.38 ± 0.02 to 0.20 ± 0.02 ($P < 0.001$).

This maneuver has been shown to reduce net sodium reabsorption and intracellular sodium activity in the *Necturus* proximal tubule (Windhager, Taylor, Maack, Lee & Lorenzen, 1982). This is thought to occur because lowering basolateral sodium reduces the sodium gradient across that membrane that is used to energize calcium extrusion by way of a sodium-calcium exchange pathway. The resultant increase in intracellular calcium activity then inhibits apical sodium entry (Windhager et al., 1982). As intracellular sodium falls, presumably intracellular potassium remains at least at control levels.

Discussion

The data reported here show that a fall in the basolateral apparent potassium transference number occurs when sodium transport is inhibited by low K^+ in the peritubular solution, ouabain, low luminal sodium, and low peritubular sodium. As discussed above, we interpret this change in transference number as indicative of a fall in the contribution of the potassium conductive pathways to the electrical properties of the membrane relative to the other conductive pathways. The problem of the presence of an electrogenic pump causing underestimates of

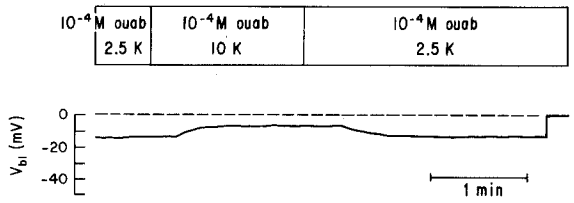

Fig. 4. Recording of the response of V_{bl} to a step increase in potassium in the presence of ouabain (*compare* Fig. 1)

potassium transference numbers has been discussed with reference to the low K^+ perfusion experiments. This is unlikely to play a role in the other groups as significant pump stimulation from the increase in peritubular K^+ during measurement of transference numbers was not likely to occur in the presence of ouabain or reduced sodium entry. Thus, in the only group in which this was a problem, cable analysis confirmed the change in membrane resistance.

A second problem arises from the fact that changes in t_K only give a measure of K^+ conductance relative to other pathways. Thus a fall in t_K could occur because of a fall in G_K or an increase in another conductive pathway. However, evidence that a fall in G_K did occur in these experiments is obtained by analysis of the driving force for potassium across the basolateral membrane. This has already been discussed in the case of the low K^+ group. In the low luminal and low peritubular sodium groups, intracellular potassium is likely to be near control levels, yet V_{bl} is diminished. Thus, the electrochemical gradient for potassium has, if anything, increased at a time when pump rate has decreased. Unless the number of potassium ions exchanged per sodium ion by the sodium-potassium pump has greatly increased, a fall in G_K must have occurred. Thus, though we know little of the regulation of the nonpotassium conductive pathways in this membrane, we consider it virtually certain that the changes in t_K reported here do reflect changes in the potassium pathway itself.

The Relationship Between G_K and Transport

We have found a reduction of t_K in the following conditions:

Lowered Peritubular Potassium

Because continuous perfusion with zero peritubular K^+ results in a progressive fall in a_K^i to very low levels, we followed 15 min of 0-K^+ perfusion with 45 min of 1-K^+ perfusion so that a steady state at higher a_K^i would be achieved. Since the electrodiffusion model of Goldman predicts that conductances are voltage and concentration dependent, we have to ask if the lowered intra- and extracellular potassium activities or the change in V_{bl} could have been responsible for the change in K^+ conductance. For a given ion with outside concentration c^0, inside concentration c^i and transmembrane voltage V, Goldman theory predicts that the ionic current, I, will be given by:

$$I = \left[\frac{Pz^2F^2V}{RT}\right]\left[\frac{c^0 - c^i e^{zFV/RT}}{1 - e^{zFV/RT}}\right]$$

where P is the permeability and z, F, R and T have their usual meanings (Schultz, 1980). Taking the partial derivative of I with respect to V (holding c^o and c^i constant) gives us the slope conductance (G) for this ionic pathway at any potential for the given concentrations. We find:

$$G = \left[\frac{Pz^2F^2}{RT}\right]\left[\frac{c^0 - (1 + (zFV/RT))c^i e^{zFV/RT}}{1 - e^{zFV/RT}} + \frac{(zFV/RT)(e^{zFV/RT})(c^o - c^i e^{zFV/RT})}{(1 - e^{zFV/RT})^2}\right].$$

Letting K^+ permeability under control conditions equal P_C, we can now calculate, using the values in Table 1, the G_K predicted by Goldman theory. This equals 47 $P_C \frac{\text{mho} \cdot \text{s}}{\text{cm}^3}$. If we assume P_C stays constant, G_K in the low K^+ situation becomes 51 P_C. Thus, despite the fall in K^+ concentration, Goldman theory predicts an increase in G_K in the low K^+ group, unless potassium permeability falls. This is because the effects of moving farther from equilibrium in this outwardly rectifying model outweighed the effects of decreases in potassium concentration. We therefore conclude that the decrease in K^+ conductance cannot be attributed to the alterations in K^+ concentration alone if the pathway conforms to electrodiffusion theory, unless these alterations actually caused a change in K^+ permeability.

We thus have a decrease in G_K associated with decreased sodium pumping, reduced V_{bl} and a_K^i and a_K^o. Presumably a_{Ca}^i is increased, as Na^+/Ca^{++} exchange is likely to be reduced in this situation. This has been demonstrated in the case of pump inhibition by ouabain in this preparation (Lorenzen, Lee & Windhager, 1982). Preliminary evidence suggests that pH falls slightly in these cells, as might be ex-

pected from the increase in a^i_{Na} (Y. Matsumura, B. Cohen, and G. Giebisch, *unpublished observations*).

Ouabain Perfusion

Ouabain caused a slow progressive fall in t_K, V_{bl} and a^i_K that tended to parallel each other in time course. In many respects this situation resembles the low K^+ perfusion cells except the pump inhibition is presumably more complete here. Others have reported similar changes in R_b from ouabain (Helman, Nagel & Fisher, 1979). In particular, the effect in frog skin is thought to be a very rapid effect followed by a slower fall that paralleled K^+ loss from the cell. Whether the rapid effect was due to a direct pump effect or a secondary effect on the potassium conductance was not clear. A large fall in a^i_K occurred in our studies, but, as noted above, when the pump is similarly inhibited by low K^+ perfusion the change in G_K is in excess of what would be predicted by electrodiffusion theory from intracellular K^+ loss alone. Also as noted above, intracellular calcium and sodium activities are increased in this situation.

Low Luminal Sodium

Reducing luminal sodium to the degree we have done will certainly cause a decrease in transport. Thus decreasing transport by reducing sodium entry has an effect on t_K similar to reducing pump rate by ouabain or low K^+. This is intriguing as in the former case a^i_{Na} will fall while in the latter it will increase (Cemerikic & Giebisch, 1980), and a^i_K does not change when sodium is lowered (Kubota et al., 1983*a*). Thus, we might expect a^i_{Ca} to fall in this group due to the effects of the increased sodium gradient across the basolateral membrane on sodium-calcium exchange. In studies in *Ambystoma* proximal tubule, lowering luminal sodium did not produce a significant fall in intracellular pH (Boron & Boulpaep, 1983).

This type of fall in G_K with block of sodium entry has been demonstrated in toad bladder (Davis & Finn, 1982*a,b*). The fall in conductance in that preparation is rapid and lags behind the fall in pump current only slightly.

Lowering Peritubular Sodium

This maneuver depolarizes V_{bl} and reduces t_K. Intracellular calcium has been shown to increase while intracellular sodium and sodium transport decrease under these conditions (Windhager et al., 1982). Thus the fall in transport appears to occur because of decreased apical sodium entry, presumably due to the elevated calcium. The mechanism of transport inhibition is therefore similar to that in the low luminal sodium experiments, but the changes in intracellular calcium activity are likely to be different in the two groups (*see* above). In a related preparation, intracellular pH falls when basolateral sodium is lowered (Boron & Boulpaep, 1983), in contrast to the results of lowering luminal sodium in that preparation.

The Mechanism of Regulation of G_K

Though there is a fall in a^i_K in low K^+ perfusion cells and ouabain-treated cells, it is clear that changes in G_K will help minimize swings in a^i_K with changes in transport rate in this preparation as it does in others (Schultz, 1981). The mechanism of this coupling between pump rate and conductance is not yet clear. It is of particular interest that decreases in G_K were noted in each experimental group despite varying changes (documented or presumed) in a^i_{Na}, a^i_K, a^i_{Ca} and a^i_{pH} in these groups. It is difficult therefore to implicate any one of these changes as the mediator of the coupling in all of the situations. A fall in V_{bl} did occur in all groups. Certainly, calcium is an excellent possibility as the mediator of the observed changes in potassium conductance during direct alterations in apical sodium entry. An increase in sodium entry might increase intracellular sodium, thereby decreasing the basolateral sodium gradient and the basolateral sodium-calcium exchange process energized by this gradient. The resultant increase in intracellular calcium activity would increase the potassium conductance. A fall in sodium entry would lead to the opposite sequence of events.

The experiments reported here suggest that factors other than calcium must also be operative. A fall in potassium conductance was found when transport was slowed by direct pump inhibition (low K^+, ouabain) or by indirect suppression of sodium entry by reduced peritubular sodium. Increases in intracellular calcium were expected in all of these situations. It is therefore likely that factors other than calcium are important in K^+ conductance regulation and that these factors can override whatever effects calcium may have. In particular, decreases in intracellular pH must be considered in this group of experiments as effects of pH changes on this K^+ pathway have been demonstrated previously (Steels & Boulpaep, 1976; Kubota, Biagi & Giebisch, 1983*b*). Alternatively, some metabolic consequence of pump inhibition, such as altered ATP/ADP ratio, could alter the calcium sensitivity

of the leak pathway. This type of metabolic control has been suspected in red cells (Romero, 1978), and changes in ATP/ADP ratio with changes in pump rate have been demonstrated in proximal tubules (Balaban, Mandel, Soltoff & Storey, 1980). It could provide a unified explanation for the responses seen in all of the experimental groups. We also cannot rule out other forms of pump-leak coupling such as a direct structural link or simultaneous addition or removal of both units.

We have demonstrated that the basolateral potassium conductance of the *Necturus* proximal tubule is directly related to transport rate when a variety of maneuvers are used to reduce that rate. As these maneuvers had varying effects on intracellular parameters, the data suggest that the signal involved is a change in some parameter related to pump turnover per se or, alternatively, that there is multifactorial regulation of this pathway.

Addendum

Recently it has been shown that ouabain causes an increase in intracellular calcium activity in frog proximal tubules (W. Wang, G. Messner, H. Oberleithner & F. Lang, *Pfluegers Arch. (submitted)*). This group also demonstrated an increase in basolateral membrane resistance and a fall in potassium transference number after ouabain application (G. Messner, H. Paulmichl, H. Oberleithner & F. Lang, *Pfluegers Arch. (submitted)*.

Dr. Brian Cohen was supported by a National Kidney Foundation Fellowship. This work was supported by U.S. Public Health Service Grants AM-17433 and AM-29848.

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Received 13 June 1983; revised 9 December 1983