J. Membrane Biol. 88, 283–292 (1985)

The Journal of
Membrane Biology

Ba^{2+}-Sensitive Potassium Permeability of the Apical Membrane in Newt Kidney Proximal Tubule

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Summary. The apical membrane K^+ permeability of the newt proximal tubular cells was examined in the doubly perfused isolated kidney by measuring the apical membrane potential change (V_a change) during alteration of luminal K^+ concentration and resultant voltage deflections caused by current pulse injection into the lumen. V_a change/decade for K^+ was 50 mV at K^+ concentration higher than 25 mM, and the resistance of the apical membrane decreased by 58% of control when luminal K^+ concentration was increased from 2.5 to 25 mM. Ba^{2+} (1 mM in the lumen) reduced V_a change/decade to 24 mV and increased the apical membrane resistance by 70%. These data support the view that Ba^{2+}-sensitive K^+ conductance exists in the apical membrane of the newt proximal tubule. Furthermore, intracellular K^+ activity measured by K^+-selective electrode was 82.4 ± 3.6 meq/liter, which was higher than that predicted from the Nernst equation for K^+ across both cell membranes. Thus, it is concluded that cell K^+ passively diffuses, at least in part, through the K^+ conductive pathway of the apical membrane.

Key Words cell membrane potential · K^+ permeability · K^+-sensitive electrode · intracellular K^+ activity · voltage divider ratio

Introduction

Intracellular K^+ activity of the proximal tubular cells has been reported to be higher than that of equilibrium across both the apical and basolateral cell membranes in bullfrog (Fujimoto & Kubota, 1976), *Necturus* (Kubota, Biagi & Giebisch, 1983*a*), rat (Edelman, Curci, Samaržija & Frömter, 1978), and rabbit (Biagi, Sohtell & Giebisch, 1981). The rate of K^+ uptake by the Na-K pump is thought to be equally balanced with K^+ efflux through the basolateral membrane which is permeable to K^+ (Biagi et al., 1981; Grasset, Gunter-Smith & Schultz, 1983; Kubota et al., 1983*a*). Such a pump-leak system may be important to maintain cell K^+ activity at a constant level. On the other hand, the role of the apical membrane with regard to K^+ efflux is not clear. There are only a few papers that discussed K^+ permeability of the apical membrane in the proximal tubule (Giebisch, 1961; Boulpaep, 1967; Frömter, 1977).

The present study investigated the properties of K^+ permeability of the apical membrane of newt proximal tubules and Ba^{2+} effects on it. For several short periods during each experiment, the lumen was perfused with a low Na^+ Ringer solution (Na^+ was replaced with equimolar choline$^+$) for two reasons: (1) Replacement of Na^+ with K^+ resulted in an underestimate of V_a change/decade for K^+ (Frömter, 1977) because the decrease of the depolarizing effect of luminal Na^+ hyperpolarizes the apical membrane (Schultz, Frizzell & Nellans, 1977). (2) Passive Na^+ leakage into the cell interior reduces the K^+ diffusion potential across the apical membrane (Giebisch, 1961; Okada, Sato & Inoue, 1975). The results indicate that there was a Ba^{2+}-sensitive K^+ permeability in the apical membrane of the newt proximal tubule.

Materials and Methods

The experiments were performed on newt kidney proximal tubular cells using micropuncture techniques. Male Japanese newts (*Triturus pyrrhogaster*) weighing about 5 g were obtained from the Nisseizai Company (Tokyo, Japan) and were kept at 8°C in an aquarium of tap water. Before use, the animals were transferred into tap water of 18°C and kept for several days. The essential steps of the preparative technique were as follows. After decapitation, both kidneys were rapidly removed and pinned on a cork board attached to the bottom of the Lucite chamber, which was continuously perfused with the normal Ringer solution (about 3 ml/min). To perfuse the peritubular capillary thoroughly, the posterior vena cava was canulated. The catheter was advanced caudally until it reached the transition between the

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Table 1. Composition of solution[a]

	Normal Ringer	Low Na^+ Ringer		
Na^+	106.2	1.2	1.2	1.2
$Choline^+$	—	105	82.5	27.5
K^+	2.5	2.5	25	80
Ca^{2+}	1.8	1.8	1.8	1.8
Mg^{2+}	1.0	1.0	1.0	1.0
$Tris^{+b}$	5.0	5.0	5.0	5.0
Cl^-	118.1	118.1	118.1	118.1
HPO_4^{2-}	0.6	0.6	0.6	0.6
$H_2PO_4^-$	0.01	0.01	0.01	0.01

[a] All concentrations in mmol · liter^{-1}
[b] Tris: Tris (hydroxymethyl)aminomethane

genital and the pelvic portion of the kidneys (Chase, 1923; Sakai & Kawahara, 1983) and was tied off together with dorsal aorta. The perfusion rate was adjusted to 0.17 ml/min. Before experiments, colored normal Ringer solution containing 0.02% Fast Green FCF was perfused and the adequacy of perfusion was checked visually. The perfusate flowed out from the cut ends of the renal portal vein. Luminal perfusion (about 0.1 μl/min) was performed with two bevelled glass micropipettes (tip OD 6-8 μm) placed in Bowman's capsule.

Table 1 shows the composition of both the luminal and peritubular perfusate. All solutions were bubbled with 100% O_2, and pH was adjusted to 7.4. The peritubular capillary was always perfused with Sol. 1 containing 5 mM D-glucose.

The following three protocols were used:

Protocol 1. Measurements of Intracellular K^+ Activity (a_K^c)

Both K^+-selective and conventional (3 M KCl-filled) electrodes were impaled into two neighboring cells in the same proximal tubule, when both sides were perfused with Sol. 1. Rapid depolarization of both electrodes responding to luminal 5 mM L-alanine indicated that both electrodes were impaled in cells of the same tubule. a_K^c was calculated from the following equation (Kubota et al., 1983*a*)

$$a_K^c = 77 \times 10^{(V_K - V_b - V_{100})/S} \quad (1)$$

where V_K and V_{100} are the voltages from the K^+ electrode both in the cell and in 100 mM KCl solution (activity coefficient of 100 mM KCl at 25°C is 0.770 (Robinson & Stokes, 1968), respectively, V_b is the basolateral membrane potential, and S is the slope constant of the K^+ electrode expressed as mV change per decade change in potassium activity in pure KCl solution.

Protocol 2. K^+ Permeability of the Apical Membrane

Changes of cell membrane potential were measured while luminal K^+ concentration was varied by using solutions 2, 3, and 4. When different solutions are used for bath and luminal perfusion, tip potentials of microelectrodes change. These were corrected with appropriate potentials measured with free-flowing KCl electrodes. The differences of tip potentials of Sols. 2, 3, and 4 *versus* the control solution (Sol. 1) were −0.9, −0.4, and +0.2 mV, respectively.

Protocol 3. The Voltage Divider Ratio

Square wave current pulses (0.5 μA, 0.4 sec duration) were injected into the lumen through a microelectrode placed at the initial part of the proximal tubule. Resultant luminal and cellular voltage deflections (ΔV_t and ΔV_b) were recorded sequentially with another microelectrode along the tubule. Voltage divider ratio ($\Delta V_a/\Delta V_b$) is defined as follows:

$$\Delta V_a/\Delta V_b \equiv \Delta V_t/\Delta V_b - 1. \quad (2)$$

Both K^+-selective and Ling-Gerard type microelectrodes were made from Pyrex® glass capillaries with filament (1.0 mm OD, 0.6 mm ID, and 90 mm length), which were pulled with a horizontal puller (PC 80, Narishige, Tokyo, Japan). The resistances and the tip potentials of 3 M KCl-filled electrodes were 15–20 MΩ and less than 5 mV in Sol. 1, respectively. K^+-selective microelectrodes were made by using ion-selective resin (IE-190, WPI) according to the methods of Edelman et al. (1978). Their properties were tested in mixtures of isotonic (100 mM) NaCl-KCl solutions before and after use. The resistance of K^+-selective electrode was $2.3 \pm 0.3 \times 10^{10}$ Ω ($n = 6$). The selectivity coefficient for Na ($k_{K,Na}$) was 0.018 ± 0.005 ($n = 7$). The average slope was 58.9 ± 0.4 mV/10-fold change in K^+ activity of pure KCl ($n = 10$). Rise time responding to changes in K^+ concentration was less than 0.5 sec.

All potential measurements were performed by using a high input impedance differential electrometer (FD223, WPI) at room temperature, 25 ± 1°C. Data were expressed as means ± SE. Statistical analysis was done with Student's t test. $P < 0.05$ was accepted for statistical significance. Unless otherwise mentioned, N indicates the number of tubules.

Results

Intracellular K^+ Activity

Intracellular K^+ activity (a_K^c) and membrane potentials were measured with K^+-selective and 3 M KCl-filled microelectrode, respectively. Mean values of a_K^c, V_b, and V_t were 82.4 ± 3.6 meq/liter ($N = 16$), −63.5 ± 2.2 mV ($N = 16$), and −1.9 ± 0.28 mV ($N = 10$), where V_b and V_t are basolateral membrane potential and transepithelial potential differences, respectively. The mean value of the apical membrane potential (V_a) obtained from $V_a = V_b - V_t$ was −61.6 ± 2.8 mV. The Nernst equilibrium potential for K^+ (E_K) across both the membranes was calculated from the following equation:

$$E_K = -\frac{RT}{F} \ln \frac{a_K^c}{a_K^o} = -59 \log \frac{a_K^c}{1.9} \quad (3)$$

where R, T, and F have their usual meanings. a_K^c and a_K^o are intracellular and extracellular K^+ activities, respectively. E_K across both membranes was -96.2 ± 1.1 mV ($N = 16$). $V_a - E_K$ and $V_b - E_K$ (magnitudes of the driving force for K^+ efflux across both cell membranes, respectively) were 34.5 ± 2.4 and 32.6 ± 2.4 mV, respectively. Therefore, a_K^c is maintained at a higher level than that predicted from the Nernst equation. These results agree with those of bullfrog (Fujimoto & Kubota, 1976), *Necturus* (Kubota et al., 1983*a*), rat (Edelman et al., 1978), and rabbit (Biagi et al., 1981) proximal tubules, indicating that if both cell membranes are permeable to K^+, K^+ will passively diffuse into extracellular solution according to the electrochemical potential difference. The relationship between V_b and a_K^c is illustrated in Fig. 1.

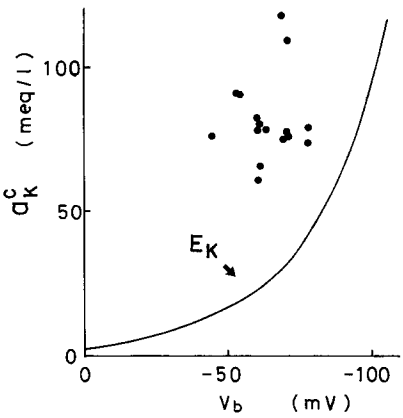

Fig. 1. Relationship between basolateral membrane potential (V_b) and intracellular K^+ activity (a_K^c). The curve represents the Nernst equilibrium potential for K^+ (E_K). Mean value of $V_b - E_K$ was 32.6 ± 2.4 mV ($N = 16$)

Apical Membrane K^+ Conductance

Figure 2 shows a typical trace of V_b and superimposed cellular voltage deflections (ΔV_b) which were induced by square wave current pulses injected into the lumen. In response to a high luminal K^+ concentration, V_b depolarized and ΔV_b increased rapidly. When the luminal perfusate was switched to the control solution (Sol. 2), V_b and ΔV_b returned to the original level. After the cellular recording, V_t and superimposed luminal voltage deflections (ΔV_t) were recorded by either advancing the electrode into the lumen or repuncturing it with the same electrode.

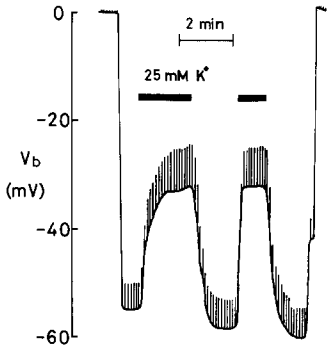

Fig. 2. A typical trace of both basolateral membrane potential (V_b) and superimposed cellular voltage deflections (ΔV_b). Note both the rapid depolarization of V_b and the increase of V_b responding to high luminal K^+ (25 mM)

Effect of Luminal Na^+ on V_a

Although Na^+ permeability of the apical membrane of the proximal tubular cells is much less than K^+ permeability (Giebisch, 1961; Frömter, 1977), substitution of K^+ for Na^+ will result in underestimation of the V_a change, because it diminishes the depolarizing effect of Na^+ on V_a. While the lumen was perfused with Sol. 1 (normal Ringer), mean values of V_b, V_t, and V_a were -59.0 ± 3.5 ($N = 9$), -2.0 ± 0.8 ($N = 10$), and -57.0 ± 4.3 ($N = 9$) mV, respectively. When luminal fluid was switched from Sol. 1 to Sol. 2 (low Na^+ Ringer), mean values of V_b, V_t, and V_a were -58.7 ± 1.8 ($N = 20$), $+6.5 \pm 0.8$ ($N = 19$), and -65.2 ± 2.0 ($N = 19$) mV, respectively. V_a hyperpolarized by 8.2 mV during low luminal Na^+ concentration but V_b did not. These results are summarized in Table 2. Thus, Na^+ was replaced with choline$^+$ in advance in the case of step increase of luminal K^+ concentration (Sol 2, 3, and 4).

K^+ Permeability

Filled circles in Fig. 3 show the relationships between membrane potentials and luminal K^+ activities. The broken line represents the voltage predicted from the Nernst equation (Eq. (3)) for step changes of K^+ activities. During these experiments, it was assumed that a_K^c was constant in spite of changes in luminal Na^+ and K^+ concentration. A change of V_a approximately obeyed the Nernstian response in the K^+ concentration range 19–62 meq/liter (25–80 mM, Fig. 3, lower panel). The slope of V_a at K^+ concentrations higher than 19 meq/liter (25 mM) was 50 mV/10-fold K^+ concentration change. The change in V_a with step-changes in luminal [K^+] is becoming less "Nernstian" at lower [K^+], indicating that there may be a finite choline$^+$ permeability (smaller than Na^+) in the apical membrane (Whittembury, Sugino & Solomon, 1961; Spring &

Table 2. Effect of luminal Na^+ on cell membrane potentials

Luminal perfusate	V_b	V_t	V_a
A.			
Normal (Sol. 1)	−59.0 ± 3.5 (9)	−2.0 ± 0.8 (10)	−57.0 ± 3.4 (9)
Low Na^+ (Sol. 2)	−58.7 ± 1.8 (20)	6.5 ± 0.8 (19)	−65.2 ± 2.0 (19)
B.			
Normal (Sol. 1)	−60.6 ± 3.8	−2.2 ± 3.1	−58.4 ± 2.8
Low Na^+ (Sol. 2)	−61.4 ± 2.3 n.s.	8.3 ± 3.4[a]	−69.7 ± 2.3[b]

Values in *B* are 7 paired tabulated data of *A*. [a] and [b] indicate $P < 0.001$ and $P < 0.02$, respectively. V_b, basolateral membrane potential; V_t, transepithelial potential differences; V_a, apical membrane potential.

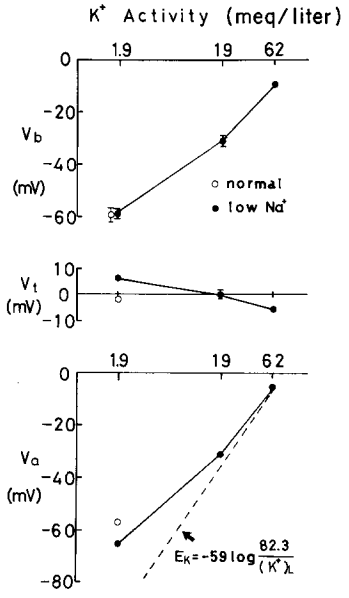

Fig. 3. Relationships between membrane potentials and luminal K^+ activity $(K^+)_L$. *Abscissa*: $(K^+)_L$. *Ordinates*: basolateral membrane potential (V_b) (upper panel), transepithelial potential difference (V_t) (middle panel), and apical membrane potential (V_a) (lower panel), respectively. Open circles (○) and filled circles (●) represent membrane potentials during normal Ringer (Sol. 1) and low Na^+ Ringer (Sol. 2, 3, and 4) in the lumen, respectively. The broken line represents the Nernst equilibrium potential for K^+. Each point represents the mean of 4–20 tubules with SE. V_a: −65.2 ± 2.0, −30.9 ± 3.0, and −5.1 ± 1.5 mV in Sol. 2, 3, 4, respectively. V_t: +6.5 ± 0.8, +0.1 ± 1.8, and −4.6 ± 0.7 mV in Sol. 2, 3, 4, respectively

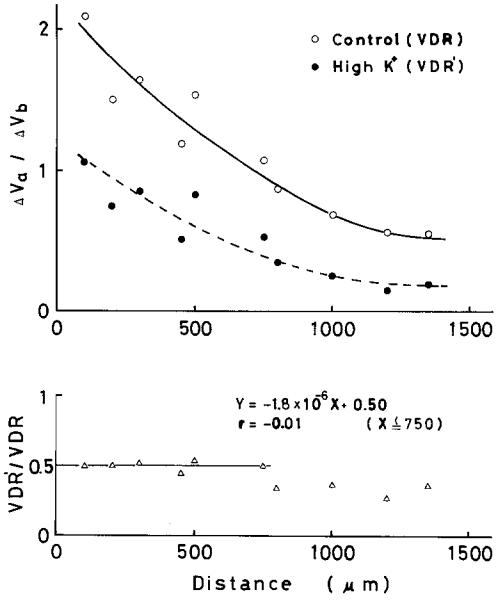

Fig. 4. Upper panel: The voltage divider ratio during both control (Sol. 2) (VDR) (○) and high luminal K^+ (Sol. 3) (VDR') (●) in the proximal tubular cells ($N = 4$). Both lines were drawn by eye. Lower panel: The ratio of VDR' to VDR. Regression line of VDR'/VDR was $Y = -1.8 \times 10^{-6} X + 0.5$ ($r = -0.01$). X = distance over which linearity is obeyed, only values below 750 μm are used. Mean value of VDR'/VDR was 0.42 ± 0.03 ($n = 10$)

Giebisch, 1977) or an anion permeability (Kubota et al., 1983*a*). Further, V_a of −5.1 ± 1.5 mV at 80 mM luminal K^+ concentration was almost equal to the E_K of −7.3 ± 1.2 mV. The change in V_t is simultaneous with that of V_b, which would indicate that the V_t change is due to an increase of luminal K^+ concentration. The middle panel of Fig. 3 shows that the paracellular shunt pathway is poorly K^+ selective (6 mV/10-fold change in luminal K^+ activity).

The Voltage Divider Ratio (VDR)

Both luminal and cellular voltage deflections (ΔV_t and ΔV_b) were recorded at various distances from the current source. The VDR ($\Delta V_a/\Delta V_b$) is shown as a function of the distance (Fig. 4, upper panel). In agreement with the observations of Hoshi, Kawahara, Yokoyama & Suenaga (1981) and Greger and Schlatter (1983), $\Delta V_a/\Delta V_b$ declined as a function of the distance. Therefore, $\Delta V_a/\Delta V_b$ is not an identical index for r_a/r_b, where r_a and r_b are unit resistances of the apical and basolateral membranes, respectively (Fig. B1 in Appendix I). Nevertheless, it should be

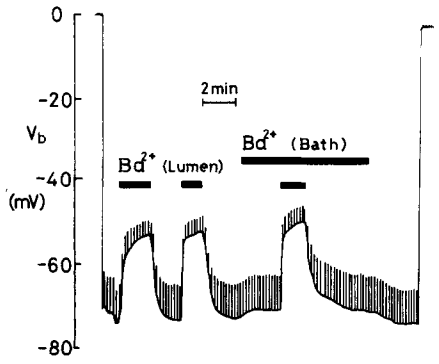

Fig. 5. A typical trace of basolateral membrane potential (V_b) and superimposed cellular voltage deflections (ΔV_b) during addition of 1 mM Ba^{2+} to either the lumen (lower bars) or bath (upper bar). ΔV_b markedly decreased responding to depolarization of V_b induced by luminal Ba^{2+}, but changed little during addition of peritubular Ba^{2+}. Note the Ba^{2+} effects on cell membrane potentials are separate and additive

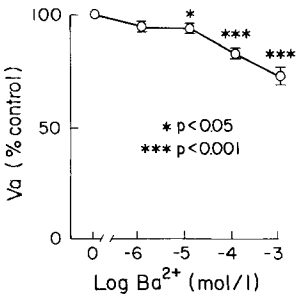

Fig. 6. Dose-response curve for Ba^{2+} inhibition of an apical membrane potential (V_a). Relative values (% control) of V_a are plotted against the logarithm of Ba^{2+} concentration added to the luminal perfusate (Sol. 1). Mean value of control (0 Ba^{2+}) was -64.3 ± 2.4 mV ($N = 24$). Each point except control represents mean ± SE of 4–7 tubules. * and *** indicate $P < 0.05$ and $P < 0.001$, respectively

noted that the data in high luminal K^+ perfusate (Sol. 3) always distributed below the control values. Since the experimental VDR (VDR′) divided by the control value (VDR) gives a reasonable estimation of the experimental-to-control ratio of the apical membrane resistance (r_a'/r_a) as discussed in Appendix II, VDR′/VDR was expressed as a function of the distance (Fig. 4, lower panel). The ratio appears to be constant up to the distance less than 750 μm. The mean value of VDR′/VDR was 0.42 ± 0.03 ($n = 10$). This indicates that the apical membrane resistance decreased by 58% of control when the luminal K^+ concentration was increased to 25 mM.

Effects of Ba^{2+} on Cell Membranes

Figure 5 shows a typical trace of 1 mM Ba^{2+} effects on V_b and ΔV_b. V_b reversibly depolarized in response to luminal and/or peritubular Ba^{2+}. The depolarizing effects of Ba^{2+} on both membranes were additive. Depolarization of V_a induced by luminal Ba^{2+} was about 7 times as large as that of V_b induced by peritubular Ba^{2+}. It suggested that a Ba^{2+}-sensitive K^+ conductance exists independently both in the apical and in the basolateral membranes, and that the apical membrane is much more sensitive to Ba^{2+} than the basolateral membrane is while luminal Na^+ concentration is low (Sol. 2). ΔV_b markedly decreased in response to the depolarization induced by luminal Ba^{2+} (*compare* with Fig. 2), but changed little when Ba^{2+} was added to the peritubular perfusate. V_t and ΔV_t were almost unchanged during luminal addition of Ba^{2+}.

Effect of Ba^{2+} on Membrane Potential

The inhibitory effect of luminal Ba^{2+} on cell membrane potentials was investigated at various concentrations. Figure 6 shows percents of control value of V_a against the logarithm of Ba^{2+} concentration. V_a significantly depolarized at Ba^{2+} concentrations above 10^{-5} M in a dose-dependent manner and decreased by $26.9 \pm 4.2\%$ at 1 mM Ba^{2+} ($N = 7$, $P < 0.001$).

Effects of Ba^{2+} on K^+ Permeability

Figure 7 shows relationships between cell membrane potentials and luminal K^+ activity during addition of 1 mM Ba^{2+} in the lumen. The broken line indicates a theoretical Nernstian slope of 59 mV. The change of V_a deviated from this line at all K^+ concentrations. The slope of V_a at K^+ concentrations higher than 25 mM was 24 mV/decade and this value was much less than that of control (50 mV/decade). The inhibitory effect on K^+ permeability below 25 mM K^+ was almost complete (3.4 mV/decade). This result agrees with the effect of Ba^{2+} on K^+ conductive pathway of cortical thick ascending limb (Greger & Schlatter, 1983).

Ba^{2+} Effects on the Voltage Divider Ratio (VDR)

As shown in the upper panel of Fig. 8, the VDR expressed as a function of the distance from the current source was higher than the control when Ba^{2+} was added to the luminal perfusate. This indicates that the apical membrane resistance increased during addition of Ba^{2+}, assuming that the basolat-

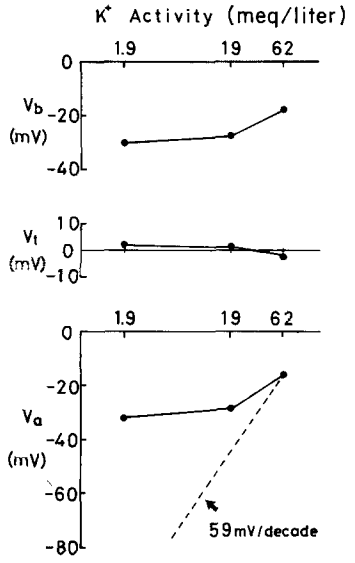

Fig. 7. Relationships between membrane potentials and luminal K^+ activity during addition of 1 mM Ba^{2+} to the luminal perfusate. V_a: -32.1 ± 1.0, -28.7 ± 1.1 and -16 ± 2.5 mV in Sol. 2, 3, 4, respectively. V_t: $+1.9 \pm 0.7$, $+1.3 \pm 0.5$, and -1.5 ± 1 mV in Sol. 2, 3, 4, respectively. (N = 5–12). *Compare* with Fig. 3

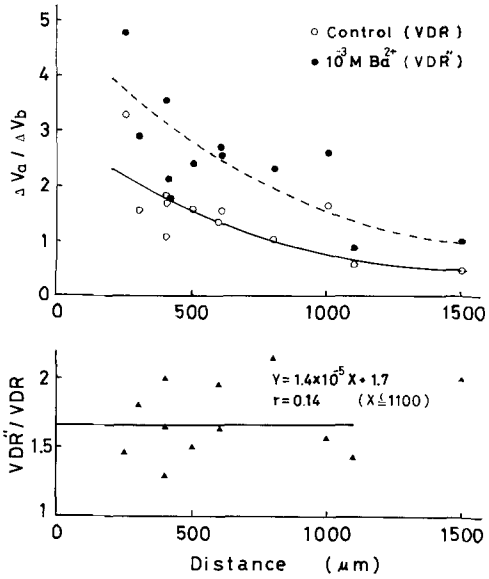

Fig. 8. Upper panel: The voltage divider ratio during both control (VDR) (○) and addition of 1 mM Ba^{2+} to the luminal perfusate (VDR") (●). Note that filled circles are always above corresponding open circles (N = 9). Lower panel: The ratio of VDR" to VDR. Regression line of VDR"/VDR was $Y = 1.4 \times 10^{-5} X + 1.7$ (r = 0.14). Values below 1100 μm are used to calculate regression line. Mean value of VDR"/VDR was 1.7 ± 0.08 (n = 12). *Compare* with Fig. 4

eral membrane resistance is constant. The ratio of VDR" (the VDR during luminal addition of Ba^{2+}) to VDR (the VDR during control solution) is shown in the lower panel of Fig. 8. The values of this ratio were always above unity ($P < 0.01$, sign test) and its regression line was almost constant in spite of increasing distance from the current source. The mean value of VDR"/VDR was 1.70 ± 0.08 (n = 12). This means that the apical membrane resistance increased by 70% during luminal addition of Ba^{2+} (Appendix II).

Discussion

Apical Membrane K^+ Permeability

The present paper provides evidence for the existence of a K^+ permeability at the apical membrane of newt proximal tubular cells. During perfusion with a low luminal Na^+ solution, the electrical response to a 10-fold K^+ concentration change was 50 mV (in the range of 25–80 mM). It was higher than those reported by Frömter (20 mV, 1977) and Boulpaep (10 mV, 1967). The above discrepancies may be caused by the differences in both luminal Na^+ concentration and the apical membrane Na^+ permeability, since a high luminal Na^+ concentration should reduce the V_a-change/decade for K^+ (Okada et al., 1975) and passive leakage of Na^+ into the cell interior should reduce K^+ diffusion potential across the apical membrane (Giebisch, 1961). Figure 9 shows a typical trace of K^+ step increase in luminal and basolateral perfusions, when both perfusates contain 82.5 mM Na^+. The mean depolarizations of V_b were 3.5 mV (lumen 25 mM K^+) and 14.1 mV (bath 25 mM K^+) (N = 2), respectively. This value of 3.5 mV is much smaller than that of 27.9 mV during low luminal Na^+ perfusion (*compare* with Fig. 2). This result indicates that depolarization of V_b in response to high luminal K^+ depends on luminal Na^+ concentration.

Since the luminal and peritubular fluids were not identical during alteration of luminal K^+ concentration change, a significant diffusion potential (E_s) will be generated across the paracellular shunt pathway (Frizzell & Schultz, 1972; Frömter & Gessner, 1974; Okada et al., 1975), which depolarizes or hyperpolarizes V_a according to its own direction. Therefore, it is important to estimate the effect of E_s on V_a in the equivalent circuit model as shown in Fig. A1 in Appendix I (Okada et al., 1975; Boulpaep, 1979). First, E_a and E_b were calculated during perfusion with identical solution on both sides and then the E_a change was estimated during alteration of the luminal ionic concentration (*see* Appendix I). Fortunately, the magnitude of the V_a change during

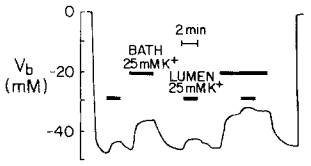

Fig. 9. A typical trace of basolateral membrane potential (V_b) in response to both luminal and basolateral high K^+ (25 mM) when the luminal perfusate contains 82.5 mM Na^+ and 22.5 mM choline$^+$. Choline$^+$ was replaced with K^+ when a step increase of K^+ was conducted

alteration of Na^+ and/or K^+ concentration in the lumen was almost equal to that of the E_a change.

The apparent K^+ and Na^+ transference numbers of the apical membrane (t'_K and t'_{Na}) can be estimated from the following equation (Boulpaep, 1979; Kubota, Biagi & Giebisch, 1983*b*)

$$t'_x = V_a \text{ change}/E_x \text{ change} \quad (4)$$

where subscript x represents K^+ and Na^+. The E_x change is the predicted change in the x equilibrium potential (59 mV). Both values (V_a change and E_x change) are estimated per 10-fold change in concentration. Then, t'_K and t'_{Na} were 0.85 ($25 \leqq K^+ \leqq 80$ mM) and 0.07 ($1.2 \leqq Na^+ \leqq 106.2$ mM), respectively. These values are well in agreement with the results of Giebisch (1961) and Frömter (1977) and comparable with the ratio of the permeability coefficient (P_{Na}/P_K) of 0.07 in duodenum (Okada et al., 1975). Furthermore, the value of V_a of −5.1 mV at 80 mM K^+ was almost equal to E_K of −7.3 mV. Thus, it is concluded that the apical membrane of the newt proximal tubular cells is markedly permeable to K^+ when the luminal Na^+ concentration is low.

Ba^{2+} Effects on the Apical Membrane K^+ Permeability

Ba^{2+} effects on K^+ permeability of the apical membrane have been studied in early distal tubule (Oberleithner et al., 1983), cortical thick ascending limb (cTAL) (Greger & Schlatter, 1983), and cortical collecting duct (O'Neil, 1983; Hunter, Lopes, Boulpaep & Giebisch, 1984; O'Neil & Sansom, 1984). The conclusion that Ba^{2+} inhibits the K^+ conductance in the present study was based on the following observation: (1) the apical membrane potential depolarized during addition of Ba^{2+} to the lumen and the depolarization of V_a by 1 mM Ba^{2+} in the lumen was much larger than that of V_b by 1 mM Ba^{2+} in the bath. (2) Ba^{2+} increased the apical membrane resistance by 70%. (3) Ba^{2+} reduced a slope of 10-fold luminal K^+ concentration change from 50 to 24 mV ($25 \leqq K^+ \leqq 80$ mM) and from 34.3 to 3.4 mV ($2.5 \leqq K^+ \leqq 25$ mM), respectively. The inhibitory effect of Ba^{2+} on V_a with step changes in luminal K^+ is K^+-dependent (Fig. 7). It suggests that Ba^{2+} competes with K^+ for a common site as mentioned in other tissues (Eaton & Brodwick, 1980; O'Neil, 1983).

The relative change of the voltage divider ratio (VDR″/VDR) revealed that Ba^{2+} (1 mM in the lumen) increased the apical membrane resistance by 70%. The value is comparable to that of gallbladder (García-Díaz, Nagel & Essig, 1983) but less than those of early distal tubule (Oberleithner et al., 1983) and cTAL (Greger & Schlatter, 1983). Since Ba^{2+} almost completely inhibits the K^+ permeability of the apical membrane as discussed above, a relatively small increase of the resistance (r_a) indicates existence of other ionic conductances, such as Cl^-, though its permeability is as low as Na^+ permeability and much less than K^+ permeability (Okada et al., 1975; Frömter, 1977). However, since the concentration of Cl^- is about 100 times higher than that of Na^+, and about 50 times higher than that of K^+ in the luminal perfusate, it is probable that Cl^- carries current injected into the lumen across the apical membrane after addition of Ba^{2+} to the lumen. Another possibility is that a secondary increase of the basolateral membrane resistance cancels the increase of VDR″/VDR. Depolarization of cell membrane potential increased the basolateral membrane resistance in the proximal tubule (Hoshi & Sakai, 1967; Lang, Messner, Wang & Oberleithner, 1983).

K^+ Transport across the Apical Membrane

The diffusional flow of K^+ out of the cell across the apical membrane (J_K) driven by the electrochemical potential difference for K^+ across this barrier ($\Delta\tilde{\mu}_K$) can be calculated from the following equations

$$J_K = G_K(\Delta\tilde{\mu}_K/F^2) \quad (5)$$

$$G_K = G - G_A \simeq G - G''* \quad (6)$$

where G, G_K, and G_A are the total electrical conductance, the partial conductance for K^+, and the conductance of other ions of the apical membrane, respectively. G'' is the conductance after addition of 1 mM Ba^{2+} to the lumen. $\Delta\tilde{\mu}_K = V_a - E_K = 34.5$ mV.

* K^+ conductance was assumed to be negligible in the apical membrane during addition of 1 mM Ba^{2+} to the lumen, because Ba^{2+} almost completely inhibited K^+ conductive pathway of the apical membrane at this dose (Fig. 7, lower panel).

Since the resistance of the apical membrane increased by 70% after addition of Ba^{2+}, $G'' = 0.59G$. Introducing this value into Eq. (6)

$$G_K = 0.41G. \quad (7)$$

If the specific resistance of the apical membrane in the present study is equal to that determined by Hoshi et al. (1981), $G_K = 0.41 \times 1/1350\ (\Omega cm^2) = 0.304\ (mS \cdot cm^{-2})$ and, from Eq. (5), $J_K = 0.304\ (mS \cdot cm^{-2}) \times 34.5\ (mV) = 0.39\ (\mu eq \cdot cm^{-2} \cdot hr^{-1})$. This value is in agreement with the rate of K^+ secretion observed in the rat proximal straight tubule while no absorption of fluid was taking place ($0.24\ \mu eq \cdot cm^{-2} \cdot hr^{-1}$, Bomsztyk & Wright, 1982).

In conclusion, these experiments have demonstrated a Ba^{2+}-sensitive K^+ permeability at the apical membrane of newt proximal tubule. Ba^{2+} in the lumen depolarizes V_a, decreases V_a change/decade for K^+, and increases the apical membrane resistance. Cell K^+ can be driven by an electrochemical potential difference ($V_a - E_K$) across the apical membrane through this K^+ conductive pathway.

I am grateful to Dr. T. Hoshi for giving me advice. I am also indebted to Dr. G. Giebisch, Dr. F. Lang, Dr. H. Oberleithner, and Dr. M. Hunter for helpful discussions. This work was supported in part by a Grant-in-Aid from the Ministry of Science, Education, and Culture of Japan.

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Received 7 August 1985

Appendix I

The Case of Luminal Perfusion with Sol. 1 ($E_s = 0$)

Since the luminal and peritubular fluids were identical, E_s is assumed to be zero. Therefore, according to the model illustrated in Fig. A1,

$$E_b = -V_b - V_t/\hat{r}_s \quad \text{(A1)}$$

$$E_a = -(\hat{r}_a + \hat{r}_s)E_b - (1 + \hat{r}_a + \hat{r}_s)V_b \quad \text{(A2)}$$

where $\hat{r}_a$ and $\hat{r}_s$ are r_a/r_b and r_s/r_b, respectively. Inserting $\hat{r}_s = 0.12$ (Maruyama & Hoshi, 1972), $V_b = -59$, and $V_t = -2$ into Eq. (A1), then $E_b = 75.7$ (mV). From Eq. (A2), in the same way, $E_a = 51.7$ (mV) because $\hat{r}_a = 0.32$ (Hoshi et al., 1981).[1]

The Case of Luminal Perfusion with Sol. 2 ($E_s > 0$)

According to the same model (Fig. A1)

$$V_t = \{r_s E_a - 75.7\, r_s + (r_a + r_b)E_s\}/(r_a + r_b + r_s) \quad \text{(A3)}$$

$$V_a = -\{(r_b + r_s)E_a + 75.7\, r_a + r_a E_s\}/(r_a + r_b + r_s) \quad \text{(A4)}$$

where $E_b = 75.7$. E_a is obtained from Eqs. (A3) and (A4)

$$E_a = -(1 + \hat{r}_a)V_a - \hat{r}_a\,(75.7 + V_t). \quad \text{(A5)}$$

Since Na^+ permeability of the apical membrane is low (Fig. 3, upper panel), r_a (Sol. 2) is safely assumed to be equal to r_a (Sol. 1). Inserting $V_a = -65.2$, $V_t = +6.5$, and $r_a = 0.32$ into Eq. (A5), then $E_a = 59.8$ (mV). E_a (Sol. 2) is larger than E_a (Sol. 1) by 8.1 mV. This value is almost equal to that of the V_a change (8.2 mV) in response to low luminal Na^+ concentration (Sol. 2).[2]

The Case of Luminal Perfusion with Sol. 3

Since r_a (Sol. 3) decreased by 58% comparing with r_a (Sol. 2) (Fig. 4, lower panel), $\hat{r}_a$ (Sol. 3) = 0.42 $\hat{r}_a$ (Sol. 2) = 0.13. Inserting $V_a = -32.1$, $V_t = +0.1$, and $\hat{r}_a = 0.13$ into Eq. (5A), then $E_a = 24.9$ (mV). E_a decreases by 34.9 mV in response to an increase of luminal K^+ concentration from 2.5 to 25 mM. This value is almost equal to that of the V_a change (34.3 mV) which occurred in response to high luminal K^+ concentration (25 mM).

The Case of Luminal Perfusion with Sol. 2 Containing 1 mM Ba^{2+}

Since r_a (Sol. 2 + 1 mM Ba^{2+}) increased by 70% comparing with r_a (Sol. 2) (Fig. 8, lower panel), $\hat{r}_a$ (Sol. 2 + 1 mM Ba^{2+}) = 0.32 × 1.7 = 0.544. Inserting $V_a = -32.1$, $V_t = 1.3$ and $\hat{r}_a = 0.544$ into Eq. (A5), then $E_a = 7.7$ (mV). E_a decreases by 52.1 (mV) (87% of control emf at the apical membrane).

[1] These estimates rely on the assumption that $E_s = 0$ under symmetrical conditions. Small differences of ion activities between the lateral intercellular space and the serosal bathing solution have been observed for this condition in other epithelia (Curci & Frömter, 1979). Using $E_a = (\hat{r}_a V_t - \hat{r}_s V_a - \hat{r}_a E_s)\hat{r}_s$ and assuming E_s varies by ±1 mV, E_a will vary by ±2.7 mV.

[2] During choline$^+$ substitution, the shunt emf is increased ($V_a = -65.2$ mV, $V_t = +6.5$ mV, $\hat{r}_a = 0.32$, $\hat{r}_s = 0.17$; $E_s = \hat{r}_s(75.7 + V_a) + (1 + \hat{r}_s)V_t = 9.4$ mV). The resulting circular current should hyperpolarize the apical membrane by only 2.0 mV ($= E_s\hat{r}_a/(1 + \hat{r}_a + \hat{r}_s)$).

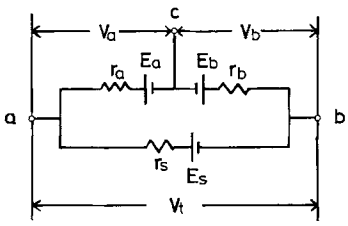

Fig. A1. An equivalent electrical circuit. r_a and r_b are the unit resistances of the apical and basolateral membranes, respectively. r_s represents a unit resistance of a paracellular shunt pathway. E_a and E_b are the electromotive force for the apical and basolateral membranes, respectively. E_s is the diffusion potential through the paracellular shunt pathway. *a*, *b*, and *c* designate the luminal solution, the peritubular solution, and cell interior, respectively.

Appendix II

Figure B1 shows a part of an equivalent circuit model of the proximal tubule. Resistances per unit length are designated by r and appropriate subscripts, apical cell membrane (r_a), basolateral cell membrane (r_b), cytoplasm and gap junction (r_c), and paracellular shunt pathway (r_s), respectively. Unit transverse currents (i_a, i_b, i_s) are defined in the same way. i_c is defined as $i_b - i_a$. When the apical membrane resistance changes in response to alteration of luminal fluid, current distribution in this circuit must change. Unit currents in test solution are denoted with prime, for example i'_a. VDR and VDR′ are the VDR during perfusion of control solution and test solution, respectively.

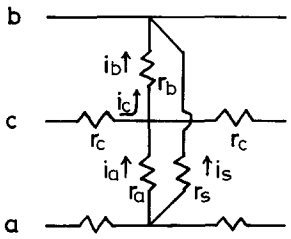

Fig. B1. Current distribution of the elementary unit of cylindrical concentric cables. The electrical source is in the lumen

$$\mathrm{VDR} = \Delta V_a/\Delta V_b = i_a r_a/(i_a + i_c) r_b \tag{B1}$$

$$\mathrm{VDR}' = \Delta V'_a/\Delta V'_b = i'_a r'_a/(i'_a + i'_c) r_b. \tag{B2}$$

Assuming that a change of current responding to change of r_a is small, i'_x is rewritten as $i_x + \Delta i_x$. Δi_x represents a minute change of i_x. Thus,

$$i'_x/i_x = (i_x + \Delta i_x)/i_x = 1 + \Delta \tag{B3}$$

where $\Delta = \Delta i_x/i_x \ll 1$. The ratio of VDR′ to VDR is as follows:

$$\mathrm{VDR}'/\mathrm{VDR} = (\Delta V'_a/\Delta V'_b)/(\Delta V_a/\Delta V_b) = \{i'_a(i_a + i_c) r'_a\}/\{i_a(i'_a + i'_c) r_a\} \tag{B4}$$

By introducing Eq. (B3) into Eq. (B4)

$$\begin{aligned} \mathrm{VDR}'/\mathrm{VDR} &= (1 + \Delta)(1 - \Delta) r'_a/r_a \\ &= (1 - \Delta^2) r'_a/r_a \\ &\simeq r'_a/r_a. \end{aligned} \tag{B5}$$