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Intracellular Potential and K^+ Activity in Rat Kidney Proximal Tubular Cells in Acidosis and K^+ Depletion

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Summary. Techniques were developed for the measurement of intracellular potentials and potassium activities in rat proximal tubule cells using double barreled K^+ liquid-ion-exchanger microelectrodes. After obtaining measurements of stable and reliable control values, the effects of K^+ depletion and metabolic and respiratory acidosis on the intracellular potential and K^+ activity in rat kidney proximal tubular cells were determined. At a peritubular membrane potential of -66.3 ± 1.3 mV (mean $\pm$ SE), intracellular K^+ activity was 65.9 ± 2.0 mEq/liter in the control rats. In metabolic acidosis [70 mg NH_4Cl/100 g body wt) the peritubular membrane potential was significantly reduced to -47.5 ± 1.9 mV, and cellular K^+ activity to 53.5 ± 2.0 mEq/liter. In contrast, in respiratory acidosis (15% CO_2) the peritubular membrane potential was significantly lowered to -46.1 ± 1.39 mV, but the cellular K^+ activity was maintained at an almost unchanged level of 63.7 ± 1.9 mEq/liter. In K^+ depleted animals (6 weeks on low K^+ diet), the peritubular membrane potential was significantly higher than in control animals, -74.8 ± 2.1 mV, and cellular K^+ activity was moderately but significantly reduced to 58.1 ± 2.7 mEq/liter. Under all conditions studied, cellular K^+ was above electrochemical equilibrium. Consequently, an active mechanism for cellular K^+ accumulation must exist at one or both cell membranes. Furthermore, peritubular HCO_3^- appears to be an important factor in maintaining normal K^+ distribution across the basolateral cell membrane.

Key words renal proximal tubule · cellular K^+ activity · metabolic acidosis · respiratory acidosis · K^+ depletion

Introduction

Under normal conditions approximately 80% of the filtered load of K^+ is reabsorbed along the proximal tubule (Giebisch, 1981). However, the route of transepithelial K^+ movement (cellular *vs.* paracellular), and the mechanism(s) of cellular K^+ transport remain controversial. In order to gain insight into the mechanisms of cellular K^+ transport and homeostasis it is necessary, as a first step, to define the electrochemical driving forces for K^+ across both the luminal and peritubular cell membranes. In this regard, measurements of intracellular potentials and K^+ activities of rat and rabbit proximal tubule cells have produced conflicting results. Studies by Edelman, Curci, Samarzija and Frömter in the rat (1979), and by Biagi, Sohtell and Giebisch in the rabbit (1981*b*) have shown that K^+ is above electrochemical equilibrium across both cell membranes. In contrast, Khuri et al. (1973) found that K^+ was in electrochemical equilibrium.

The present study was done to re-examine and to extend studies on the distribution of K^+ across the cell membranes of rat proximal tubules. To this end intracellular potentials and K^+ activities were measured using double-barreled K^+-sensitive liquid ion-exchanger microelectrodes. Since dietary potassium restriction and acute acidosis can lead to cellular K^+ loss (Gennari & Cohen, 1975; Linas et al., 1979), we also examined the effects of K^+ depletion and acute metabolic and respiratory acidosis on cellular K^+ distribution. As will be shown, K^+ is above electrochemical equilibrium under all conditions studied. Consequently an active mechanism for cellular K^+ accumulation must exist at one or both of the cell borders. Furthermore, changes in the peritubular [HCO_3^-] appear to play an important role in maintaining the normal distribution of K^+ across this membrane.

Materials and Methods

Experiments were performed on male Sprague-Dawley rats weighing 190 to 250 g. All animals were deprived of food the night prior to study (18 hr), but allowed free access to water. The rats were prepared for micropuncture as described previously from this laboratory (Wright, Strieder, Fowler & Giebisch 1971). In brief, the animals were anesthetized with Inactin (Promonta Corp.; Hamburg, Germany), 100 mg/kg body weight

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Table 1. Plasma composition of rats during changes in acid-base and K^+ balance

Group	pH	$P_a CO_2$ (mm Hg)	$[HCO_3^-]$ (mEq/liter)	$[K^+]$ (meq/liter)
Control ($n=7$)	7.38±0.02	41.0±1.5	23.7±1.1	4.1±0.3
Metabolic acidosis ($n=8$)	7.13±0.01[c]	29.9±3.0[b]	9.7±0.8[c]	5.3±0.3[a]
Respiratory acidosis ($n=6$)	7.01±0.01[c]	110.3±3.6[c]	27.1±0.9[a]	4.9±0.4
K depletion ($n=11$)	7.51±0.02[c]	43.2±2.3	33.4±0.9[c]	2.0±0.2[c]

Values are means±SE. n=number of rats. [a] $P<0.05$. [b] $P<0.01$. [c] $P<0.001$.

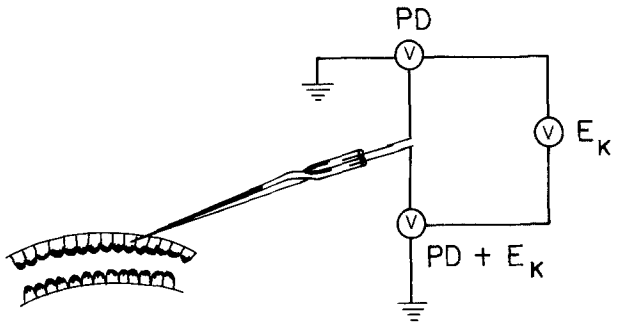

Fig. 1. Electrical schematic for recording intracellular ion activities with double barrel microelectrode. *PD* represents the basolateral membrane potential, $PD+E_K$ the potential recorded by the ion-sensitive barrel, and E_K the differential recording between both barrels of the electrode

(BW) i.p., and placed on a thermostatically controlled heating board to maintain body temperature at 38 °C. A tracheostomy was performed and catheters were placed in the carotid artery and jugular vein. Each animal received isotonic saline (1–1.5 ml/100 g BW per hour) during the experiment. The left kidney was exposed through a flank incision and immobilized in a Lucite cup with 3 g% Ringer agar. The decapsuled kidney surface was superfused with warm (37–38 °C) Ringer of the following composition (in mmol/liter): sodium, 144; potassium, 5; calcium, 1.8; magnesium, 1; chloride, 123.6; bicarbonate, 25; phosphate, 2; sulphate, 1; acetate, 2; acetate, 2; D-glucose, 5; alanine, 5; and urea, 3.3. The superfused Ringer was gassed continuously with 5% CO_2–20% O_2 (balance N_2), and had a pH of 7.4 In K^+-depleted animals the superfused Ringer was modified as follows: bicarbonate was elevated to 35 mM and potassium reduced to 2 mM (substitution with NaCl). In animals undergoing metabolic acidosis, the superfusion fluid was modified to contain 10 mM $NaHCO_3$ (substitution with NaCl), and in respiratory acidosis Ringer's solution was used in which 15% CO_2–20% O_2 (balance N_2) replaced the control gas mixture. Proximal tubules were identified with 0.05 ml of 5% FD & C green (Keystone Chemical Co., Chicago, Ill.) in isotonic saline infused via the jugular vein. Blood for analysis was obtained from a carotid artery; blood pH and PCO_2 were measured at 37 °C with a pH blood gas analyzer (Model 213, Instrumentation Laboratory Inc., Lexington, Mass.), and potassium was determined by flame photometry with lithium as the internal standard (Model 143, Instrumentation Laboratory Inc., Lexington, Mass.). Plasma bicarbonate concentrations were calculated by the Henderson-Hasselbalch equation using a pK=6.1. The plasma composition of the rats in each experimental group is summarized in Table 1.

K^+ depletion was induced by feeding the animals with a K^+-deficient diet (ICN Pharmaceuticals, Cleveland, Oh.) for six weeks prior to the experiments. Metabolic acidosis was achieved by giving orally 70 mg NH_4Cl/100 g BW, 30 min prior to the induction of anesthesia.

Intracellular K^+ activities were measured with double barreled microelectrodes as shown in Fig. 1. Impalement of the tubule cells was facilitated by keeping the microelectrode at a shallow angle (30°) to the kidney surface. The reason for this is not known, but a similar observation has been reported for studies of rabbit proximal tubules perfused *in vitro* (Biagi et al., 1981*a*, *b*). Other precautions were also taken to improve the success rate at which stable impalements could be obtained and maintained. Care was taken to insure that the diaphram of the rat did not contact the Lucite kidney cup during inspiration. Additionally it was found that food deprivation the night prior to study tended to decrease gut motility, thereby further minimizing movement of the kidney. Despite these precautions, it was not possible to eliminated the pulsations of the kidney during the cardiac cycle. Consequently, the microelectrode was connected to its holder with a small piece of silicone tubing (Sato, 1977), thus allowing it to "float" with the pulsatile excursions of the kidney.

The electrodes and their manufacture were similar to those described by Fujimoto and Kubota (1976), and by Biagi et al. (1981*b*). In brief, thick walled (OD=1 mm; ID=0.5 mm) borosilicate capillary tubing with an internal fiber (Frederick Haer Co., Brunswick, Me.) was first washed in sulfo-chromic acid and rinsed repeatedly with distilled water in order to minimize the development of tip potentials (Okada & Inouye, 1975). Using a horizontal puller (Model PD-5, Narishegi Instrument Lab, Tokyo, Japan), double barreled electrodes were constructed with tip diameters less than 0.5 μm. To siliconize the liquid ion-exchanger barrel, the shank of the reference barrel was first filled with acetone and the electrode tip held in 0.2% silicone/acetone solution (DC1107, Dow Corning Co., Midland, Mich.) for approximately 10 sec. The electrode was heated on a hot-plate (300 °C) for 10 min. A small drop of liquid ion-exchange resin (Corning 477317, Dow Corning Co., Midland, Mich.) was introduced down the shank of the siliconized barrel and by capillarity filled the electrode tip to a height of 100–200 μm. This barrel was then back-filled with 0.5 M KCl, while the reference barrel was filled with 1 M NaCl. Each barrel of the electrode was connected via Ag–AgCl half cells to a high input impedance dual electrometer (Model F223, W.P. Instruments, Hamden, Conn.), and the measured voltages recorded on a strip-chart recorder (Model 220, Gould Incorporated, Cleveland, Oh.).

Electrodes were calibrated in Ringer or in pure solutions of KCl (154, 100, 77, and 10 mM) and NaCl (154 and 100 mM). The K^+ activity of the calibration solutions were calculated using a modification of the Debye-Hückel equation (Fujimoto & Kubota, 1976; Robinson & Stokes, 1965). Since the temperature sensitivity of these electrodes is small (Fujimoto & Kubota, 1976; Edelman et al., 1978) all calibrations were done at room temperature. In 16 representative electrodes the mean slope was 58.3 ± 0.5 mV per tenfold change in $[K^+]$, and the selectivity of K^+ over Na^+ was 30.2 ± 2.4. The input resistance of both barrels was tested in Ringer and averaged 126 ± 2 MΩ and $3.7 \pm 0.3\ 10^{10}$ Ω for the reference and ion-selective sides, respectively.

The intracellular ion activity (a_i^i) was calculated according to Walker (1971).

$$a_i^i + K_{ij} A_j^i = (a_i^o K_{ij} A_j^o) \exp\left[\frac{(\Delta E - Em) Z_i F}{nRT}\right]$$

where: a_i^i and a_i^o are the activities of the ion (K^+) in the intracellular and extracellular fluid, respectively; A_j^i and A_j^o are the activities of the interfering ion (Na^+) in the intracellular and extracellular fluid, respectively; K_{ij} is the selectivity coefficient; ΔE and Em are the change in the measured potential of the ion-selective and reference barrels of the electrode upon cell impalement, n is a correction factor for non-ideal slopes, and Z_i, F, R, and T have their usual meanings. In all cases the intracellular K^+ activity was determined from calibration curves obtained in pure KCl solutions.

All results are expressed as mean ± SE. Comparison between group means was made using a paired "Student's t" analysis, or by one-way analysis of variance. P values less than 0.05 were considered significant.

Results

Cellular Impalements

As shown in Fig. 2, impalement of a proximal tubule cell by the microelectrode resulted in an abrupt change in the potential measured by both the reference (PD) and ion-sensitive (E_K) barrel of the electrode. In the majority of cases the measured potentials would decay back toward baseline. Impalements of this type were not accepted. However, impalements could be obtained in which the measured potentials were stable for at least 10–30 sec, or on occasion for several minutes (*see* Fig. 2). Only these stable impalements were summarized. In addition it was necessary that: (1) upon withdrawal of the microelectrode from the cell the measured potentials returned rapidly to the baseline and (2) the electrode, after use, was not plugged and when calibrated yielded a good slope and high selectivity for K^+ over Na^+.

The above criteria were met in 21 tubules of control rats, and the frequency distribution of the intracellular potential and K^+ activity (a_K^i) is shown in Fig. 3. The mean cell PD was -66.3 ± 1.3 mV, and a_K^i averaged 65.9 ± 2.0 mEq/liter. No correlation between cell PD and a_K^i was found ($r = 0.23$), which is similar to that reported for *Necturus* proximal tubule cells (Kubota, 1980).

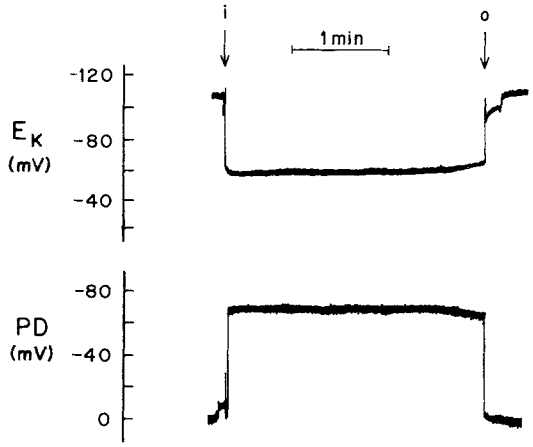

Fig. 2. Long term recording of intracellular potential and K^+ activity. In this cell the basolateral PD was −70 mV and K^+ activity was 60 meq/liter

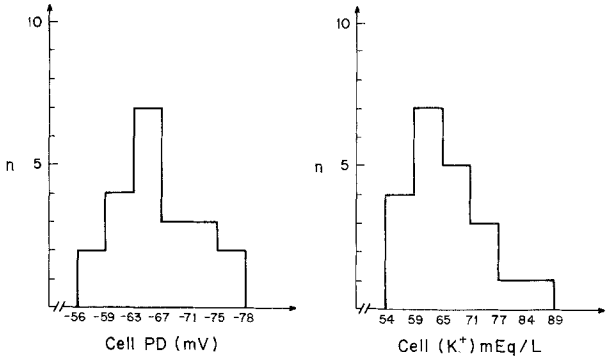

Fig. 3. Frequency distribution of the cell membrane potential (PD) and the intracellular K^+ activity in control rats

At present it is not possible, in rat proximal tubule cells, to assess microelectrode-induced cell damage as has been done in other systems (Lindeman, 1975; Lewis, Wills & Eaton 1978; Nelson, Ehrenfield & Lindemann, 1978). However, in an attempt to estimate impalement damage by the double-barreled electrodes, we compared the measured cell PD with values obtained with conventional 1 M KCL-filled microelectrodes. In all animal groups the values of cell PD measured with double-barreled electrodes were not different from those obtained with conventional single barreled electrodes (*see* Table 2). This was taken as evidence that cell damage, if present, was minimal, since it seemed unlikely that both types of electrodes would produce similar degrees of impalement damage.

Effect of Acidosis and K^+ Depletion

The effects of metabolic and respiratory acidosis and K^+ depletion on plasma composition are summarized in Table 1. Infusion of NH_4Cl (70 mg/

Table 2. Comparison of cell PD measured with double-barreled and single-barreled microelectrodes

	Control	Metabolic acidosis	Respiratory acidosis	K^+ depletion
Single-barrel (*n*)	-69.4 ± 1.4 (13)	-47.3 ± 1.4 (17)	-49.2 ± 2.1 (12)	-75.4 ± 1.6 (23)
Double-barrel (*n*)	-66.3 ± 1.3 (21)	-47.5 ± 1.9 (13)	-46.1 ± 1.4 (16)	-74.8 ± 2.1 (16)
P	>0.10	>0.50	>0.20	>0.50

Values are mean (mV) ± SE. *n* = number of measurements

Table 3. Peritubular membrane potentials (PD), cellular K^+ activities (a^i_K), and K^+ equilibrium potentials (ε_K) in the control and experimental groups of the rats

Group	PD (mV)	a^i_K (meq/liter)	ε_K (mV)[a]
Control ($n=21$)	-66.3 ± 1.3	65.9 ± 2.0	-82.6 ± 0.8
Metabolic acidosis ($n=13$)	-47.5 ± 1.9[c]	53.5 ± 2.0[c]	-71.4 ± 1.0[c]
Respiratory acidosis ($n=16$)	-46.1 ± 1.4[c]	63.7 ± 1.9	-76.1 ± 0.8[c]
K depletion	-74.8 ± 2.1[c]	58.1 ± 2.7[b]	-97.6 ± 1.2[c]

Values are mean ± SE. *n* = number of measurements.
[a] Control $a^o_K = 3$ meq/liter; acidosis $a^o_K = 3.7$ meq/liter; K depletion $a^o_K = 1.5$ meq/liter. [b] $P<0.05$. [c] $P<0.001$.

100 g BW) and respiration of a high CO_2 gas mixture (15% CO_2/20% O_2/balance N_2) produced a marked acidosis and a moderate rise in plasma $[K^+]$. In both groups of animals the intracellular PD was depolarized from a control value of -66.3 ± 1.3 mV to values of -47.5 ± 1.9 and -46.1 ± 1.4 mV during metabolic and respiratory acidosis, respectively. A fall in cell a^i_K was observed with metabolic acidosis, a^i_K averaging 53.5 ± 2.0 meq/ liter in acidotic rats, and 65.9 ± 2.0 meq/ liter in control rats. In contrast, no change in cell a^i_K occured with respiratory acidosis. These results are summarized in Table 3. In K^+-depleted rats plasma $[K^+]$ was decreased and the animals became alkalotic. The intracellular PD increased significantly to a value of -74.8 ± 2.1 mV, and a^i_K fell moderately to 58.1 ± 2.8 meq/liter (*see* Table 3).

In all groups a^i_K was above electrochemical equilibrium. The K^+ equilibrium potentials calculated for each group of animals:

$$\varepsilon_K = 61.7 \log \frac{a^o_K}{a^i_K}$$

are also summarized in Table 3. It should be noted that in both metabolic and respiratory acidosis the change in basolateral membrane PD was greater than the concurrent change in ε_K. In contrast, during K^+ depletion the change in PD was less than the change in ε_K.

Discussion

The results of the present study clearly demonstrate that K^+ is above electrochemical equilibrium across the peritubular cell membrane of rat proximal tubule cells. This is in accord with the results of Edelman et al. (1978), but contrary to those of Khuri et al. (1974). Furthermore, K^+ remained above equilibrium in all experimental groups. Thus K^+ uptake into the cell must occur via an active mechanism as originally postulated by Whittembury (1965) and Whittembury and Proverbio (1970) from isotopic uptake studies in kidney slices. Recent studies in the rabbit proximal straight tubule (Biagi et al. 1981*b*), have also shown active cellular uptake of K^+ dependent upon the activity of the Na^+-K^+-ATPase. Although we have not examined the role of the Na^+-K^+-ATPase in regulating intracellular K^+ in rat proximal tubule, it is likely that a similar relationship exists.

The rat proximal tubule has a transepithelial potential difference near 0 mV (Frömter & Gessner, 1974), and does not maintain large transepithelial chemical gradients for K^+ (Malnic et al., 1964). Consequently, it seems certain that K^+ is above electrochemical equilibrium across the apical cell membrane as well.

Recent evidence indicates that the major fraction of proximal tubule K^+ reabsorption is dependent upon the rate of fluid reabsorption (Bomsztyk & Wright, 1982), and presumably occurs via a

paracellular route. However, TF/P ratios for potassium can be less than 1.0, and even less than the simultaneously measured ratios for inulin (Malnic, Klose & Giebisch 1964). Accordingly, some "active" component of proximal tubule K^+ reabsorption must in addition be present. Since K^+ appears to be above electrochemical equilibrium across the apical cell membrane, an active luminal uptake mechanism must also exist. Such an uptake mechanism has been postulated by others (Edelman et al., 1979), and thought also to be present in proximal tubule cells of the bullfrog kidney (Fujimoto, Naito & Kubota, 1980).

From the measured K^+ activity in control rats (66 meq/liter) and assuming the activity coefficient for K^+ inside the cell is the same as in the extracellular fluid, the expected free intracellular $[K^+]$ would be 90 meq/liter. This value is considerably below the 144 meq/liter value determined by electron microprobe analysis (Beck et al., 1980). Whether this represents intracellular binding or compartmentalization of K^+, as suggested for *Necturus* proximal tubules (Kubota, Biagi & Giebisch, 1980), or simply a lower activity coefficient for K^+ in the intracellular fluid cannot be determined.

The origin of the basolateral membrane PD in the rat proximal convoluted tubule is not completely understood. K^+ appears to be the dominant conductive ion species (Frömter, Müller & Wick, 1971), but a significant HCO_3^- conductance can also be demonstrated (Burckhardt & Frömter, 1980). In addition, there is evidence that the Na^+-K^+-ATPase driven cation exchange pump is electrogenic (Frömter & Gessner, 1975). Taking these findings together, the basolateral membrane can be tentatively modeled as the parallel sum of an electrogenic Na^+-K^+ pump, and conductive pathways for both K^+ and HCO_3^-. Accordingly, the magnitude of the measured PD would reflect the composite emf (ε_b) of this membrane,

$$\varepsilon_b = \frac{\varepsilon_{pump}\, g_{pump}}{\Sigma g} + \frac{\varepsilon_K\, g_K^+}{\Sigma g} + \frac{\varepsilon_{HCO_3^-}\, g_{HCO_3^-}}{\Sigma g}$$

(where the respective ε and g values are the equivalent emfs and conductances of each pathway) and the voltage drops associated with intraepithelial current flow (Boulapep & Sackin, 1979).

Given that presently available techniques do not allow precise determination of these various membrane parameters, interpretation of the results of the present study must remain speculative. However, if it is assumed that the basolateral membrane is dominated by a high K^+ conductance (Frömter et al., 1971), then several interesting points regarding the regulation of intracellular K^+ are apparent.

Both metabolic and respiratory acidosis cause a depolarization of the basolateral membrane PD and a decrease in the magnitude of ε_K. Since the fall in PD was in excess of that expected from the decrease in ε_K, it seems likely that the K^+ conductance of the membrane also fell. In this regard, similar effects of extracellular acidosis on both membrane PD and K^+ conductance have been reported in *Necturus* proximal tubules (Steels & Boulpaep, 1976), and in proximal straight tubules of the rabbit (Bello-Reuss, 1982; Biagi et al., 1981 *a*, *b*).

Metabolic acidosis caused a marked fall in intracellular K^+ activity. Similar results have been observed in proximal tubules of *Necturus* (Kubota et al., 1980). In contrast, rapid changes in peritubular $[HCO_3]$/pH in rabbit proximal straight tubules perfused *in vitro* have no effect on intracellular K^+ activity (Biagi et al., 1981 *b*). In this latter study the tubules were exposed to an acid environment for only a few minutes, whereas in the present study, as well as in the study by Kubota and coworkers (1980), the acidosis was prolonged over several hours. The mechanism(s) responsible for the fall in intracellular K^+ during prolonged extracellular acidosis is not known, but may reflect changes in Na^+-K^+-ATPase activity (Brown, Cohen & Noble, 1978), and/or long-term changes in membrane permeability secondary to changes in intracellular pH (Boron & Boulpaep, 1981), and intracellular Ca^{2+} (Taylor & Windhager, 1979).

In contrast to the effects of metabolic acidosis, respiratory acidosis did not appreciably alter intracellular K^+ activity. The fact that extracellular $[HCO_3^-]$ was elevated in respiratory acidosis (27.1 meq/liter) as compared to metabolic acidosis (9.7 meq/liter), suggests that in some manner extracellular HCO_3^- is important in the regulation of intracellular K^+. This effect of extracellular HCO_3^- on intracellular K^+ activity may be related to changes in intracellular pH, since in many systems intracellular pH regulation is dependent upon the presence of HCO_3^- (Roos & Boron, 1981). Clearly, measurements of intracellular pH during prolonged metabolic and respiratory acidosis are needed for more complete understanding of the relationship of intracellular K^+ homeostasis to extracellular $[HCO_3^-]$.

With K^+ depletion both the basolateral membrane PD and ε_K hyperpolarized. The net effect was an increase in the driving force for cellular K^+ efflux ($PD-\varepsilon_K$) from 16.3 to 22.8 mV. This

could in part account for the observed fall in a^i_K. However, secondary effects related to the associated alkalosis (plasma $[HCO_3^-] = 33.4$ meq/liter and pH = 7.51) cannot be ruled out.

The results of the present study also provide some insight into the possible mechanism by which metabolic acidosis inhibits proximal tubular Na^+ and fluid reabsorption (Malnic, Mello Aires & Vieira, 1970; Levine & Nash, 1973; Dubb, Goldberg & Agus, 1977; Wilcox, 1980). The observed fall in basolateral membrane PD would be expected to reduce the electrochemical driving force for sodium entry across the luminal cell membrane and thus reduce the transepithelial transport of Na^+ (Spring & Giebisch, 1977). Similarly, the exchange of Ca^{2+} for Na^+ at the basolateral cell membrane would be reduced, leading to a rise in intracellular $[Ca^{2+}]$, which in turn would further reduce Na^+ entry across the luminal membrane (Taylor & Windhager, 1979). Finally, an effect of acidosis on the $Na^+ - K^+$-ATPase, either directly or secondary to changes in the intracellular composition (Ca^{2+}, K^+, Na^+, etc.) could be important (Brown et al., 1980).

In summary, the results of the present study confirm that in rat proximal tubule cells K^+ is above electrochemical equilibrium and hence actively accumulated into the cell. The effects of metabolic and respiratory acidosis also suggest that extracellular HCO_3^- either directly or indirectly (via changes in intracellular pH) can influence cellular K^+ activity.

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