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Intracellular Potassium Activity Measurements in Single Proximal Tubules of *Necturus* Kidney

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Summary. The intracellular potential (E_M) and potassium activity (a^i_K) of *Necturus* proximal tubule were measured with double barreled liquid ion-exchange microelectrodes. Axial heterogeneity along the length of the accessible proximal tubule was observed, with E_M and a^i_K averaging -63.6 ± 6.0 mV and 59.6 ± 8.5 meq/liter in early segments, and -75.4 ± 6.9 mV and 71.5 ± 7.8 meq/liter in late segments. In both segments a^i_K was above electrochemical equlibrium and thus actively accumulated within the cells. Increasing extracellular $[K^+]$ in increments from 2.5 to 50.0 meq/liter caused E_M to depolarize progressively from -63.9 ± 5.8 to -15.8 ± 3.8 mV, while a^i_K increased only slightly from 63.1 ± 7.0 to 69.0 ± 8.7 meq/liter. The response of E_M to increasing extracellular $[K^+]$ was reduced when extracellular $[Na^+]$ was decreased from 101 to 13 meq/liter. Treatment of tubules with ouabain for 1–2 hr caused a dose-dependent depolarization of the cell potential and a fall in intracellular K^+. With 10^{-4} M ouabain E_M decreased from -61.1 ± 7.6 to -28.1 ± 5.6 mV, and a^i_K decreased from 62.7 ± 5.7 to 10.2 ± 4.0 meq/liter. However, when sodium entry into tubule cells was curtailed by perfusion with low-sodium solutions, or by replacement of chloride with a poorly permeant anion, cellular potassium activity remained unchanged. Taken together, the results of these studies clearly demonstrate that K^+ is actively accumulated within the cells of the *Necturus* proximal tubule and that this accumulation is dependent upon Na^+-K^+-ATPase. In addition, the basolateral cell membrane has a relatively large K^+ conductive pathway, which is subject to modulation by extracellular sodium. Finally, significant axial heterogeneity of the peritubular potential and of a^i_K along the proximal tubule were noted.

Key Words potassium activity · proximal tubule cell · membrane potential · K^+-conductance · ouabain

Introduction

The basolateral membrane of the renal proximal tubule cell is characterized by a high permeability to potassium and the presence of a sodium-potassium exchange pump driven by ATP hydrolysis (Whittembury, 1971; Biagi, Kubota, Sohtell & Giebisch, 1981*a*; Biagi, Sohtell & Giebisch, 1981*b*; Sackin & Boulpaep, 1981*a*; Bello-Reuss, 1982). Such a pump-leak system serves not only to establish the principal intracellular ion composition of the cell but also functions as a key mechanism for net transepithelial sodium transport. It has been the purpose of this investigation to examine several aspects of this dual function – maintenance of intracellular ion composition and active transepithelial sodium transport – through the measurement of intracellular electrical potential (E_m) and potassium activity (a^i_K).

Clearly, the level of intracellular potassium activity and the basolateral membrane potential reflect not only the activity of the basolateral exchange pump but also the relative passive permeabilities of this membrane barrier. To examine these properties, extracellular potassium activity was varied over a wide range and the relationship between the basolateral membrane potential (E_M) and the Nernst equilibrium potential for potassium (ε_K) across the basolateral membrane examined. In addition, chemical determinations of intracellular potassium concentrations were made. This allowed calculation of an apparent intracellular potassium activity coefficient, which could be compared with previous estimates of this parameter (Khuri et al., 1972, 1978, 1979; Kimura & Spring, 1979).

Alterations in net sodium transport, by alterations of peritubular sodium-potassium exchange, might also lead to changes in intracellular potassium activity. With this in mind, we have measured intracellular K^+ activity under conditions that have been shown previously to reduce sharply the rate of net transepithelial sodium transport (Whittembury, 1971; Giebisch, Sullivan & Whittembury, 1973; Spring & Giebisch, 1977*a*, *b*). Three such

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maneuvers were performed, including: i) lowering both luminal and peritubular sodium concentration; ii) replacement of chloride by a less permeant anion; and iii) addition of ouabain to the control peritubular perfusion solution.

Finally, the question of axial heterogeneity along the length of the *Necturus* proximal tubule was addressed. Axial heterogeneity has been clearly established in the mammalian proximal tubule (Biagi et al., 1981*a*; Jacobson, 1981), and also demonstrated in the *Necturus* proximal tubule with regard to fluid absorption and organic solute transport (Forster, Steels & Boulpaep, 1980). In the present studies the intracellular potential and potassium activity of early and late portions of the proximal tubule were compared.

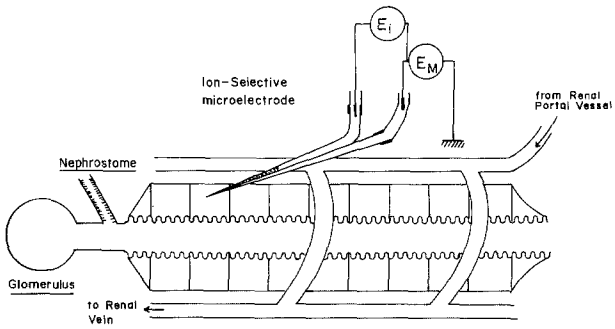

Fig. 1. Schematic for ion-selective microelectrode studies of *Necturus* proximal tubule. E_M represents the basolateral membrane potential measured by the reference half of the double barreled electrode, while E_i is the potential of the ion-selective barrel corrected for the membrane potential

Materials and Methods

The Doubly Perfused Necturus Kidney in Vitro

Experiments were carried out in the doubly perfused *Necturus* kidney, which was prepared as described in detail in a previous publication from this laboratory (Giebisch et al., 1973). A schematic of this experimental model is presented in Fig. 1. Normal aortic perfusion flow rate was 1.5 ml/min and renal portal vein flow rate was 1.0 ml/min. In experiments in which peritubular perfusion was changed at relatively high speed, the renal portal vein was perfused at 3 ml/min while the aortic perfusion rate was maintained at 3.5 ml/min. In all experiments, solutions of the same composition were used to perfuse the aortic and portal circulations.

Control perfusion fluid had the following composition in mmol/liter: NaCl, 90; $NaHCO_3$, 10; Na_2HPO_4, 0.43; NaH_2PO_4, 0.07; $MgCl_2$, 1.0; $CaCl_2$, 2.5; KCl, 2.5; glucose, 2.2; polyvinylpyrrolidone (PVP), 15 g/liter; and 2000 units/liter heparin. Modifications of the control solution were made in the following manner. Extracellular potassium concentration was elevated by replacing increasing fractions of sodium and potassium chloride by potassium and sodium isethionate such that the product $[K^+] \times [Cl^-]$ remained constant. The final potassium concentration of these solutions was 5, 10, 20, 50 and 90 meq/liter. Sodium concentration was lowered from 101 meq/liter to 33 and 19 meq/liter by replacement of NaCl with mannitol, a maneuver which has been shown to inhibit net sodium transport (Spring & Giebisch, 1977*a*). In studies where the $[K^+]$ was raised in the presence of low $[Na^+]$, KCl replaced NaCl in the 33 mM NaCl/mannitol solution such that the final $[K^+]$ was 2.5, 10.0, and 22.5 meq/liter, while the final $[Na^+]$ was 33.0, 26.5, and 13.0 meq/liter respectively. Chloride concentration was lowered by replacing NaCl with sodium cyclamate, a poorly permeant anion which also reduces net sodium transport in the perfused *Necturus* kidney (Giebisch et al., 1973). Finally, ouabain (Sigma Chemical Co., St. Louis, Mo.) was added to control solutions over the concentration range of 10^{-6} to 10^{-4} M. In contrast to the experiments in which extracellular potassium was elevated by rapid perfusion of the peritubular spaces, steady-state measurements during reduced Na^+, Cl^-, and during ouabain perfusions were made after 1–2 hr of perfusion with the experimental solutions. All solutions were gassed with a mixture of 1% CO_2–99% O_2 giving a pH = 7.60. The total osmolality of all solutions was between 210–215 mOsm/liter.

Electrical Methods

Single-barreled microelectrode measurements of the basolateral membrane potential (E_m) were made using glass capillaries containing an internal fiber (Frederick Haer, Brunswick, Me. OD = 1.2 mm). Resistances were in the range of 100–200 MΩ, when filled with either 1.0 M NaCl or 0.5 M KCl. It was found that, providing the tip potential was less than 5 mV, there was no significant difference between cell potential measurements made with KCl or NaCl filled microelectrodes. Consequently, in using K^+ selective double-barreled electrodes, 1.0 M NaCl was used as the filling solution in the reference barrel.

Double-barreled potassium-selective microelectrodes, similar to those originally described by Khuri, Hajjar and Agulian (1972) were prepared and calibrated with minor modifications as described by Fujimoto and Kubota (1976). Briefly, following dichromate-H_2SO_4 acid washing and thorugh rinsing with distilled water, two glass capillaries containing a solid glass fiber (Frederick Haer, OD = 1.0 mm) were fused, twisted, and pulled to a single shank double-barreled microelectrode (tip diameter less than 1 μm). One barrel was filled from the shank with a mixture of acetone and distilled water (volume ratio 20–50:1). The microelectrode tip was then dipped in siliconizing solution (0.5% Dow Corning 1107 Fluid dissolved in acetone) for 3–5 sec and heated on a hot plate at 300 °C for 10–15 min. Potassium-selective liquid ion exchanger (Corning 477317) was introduced into the shank of the silconized barrel. After the resin had migrated to and filled the tip, the back of the electrode was filled with 1.0 M KCl. As discussed previously, the reference barrel was filled with 1.0 M NaCl. Completed double-barreled electrodes were examined microscopically and discarded if the ion exchanger had been displaced from the tip by the filling solution. After filling, the electrode tips were beveled with a microelectrode grinder (WP Instruments, Hamden, Ct., Model 1300) while monitoring the resistance of the reference electrode (*see* below). Electrodes were stored in 1.0 M NaCl solution until use. Fresh electrodes were made each day, however, since it was found that the tip potentials of the reference electrodes increased with storage time (days) and that this was accompanied by a loss in selectivity of the ion-exchanger barrel.

Several criteria were satisfied for the use of a double-barreled electrode. These included: i) the resistance of the reference barrel filled with 1.0 M NaCl was in the range of 80–150 MΩ; ii) the tip size of the electrodes was 0.5–1.0 μm; iii) the tip potential of the reference electrode was less than 5 mV; iv) the slope constant of the ion exchanger barrel was 55–62 mV/

decade K^+ activity; and v) the response time to a step change in potassium activity was greater than 95% complete within 1 sec. Selectivity ratios of the electrode for potassium over sodium were greater than 20:1.

All potential measurements were made using a high impedance differential electrometer ($10^{15}\,\Omega$, W.P. Instruments, Hamden, Ct., model F223A). As illustrated in Fig. 1, the ion-selective electrode measured both basolateral membrane potential (E_m) and a voltage dependent upon K^+ activity in the cell (E_K). Subtraction of the membrane potential from the total ion-selective electrode voltage gave the potassium dependent signal. All voltages were referenced to a ground electrode which consisted of a Ag-AgCl half-cell filled with saturated KCl, and contacted to the peritoneal cavity by a 3-M KCl-Agar bridge. Measured voltages were displayed on panel meters and recorded on a four-channel pen recorder (Gulton Industries, R.I., model TR446).

Calculation of Intracellular Potassium Activity

Intracellular potassium activity was calculated using the following equation:

$$a^i_K = 76.7 \times 10^{(E_{100} - \Delta E_K)/\alpha}$$

where E_{100} is the voltage from the K^+ electrode in 100 mM KCl (K activity = 76.7 mM), ΔE_K is the voltage difference between E_K (intracellular voltage of the K^+ electrode) and E_M (peritubular membrane potential) and α is the slope constant of the K^+ electrode expressed as mV change per decade change in potassium activity. The intracellular recording was accepted only when both E_K and E_M were stable for more than 30 sec.

Determination of Intracellular Sodium and Potassium Concentrations

Intracellular sodium and potassium concentrations were measured following experiments in which extracellular potassium concentration had been elevated or ouabain had been perfused. After the determination of intracellular activities, the aortic and portal perfusates were changed to include 0.1 μCi/ml of either ^{14}C-inulin or ^{14}C-polyethylene glycol (mol wt ≃ 4,000). Perfusion rates were maintained at 1.5 and 1.0 ml/min, respectively, for 45 to 50 min. The venous effluent was sampled at 5-min intervals to determine the steady-state isotope concentration in the fluid bathing the kidney. Effluent concentrations (cpm/min/ml) were constant after approximately 15 min of perfusion.

At the end of the equilibration period, the kidneys were rapidly removed and cut into four equal pieces. Each piece was gently blotted on Whattman filter paper # 1 and immediately weighed. The tissue was then dried overnight in an oven at 110 °C and reweighed. The difference between the wet and dry weights was taken as the total tissue water.

To extract sodium, potassium, and isotope content, each tissue piece was placed in 10 ml of an extraction fluid containing 15 mM HCl and 70 mM HNO_3. Incubation lasted for 48 hr. Sodium and potassium concentrations in the extraction fluid were read directly by flame photometry (Instrumentation Laboratories, model # 143) against known standards mixed in extraction fluid. Isotope content of the extraction fluid was measured by liquid scintillation counting (Searle Analytic) and referenced to aliquots of venous effluent which had been diluted in an equal volume of nonlabeled extraction fluid. The extracellular fluid volume was calculated from the inulin and polyethylene glycol counts.

Calculations of intracellular ion concentrations were made from the separate determinations of intracellular fluid volume and intracellular ion content. Both parameters were estimated as the difference between total tissue content of water or ion and that associated with extracellular fluid. Extracellular fluid was assumed to have the same electrolyte composition as the aortic and portal perfusion solutions.

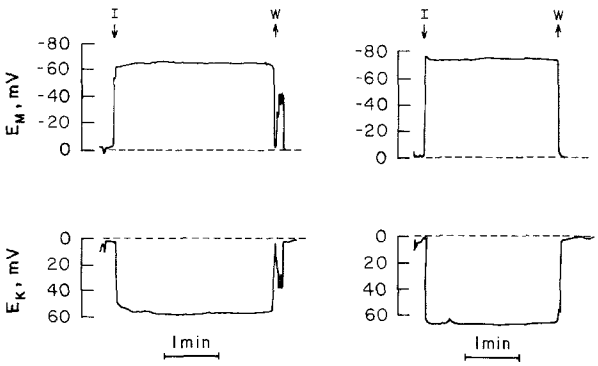

Fig. 2. Typical strip-chart records of membrane potential (E_M) and the potassium-dependent potential (E_K). Examples from the early (*left*) and late (*right*) segments of the proximal tubule are shown

Values are reported as mean ±SE. Differences between group means were analyzed by Student's t test, with $P < 0.05$ considered significant. The standard error of the Nernst equilibrium potential for K^+ (ε_K) was calculated according to Eisenberg and Gage (1969) as used by Giebisch, Malnic, DeMello & DeMello-Aires (1977).

Results

Intracellular Potassium Activity in Early and Late Tubule Segments

Figure 2 illustrates typical intracellular recordings of basolateral membrane potential and intracellular potassium activity as measured with the double barreled microelectrode. Punctures from both early and late tubular segments are shown. Stable recordings lasting several minutes could frequently be obtained. In addition, the rapid response of the potassium selective barrel to the change from extracellular to intracellular activity on insertion and withdrawal from the cell is apparent.

The results obtained from punctures made in early and late proximal tubule segments are summarized in Table 1. Mean values of both PD and a^i_K in the late tubule segments (−75.4 mV and 71.5 meq/liter) were signicantly higher than those found in the early segment (−63.6 mV and 59.6 meq/liter), thus providing evidence for axial heterogeneity along the length of the accessible portion of the proximal tubule. In both cases, however, the calculated Nernst equlibrium potential for potassium (ε_K) across the basolateral membrane was more negative than the membrane potential difference. Therefore, in both tubular seg-

Table 1. Intracellular potentials and potassium activities in early and late segments of *Necturus* proximal tubules

	E_M (mV)	a^i_K (meq/liter)	ε_K (mV)
Early segment ($n=25$)	-63.6 ± 1.2	59.6 ± 1.7	-86.8 ± 0.7
Late segment ($n=10$)	-75.4 ± 2.2	71.5 ± 2.5	-91.4 ± 0.9
p	<0.001	<0.001	<0.001

Values are mean $\pm$SEM. ε_K was calculated assuming $a^e_K=1.8$ meq/liter.

ments intracellular potassium activity is maintained at a higher activity than that predicted for passive distribution. The magnitude of the driving force for potassium efflux from the cell (PD-ε_K) was 23.2 mV in the early segment and 16.0 mV in the late segment. In the steady state, this driving force must be balanced by the active transport of potassium into the cell. Similar results have also been obtained in the perfused bullfrog kidney (Kubota, Honda, Kotera & Fujimoto, 1980) and the rat proximal tubule (Edelman, Curci, Samarzija & Frömter, 1978; Cemerikić, Wilcox & Giebisch, 1982).

Axial heterogeneity along the *Necturus* proximal tubule has also been described by Forster et al. (1980), where they found that net fluid reabsorption was significantly higher in the early convoluted segment than in the later straight segment. Furthermore, the transepithelial specific resistance was also significantly lower in the early than in the late segment. The present study extends the description of axial heterogeneity to the cellular level. Not only were the values of intracellular potential and K^+ activity different in early and late segments, but the effects of extracellular pH and $[Cl^-]$ on the cell PD of these segments varied (*see* Fig. 3). In the early segment reducing bath pH from 7.6 to 7.0 resulted in a large ($\sim$30 mV) and sustained depolarization of E_M. The cell rapidly repolarized upon return to pH 7.6. Reduction in bath $[Cl^-]$ from 90 to 36 meq/liter in the same cell produced no change or a slight hyperpolarization of E_M. In contrast, when similar bath solution changes were made in late segments, E_m showed a much smaller depolarization with acid bath (7 mV) and a significant depolarization of E_M ($\sim$10 mV) following bath perfusion with low chloride Ringer. The change in E_M following low $[Cl^-]$ bath suggests that the basolateral membrane of the early segment is relatively impermeable to chloride while a significant Cl^- conductance could be demonstrated in late tubular segments. A more detailed study of bath pH effects will be presented in the companion paper (Kubota, Biagi & Giebisch, 1982).

Increasing Extracellular Potassium Activity

When kidneys were perfused for 1–2 hr with solutions of different extracellular potassium concentrations at constant $K^+ \times Cl^-$ product, the mean peritubular membrane PD and ε_K were determined as a function of the external potassium activity, a^e_K. All values were obtained using double barreled potassium selective microelectrodes. Thus, ε_K could be directly calculated from the ratio of intracellular and peritubular potassium activities. A summary of results is given in Table 2 and Fig. 4. As seen in Table 2, when extracellular potassium was raised there was little or no change in intracellular K^+ activity. Only at the highest a^e_K of 38 meq/liter was there a small but significant increase in a^i_K. Accordingly, as a^e_K was increased, ε_K became less negative, and this change was closely followed by a depolarization of the basolateral potential (Fig. 4). This is clearly in agreement with the predicted changes in potential across a potassium-selective membrane as extracellular potassium is raised. As a^e_K was increased the electrochemical gradient for potassium efflux from the cell decreased to the point where at $a^e_K=38$ meq/liter the gradient was not different from zero (i.e., potassium distribution was at equilibrium across the basolateral membrane).

Intracellular Potassium and Sodium Concentrations

Chemical determination of potassium and sodium *concentrations* in the perfused kidney cells are summarized in Fig. 5. Under control conditions ($[K^+]_o=2.5$ meq/liter) the measured intracellular $[K^+]$ was 133.6 ± 13.9 meq/liter and intracellular $[Na^+]$ was 20.3 ± 8.5 meq/liter. No consistent change in either intracellular potassium or sodium concentrations were observed when external potassium was raised progressively to 90 meq/liter. Clearly, the activity values of K^+ are considerably less than the chemical concentrations of K^+. A similar relationship between K^+ activity values and chemical concentrations of K^+ have been reported by others (Khuri et al., 1975, 1978).

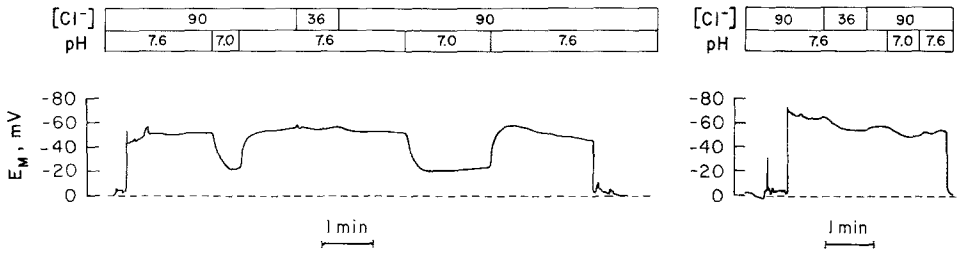

Fig. 3. Response of early (*left*) and late (*right*) segments of the proximal tubule to changes in peritubular pH and $[Cl^-]$. Note the large depolarization of E_M in early segments as peritubular pH is lowered by reducing $[HCO_3^-]$ at constant PCO_2. This is not seen in late segments. Reducing the peritubular $[Cl^-]$ to 36 mM has no effect in early segments but causes a slight depolarization of E_M in late segments

Table 2. Effect of extracellular $[K^+]$ on intracellular potentials and potassium activities in early proximal tubule segments

$[K^+]$ (meq/liter)	E_M (mV)	a_K^i (meq/liter)	ε_K (mV)
2.5 ($n=17$)	-63.9 ± 1.4	63.1 ± 1.7	-88.1 ± 0.7
5.0 ($n=19$)	-60.4 ± 1.4	64.6 ± 2.2	$-71.3^a\pm0.9$
10.0 ($n=13$)	$-48.4^a\pm1.5$	67.5 ± 3.1	$-54.7^a\pm1.1$
20.0 ($n=21$)	$-34.4^a\pm0.6$	66.8 ± 1.4	$-37.2^a\pm0.5$
50.0 ($n=21$)	$-15.8^a\pm0.8$	$69.0^b\pm1.9$	$-14.5^a\pm0.7$

Values are mean ±SEM. Extracellular K^+ activity was calculated assuming $\gamma=0.73$. P values represent comparisons to values at $[K^+]=2.5$ meq/liter.
[a] $P<0.001$. [b] $P<0.05$.

Effect of Reducing Luminal and Peritubular Sodium and Chloride Concentrations

Studies on the isolated perfused *Necturus* kidney have previously shown that net sodium transport is sharply reduced by lowering both luminal and peritubular sodium concentration, and by substitution of Cl^- with the less permeant anion cyclamate (Spring & Giebisch, 1977*a*, *b*). Table 3 summarizes the effects of these maneuvers on basolateral membrane potential and intracellular potassium activity of early proximal tubule cells.

Reducing extracellular sodium concentration to 19 mM resulted in a progressive depolarization of the basolateral PD from the control value of −60.3 mV to a value of −48.4 mV (30–60 min) and −34.5 mV (60–120 min). In contrast, there was no significant change in intracellular potassium activity from the control value of 58.9 meq/liter. As a consequence, the calculated ε_K across the basolateral membrane also remained constant (86 mV) while the electrochemical gradient for potassium efflux increased from a control value of 24.9 mV to a maximum value of 51.5 mV. Reduction of extracellular $[Na^+]$ to 33 meq/liter produced similar results.

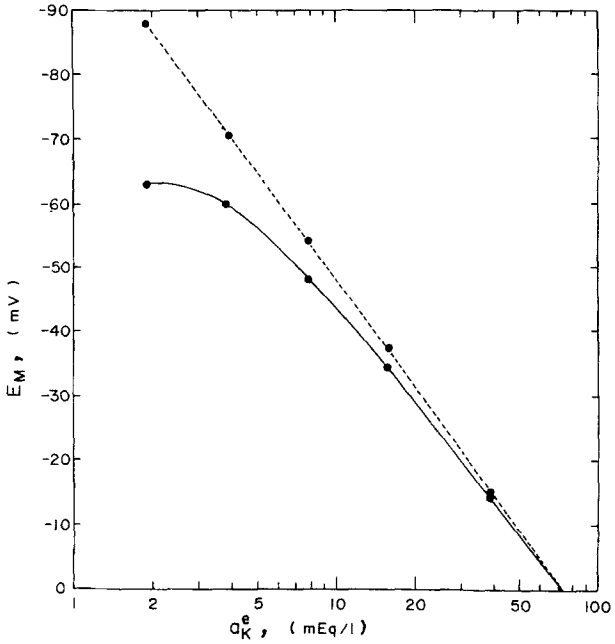

Fig. 4. Dependency of E_M on extracellular K^+ activity (a_K^e). The dashed line represents the calculated values of the Nernst equilibrium potential for K^+ (ε_K)

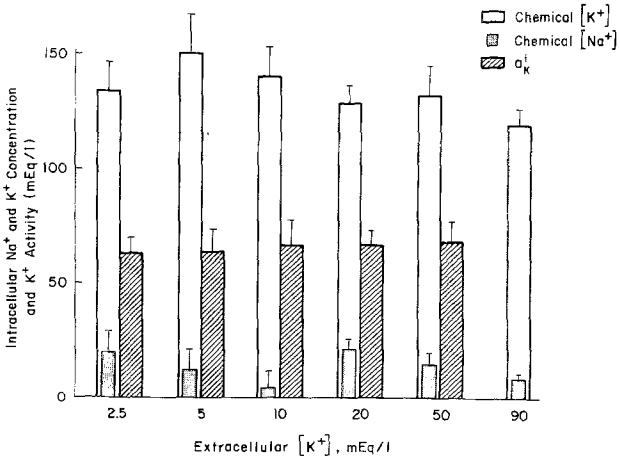

Fig. 5. Comparison of the chemical concentrations of Na^+ and K^+, and intracellular K^+ activity (a_K^i) at different levels of extracellular K^+

Table 3. Effect of extracellular $[Na^+]$ and $[Cl^-]$ on intracellular potentials and potassium activities in early proximal tubule segments

	E_M (mV)	a^i_K (meq/liter)	ε_K (mV)
19 mM NaCl			
Control ($n=11$)	-60.3 ± 1.1	58.9 ± 2.1	-86.3 ± 0.9
30–60 min ($n=7$)	-48.4 ± 1.5	53.7 ± 2.3	-87.2 ± 1.1
>60 min ($n=18$)	-34.5 ± 1.1	60.7 ± 2.0	-85.7 ± 0.9
33 mM NaCl			
>60 min ($n=17$)	-46.4 ± 1.5	58.4 ± 1.5	-86.3 ± 0.7
Na-Cyclamate			
Control ($n=25$)	-63.6 ± 1.2	59.6 ± 1.7	-86.6 ± 0.7
>60 min ($n=30$)	-73.7 ± 1.3	69.9 ± 1.4	-90.7 ± 0.5

Values are mean ±SEM. ε_K was calculated assuming $a^e_K = 1.8$ meq/liter.

[a] Control values represent determination made prior to a reduction of extracellular NaCl. The control solution contained 101 meq/liter of Na^+ and 95 meq/liter of Cl^-.

Table 4. Effect of extracellular $[Na^+]$ and $[K^+]$ on intracellular potential and potassium activity in early proximal tubule segments

$[Na^+]$ (meq/liter)	$[K^+]$ (meq/liter)	E_M (mV)	a^i_K (meq/liter)	ε_K (mV)
101.0 ($n=21$)	2.5	-60.5 ± 1.1	59.3 ± 1.7	-86.7 ± 0.7
33.0 ($n=17$)	2.5	-46.4 ± 1.5	58.4 ± 1.5	-85.0 ± 0.7
26.5 ($n=8$)	10.0	-40.9 ± 1.2	66.7 ± 2.2	-53.4 ± 0.9
13.0 ($n=12$)	22.5	-32.0 ± 0.6	67.5 ± 2.4	-33.3 ± 0.9

Values are mean ±SEM.
Extracellular K^+ activity were calculated assuming $\gamma = 0.73$.

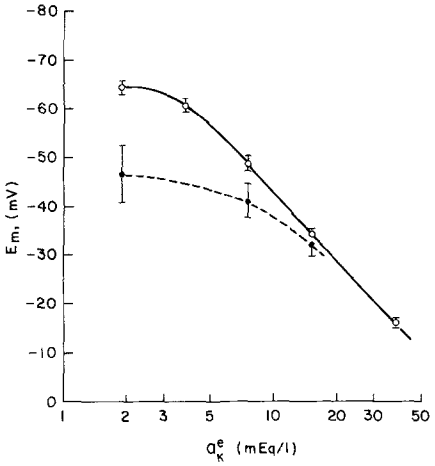

Fig. 6. Dependency of E_M on extracellular K^+ activity a^e_K. The effect of reducing extracellular $[Na^+]$ from 101 meq/liter (open circles) to 13 meq/liter (closed circles) is shown

Substitution of chloride with the less permeant anion cyclamate resulted in the opposite changes in E_M and a^i_K. Following 60 min of cyclamate perfusion the E_M had hyperpolarized from -63.6 to -73.7 mV and a^i_K had increased from 59.6 to 69.9 meq/liter. These changes resulted in only small differences in the calculated ε_K and electrochemical gradient (23.3 *vs.* 17.1 mV) across the basolateral membrane.

Increasing a^e_K at Low Extracellular Sodium

The results presented in Table 3 show that the basolateral potential depolarizes as extracellular sodium is reduced. In these studies the extracellular $[K^+]$ was held constant (2.5 meq/liter), while NaCl was replaced by mannitol (*see* Results). This change in E_M is opposite of that expected if a conductive pathway(s) for Na^+ existed at either cell border (i.e., decreasing extracellular sodium should hyperpolarize the E_M). To examine the possibility that the depolarization with low external $[Na^+]$ is due to a change in basolateral membrane potassium conductance, kidneys were perfused with low $[Na^+]$ solutions and simultaneously extracellular potassium was raised. When extracellular sodium was low, increasing extracellular potassium resulted in a depolarization of the PD and a significant increase in intracellular potassium activity (*see* Table 4). Furthermore, when the extracellular $[Na^+]$ was lowered to 13 meq/liter, the slope of the relationship between extracellular K^+ activity and basolateral membrane potential was less than that observed at an extracellular $[Na^+]$ of 101 meq/liter (*see* Fig. 6). Thus it appeared that by reducing extracellular $[Na^+]$ the equivalent conductance of the basolateral membrane to K^+ was reduced. This apparent decrease in the basolateral membrane K^+ conductance could account for the observed depolarization of E_m when extracellular Na^+ was reduced.

The Effect of Ouabain on Cell PD and Ion Content

The effect of peritubular ouabain was examined over the concentration range of 10^{-6} to 10^{-4} M ouabain. In all experiments the tissue was treated with ouabain for 1–2 hr. These results are summarized in Table 5. At all ouabain concentrations, there was a dose-dependent fall in a^i_K. The highest dose of ouabain, 10^{-4} M, resulted in a fall in a^i_K

Table 5. Effect of ouabain on the intracellular potential and potassium activity in early proximal tubule segments

	E_M (mV)	a^i_K (meq/liter)	ε_K (mV)	Cell [Na^+] (meq/liter)	Cell [K^+] (meq/liter)
Control					
($n=37$)	−61.1 ±1.3	62.7 ±0.9	−87.9 ±0.4	20.3 ±1.5	133.6 ±2.3
Ouabain					
10^{-6} M ($n=8$)	−57.1 ±1.5	54.0[b] ±4.4	−83.7[b] ±2.1	52.8[a] ±1.0	114.9[c] ±1.4
10^{-5} M ($n=15$)	−49.4[a] ±1.7	33.6[a] ±2.1	−71.4[a] ±1.7	61.5[a] ±2.1	95.1[a] ±2.1
10^{-4} M ($n=9$)	−28.1[a] ±1.9	10.2[a] ±1.3	−40.8[a] ±2.7	77.9[a] ±1.5	83.9[a] ±1.8

Values are mean ±SEM. ε_K was calculated assuming $a^e_K = 1.8$ meq/liter.
[a] $P<0.001$
[b] $P<0.005$.
[c] $P<0.025$.

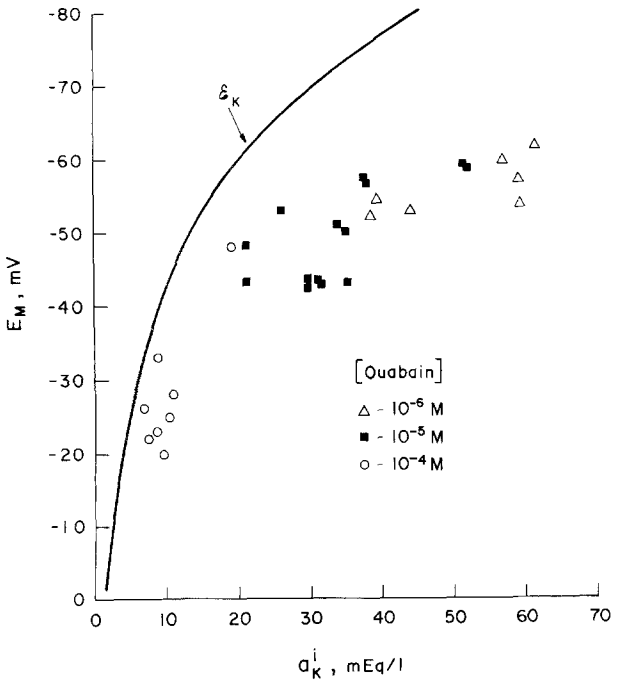

Fig. 7. Relationship between membrane potential (E_M) and the intracellular potassium activity (a^i_K) at different concentrations of peritubular ouabain. The solid line represents the Nernst equilibrium potential for K^+ (ε_K)

from 62.7 to 10.2 meq/liter and in a depolarization of E_M from −61.1 to −28.1 mV. Figure 7 illustrates the relationship between the basolateral membrane potential (E_M) and a^i_K at various concentrations of ouabain. Clearly, at the higher ouabain doses, the E_M approaches the calculated Nernst equilibrium potential for K^+, suggesting that passive membrane properties are sufficient to account for the K^+ distribution across the basolateral membrane. In addition, chemically measured potassium concentrations paralleled the change in activity, falling from a control value of 134 meq/liter to a low of 83.9 meq/liter. Accompanying these changes was an increase in intracellular sodium concentration from 20.3 meq/liter to 77.9 meq/liter.

Discussion

In this study we have addressed several questions regarding the distribution of potassium ions across the peritubular cell membrane. One consistent finding is the observation that under control conditions, intracellular potassium ions are not in electrochemical equilibrium across the basolateral membrane. Intracellular K^+ activity (~65 meq/liter) is higher than that predicted on the basis of the basolateral membrane potential to the extent that there is a gradient for potassium efflux out of the cell equivalent to a driving force of approximately 25 mV (Tables 1 and 2). Under steady-state conditions when potassium efflux must equal potassium influx and a^i_K is constant, the net passive efflux of K^+ must be balanced by the active transport of K^+ into the cell. Thus, as has been demonstrated in a number of renal tissues (Biagi et al., 1981*b*; Sackin & Boulpaep, 1981*b*; Cemerikić et al., 1982), the level of intracellular potassium activity is set by a pump-leak system where the net passive fluxes (efflux) are balanced by active pump fluxes (influx) to obtain steady-state condition.

The sensitivity of intracellular potassium activity to different doses of ouabain (Fig. 7 and Table 5) is clearly in support of this conclusion. The specificity of ouabain inhibition of Na^+-K^+ ATPase is well known, and the low level to which intracellular potassium activity falls, i.e., to near equilibrium values, following exposure to 10^{-4} M ouabain, suggests that basolateral Na^+-K^+ pumping is the principal mechanism responsible for intracellular accumulation of K^+. The observation that K^+ does not reach equilibrium values following long exposures to relatively high doses of ouabain may suggest additional mechanisms, as yet unidentified, which may play a minor role in the regulation of intracellular potassium activity.

Chemical determinations of intracellular sodium and potassium concentrations provide additional support for a primary role of the Na^+-K^+

pump in determining intracellular ion composition in *Necturus* proximal tubule. Consistent with Na^+-K^+ pumping is the observation that intracellular sodium concentration is low (10–20 meq/liter) while potassium is high (130 meq/liter). Inhibition of the pump with ouabain results in reciprocal changes in these concentrations, with an increase in sodium and a decrease in potassium concentration. It is interesting to note that the changes in $[Na^+]$ (+58 meq/liter) and $[K^+]$ (−50 meq/liter), were almost identical, indicating that the sum of intracellular cation concentrations is constant.

In agreement with previously published results (Giebisch, 1961; Boulpaep, 1967; Whittembury, 1971), a second consistent finding in our experiments was that the basolateral membrane is selective for potassium. Thus when extracellular potassium concentration is raised the basolateral membrane potential depolarizes as would be predicted by changes in the Nernst equilibrium potential for potassium across this membrane.

The results presented in Fig. 4 and Table 2 show clearly that intracellular K^+ does not change greatly when external K^+ is raised as high as 50 mM. In addition, the direct intracellular activity measurements allow ε_K to be calculated and compared with the basolateral membrane potential, E_M as is done in Fig. 4. Clearly, as the external potassium is raised the basolateral membrane behaves more and more as a pure potassium electrode, (i.e., the contribution of other permeant ions to the membrane potential becomes less and less). This result would be expected for a membrane which is principally K^+ selective under conditions of normal external K^+, since raising external $[K^+]$ would increase the potassium conductance so that the membrane would become dominated by the ratio of internal/external K^+ activities. Additional support for this conclusion can be made by extrapolating the ε_K and E_M lines in Fig. 4, to the abscissa. At this point PD = 0, and since the membrane is dominated by the K^+ activity ratio, the ratio of internal to external activity should approach one. The extrapolated a^i_K at PD = 0 in Fig. 4 is 69 mM, a value not different from directly measured a^i_K values over the entire range of external K^+ activities (Table 2).

Finally, it was of interest to examine the effects of several experimental maneuvers known to inhibit net volume reabsorption on a^i_K. Since normal functioning of a Na^+-K^+ pump is required for proximal tubule sodium transport in the *Necturus* (Giebisch et al., 1973), it might be expected that changes in the rate of net sodium transport could affect a^i_K. Clearly for the case of ouabain inhibition this is true (Fig. 7 and Table 5). Inhibition results in a fall in a^i_K, and as shown by chemical analysis intracellular sodium concentration shows reciprocal changes. Thus, direct pump inhibition results in a marked fall in intracellular K^+ and rise in intracellular Na^+.

In sharp contrast to the ouabain results is the observation made in the low Na^+ and low Cl^- experiments (Table 4). These experiments show either no change (low Na^+) or a small increase in cell K^+ activity (low Cl^-), yet both maneuvers have been shown to result in significant inhibition of net sodium transport by reducing Na^+ delivery to the peritubular active pump mechanism (Spring & Giebisch, 1977*a*, *b*). Considering the mechanism by which cellular K^+ remained unchanged, or was even slightly increased, following diminished entry of sodium ions across the luminal cell membrane, at least two possibilities have to be considered.

First, it is possible to consider the transepithelial transport of Na^+ by the *Necturus* proximal tubule within the framework of a two-barrier model similar to the concepts developed by Koefoed-Johnsen and Ussing (1958). Accordingly, the luminal cell membrane is the main site of sodium entry, largely by a sodium chloride cotransport system (Spring & Kimura, 1978), whereas the peritubular cell membrane has a low sodium permeability (Cemerikić & Giebisch, 1981), and actively extrudes sodium by an ATPase driven sodium-potassium exchange pump (Giebisch, 1961; Whittembury, Sugino & Solomon, 1961). The K^+ actively pumped into the cell during Na^+ extrusion would recycle across the basolateral cell membrane down its electrochemical gradient. Thus, restricting sodium entry across the luminal cell membrane (luminal perfusion with low sodium or cyclamate), despite the ensuing fall in pump activity, would be expected to restrict K^+ efflux across the basolateral cell membrane. In addition, restricting NaCl entry into the cell would be expected to cause cell shrinkage (Ericson & Spring, 1982), which in turn could result in a rise in intracellular $[K^+]$. In experiments on the isolated frog skin, Rick, Dörge, von Arnim and Thurau (1978) have also observed that the effects of pump inhibition upon intracellular $[K^+]$ depend critically upon the availability of sodium in the mucosal (luminal) solution. Thus, whereas ouabain normally led to a sharp fall of cellular potassium concentration, these effects were totally abolished when the outside medium was replaced with sodium-free solutions.

A second possibility involves the interesting

suggestion that there exists a parallel relation between the rate of peritubular sodium extrusion and the conductance of that barrier to potassium ions. This topic has recently been reviewed by Schultz (1981). Although the precise mechanism of such a relationship is not clear, such an effect would obviously protect the intracellular potassium concentration from changing during changes in the rate of pump-mediated net sodium transport. With respect to the results of the present study, a fall in peritubular pump activity would lower peritubular potassium permeability, and thus by restricting potassium loss maintain cell potassium activity at control levels. It is of interest that both the absolute and relative potassium conductance of the peritubular cell membrane has been reported to fall when peritubular sodium-potassium exchange has been compromised by perfusion of the *Necturus* kidney with low-K^+ solutions (Matsumura, Guggino & Giebisch, 1982).

Reducing extracellular Na^+ also results in a depolarization of the basolateral membrane and the transepithelial potential (*see* Table 3, and Kimura & Spring, 1979; Spring & Giebisch, 1977*a*, *b*). Consideration of the mechanism underlying the basolateral potential changes in low Na^+ solutions suggests that alterations in basolateral ionic permselectivity may be involved. Although the mechanism(s) by which extracellular Na^+ could alter the ionic permselectivity of the basolateral cell membrane is unknown, it is important to note that changes in peritubular and cell pH have been shown to affect basolateral potassium permeability (Steels & Boulpaep, 1976; Biagi et al., 1981*a*, *b*; Boron & Boulpaep, 1981), and that a Na^+-H^+ exchange is present in amphibian proximal tubules (Boron & Boulpaep, 1981). Thus reducing external [Na^+] may result in a sequence of events involving a decreased Na^+-H^+ exchange, a fall in intracellular pH, a reduction of K^+ permeability and, finally, depolarization of the basolateral membrane potential. Alternatively, and in addition, changes in cytosolic Ca^{2+} could modulate the basolateral membrane K^+ conductance. Accordingly, the following scheme can be proposed. Reducing extracellular [Na^+] would inhibit basolateral membrane Na^+-Ca^{2+} exchange leading to a rise in cytosolic Ca^{2+} levels (Taylor & Windhager, 1979). This rise in intracellular Ca^{2+} could in turn reduce the K^+ conductance of this membrane. The above considerations clearly underscore the complex interrelationships between extracellular [Na^+], cellular ion homeostasis, and various cellular ion transport systems.

Similarly, the effects of reducing extracellular [Cl^-] are complex and at present incompletely understood. Cl^- substitution may limit Na^+ entry into the cell by reducing the transport via the Na^+-Cl^- cotransport system in the luminal membrane (Spring & Kimura, 1978; Kimura & Spring, 1979). Again, the failure to observe a fall in cell K^+ activity (Table 5) may be due to the sharp curtailment of cellular sodium-potassium exchange (*see* above). As with low [Na^+], decreasing external [Cl^-] results in a change in peritubular E_M opposite to that predicted by passive electrodiffusion, implying the involvement of additional mechanisms, such as altered Cl^--HCO_3^- exchange across the basolateral membrane, and secondary effects on cell pH (Guggino et al., 1982*a*, *b*).

In conclusion, our results of simultaneous measurements of cell potential and ion activities demonstrate that K^+ is actively transported into and accumulated within the cell compartment by the Na^+-K^+ pump. Whereas the level of potassium within the cell drops sharply with direct pump inhibition, it is not affected by maneuvers that primarily curtail sodium delivery to the pump site. Increasing external [K^+] clearly demonstrates the potassium selectivity of the peritubular cell barrier, and experiments in low Na^+ solutions indicate a significant reduction in potassium selectivity.

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[1] The values of a_K^i were not corrected for interference due to elevated intracellular Na^+ content. Because of the high selectivity of the microelectrodes for K^+ over Na^+ (>20:1) the errors in the values of a_K^i were expected to be less than 1–2 meq/liter.

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