J. Membrane Biol. 73, 61–68 (1983)

The Journal of
Membrane Biology

Effects of Acid Base Disturbances on Basolateral Membrane Potential and Intracellular Potassium Activity in the Proximal Tubule of *Necturus*

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Summary. The effects of extracellular acid-base disturbances on intracellular potential (E_m) and potassium activity (a_K^i) in the early proximal tubule of *Necturus* were examined. Using conventional and double barreled potassium ion selective microelectrodes it was possible to measure both the transient and steady-state responses to various states of extracellular acidosis and alkalosis. The results show that (i) when extracellular [HCO_3^-] is varied at constant pCO_2, E_m and a_K^i decrease in acidosis and increase in alkalosis. The greatest sensitivity in E_m is between pH 7.6 and 6.8 with apparent saturation above and below these extremes; (ii) decreased [HCO_3^-] at constant pH = 7.6 also causes a depolarization of E_m and reduces a_K^i, suggesting a major effect of extracellular [HCO_3^-] on intracellular potential and a_K^i; (iii) rapid perfusions and transient ΔE_m analysis suggest a high basolateral conductance for K^+ and HCO_3^- and a low Cl^- conductance; (iv) increasing extracellular [K^+] decreases the response of both E_m and a_K^i to reduced [HCO_3^-] at constant pCO_2. The results of this study demonstrate the important role of extracellular pH and/or [HCO_3^-] on the maintenance of cellular K^+ homeostasis.

Key Words acidosis · alkalosis · pH · bicarbonate

Introduction

Disturbances in whole body acid-base balance have been shown to influence the level of intracellular potassium activity in a number of tissues (Miller, Tyson & Relman, 1963; Struyvenberg, Morrison & Relman, 1968; Adler & Farley, 1977). Extracellular acidosis produces a fall in intracellular potassium, while extracellular alkalosis results in an increase in cell potassium. These changes are sufficient to be reflected by the opposite changes in plasma potassium levels (Simmons & Avedon, 1959; Malnic, Mello-Aires, & Giebisch). With regard to kidney function, changes in body potassium distribution may influence specific transport functions in different nephron segments. For example, acidosis is known to inhibit both proximal tubular fluid reabsorption and K^+ secretion in the distal tubule (Malnic et al., 1971; Stanton & Giebisch, 1982).

More recent data obtained in rat, rabbit, and amphibian proximal tubule segments have shown that both intracellular potassium activity and the basolateral membrane potential can be influenced by changes in the acid-base status of both the extracellular and intracellular environments (Anagnostopoulos, 1972; Frömter & Sato, 1976; Steels & Boulpaep, 1976; Boron & Boulpaep, 1980, 1981; Biagi, Kubota, Sohtell & Giebisch, 1981*a*; Biagi, Sohtell & Giebisch, 1981b; Cemerikić, Wilcox & Giebisch, 1982). Thus, there is evidence for interaction between those membrane parameters which establish the basolateral membrane potential (both permeabilities and ionic distributions across the cell membranes) and the levels of intra- and extracellular K^+, pH, and/or HCO_3^-.

It was the purpose of the present study to examine how acid base parameters (pH, HCO_3^-, pCO_2) influence the steady-state values of intracellular potassium activity (a_K^i) and the basolateral membrane potential (E_m) in the proximal tubule of *Necturus*. The strategy was to measure a_K^i and E_m under conditions which duplicate the states of metabolic acidosis and alkalosis. In addition, the influence of extracellular potassium concentration on the effects of acid-base parameters was examined. All studies were performed using double barreled potassium selective microelectrodes which allowed the simultaneous measurement of both basolateral membrane potential and intracellular K^+ activity.

Materials and Methods

Experiments were carried out in the doubly perfused *Necturus* kidney *in vitro* according to techniques described previously

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from this laboratory (Giebisch, Sullivan & Whittembury 1973; Kubota, Biagi & Giebisch, 1983). Control perfusion fluid had the following composition in mmol/liter: NaCl, 90; $NaHCO_3$, 10; Na_2HPO_4, 0.43; $NaHPO_4$, 0.07; $MgCl_2$, 1.0; $CaCl_2$, 1.8; KCl, 2.5; glucose, 2.2; PVP (polyvinylpyrrolidone), 15 g/liter; and heparin 2000 units/liter. Changes in pH from control (pH = 7.60) were made at constant pCO_2 (1%) either by lowering the bicarbonate concentration from 10 to 2.0 mM (pH = 6.83) or to 1.0 mM (pH = 6.4), or by raising the bicarbonate concentration to 30 mM (pH 8.15). Chloride was used as the bicarbonate substitute. To maintain pH constant at different bicarbonate concentrations, the pCO_2 of the solution was adjusted as required. Extracellular potassium concentration was raised to 5, 10, or 20.5 mM by replacing NaCl with KCl. The control solution was gassed with a mixture of 1% CO_2/99% O_2 (pH = 7.60) and had a total osmolality between 210–215 mOsm/liter.

Electrical Methods

The electrical methods were identical to those reported in the companion paper (Kubota et al., 1983). In some experiments measurement of the basolateral membrane potential (E_m) was made using single-barreled glass microelectrodes filled with 0.5 M KCl. The glass capillaries contained an internal fiber for easy filling (Frederick Haer, New Brunswick, Me., OD = 1.2 mm), and had resistance in the range of 100–200 MΩ, when tested in Ringer.

Measurements of E_m and of intracellular potassium activities were made simultaneously using double-barreled potassium selective microelectrodes. The methods of fabrication and calibration have been described in detail previously (Fujimoto & Kubota, 1976; Kubota et al., 1983). Intracellular potassium activity (a^i_K) was calculated from the following equation:

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

where E_{100} is the voltage from the output of the potassium selective barrel in 100 mM KCl solution (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 electrode expressed as mV change in E_K per decade change in potassium activity. A stability criterion of 30 sec was used for both E_m and E_K before any measurement was accepted.

A high impedance differential electrometer (10^{15} Ω, W.P. Instruments, Hamden, Ct., model F233A) was used for all potential measurements and the outputs were recorded on a four-channel pen recorder (Gulton Industries, East Greenwich, R.I., model TR44G). All potentials were referenced to ground via a 3 M KCl agar bridge in contact with the peritoneal cavity.

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

Results

Steady-State Measurements; Luminal and Peritubular Perfusion

The mean values of E_m and a^i_K measured with double-barreled electrodes following 1 hr of perfusion with solutions of different pH are illustrated in Fig. 1 and the values summarized in Table 1. Over the range of pH = 6.4 to 8.15 the relationship between E_m and pH_e was sigmoidal showing the largest sensitivity in the range of $pH_e = 6.8$ to 7.6. Over the whole pH range the value of a^i_K decreased by 18.3 mEq/liter when the pH_e was lowered from 8.15 to 6.40. Although the depolarization of E_m

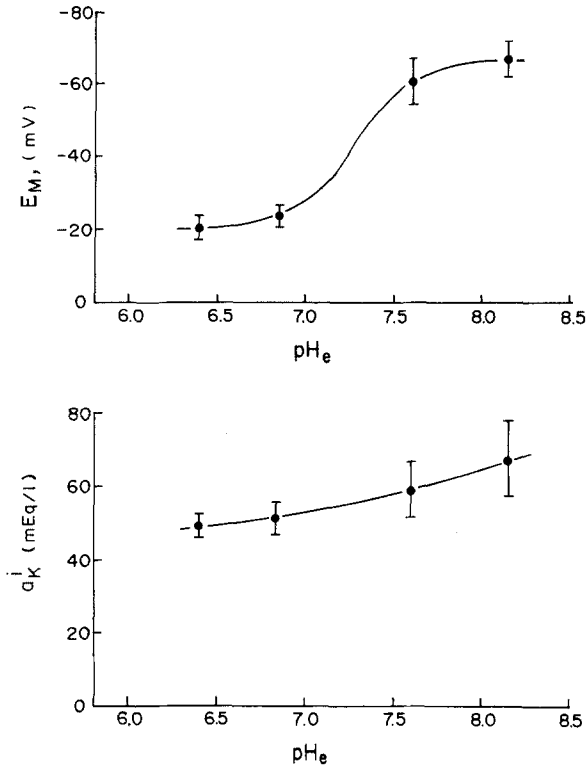

Fig. 1. Relationship of the basolateral membrane potential (E_m) and the intracellular K^+ activity (a^i_K) to the pH of the extracellular fluid. Values represent steady-state values measured at least 1 hr following exposure to the various pH perfusion solutions

Table 1. Effect of extracellular pH on intracellular potential and potassium activities in early proximal tubule segments

pH	CO_2 (vol%)	$[HCO_3^-]$ (meq/liter)	E_m (mV)	a^i_K (meq/liter)	ε_K (mV)
8.15 ($n=29$)	1%	30	−67.2[a] ±0.8	67.4[c] ±1.8	−89.9[b] ±0.7
7.60 ($n=21$)	1%	10	−60.7 ±1.1	59.4 ±1.7	−86.7 ±0.7
6.83 ($n=11$)	1%	2	−23.7[a] ±0.8	51.9[b] ±1.5	−83.3[b] ±0.8
6.40 ($n=7$)	1%	1	−19.9[a] ±1.0	49.1[b] ±1.4	−81.9[a] ±0.8

Values are mean ±SEM. ε_K was calculated assuming a^e_K = 1.8 meq/liter. P values represent comparisons to pH = 7.60.
[a] $P < 0.001$. [b] $P < 0.005$.

Table 2. Effect of extracellular HCO_3^- and pCO_2 on intracellular potential and potassium activity in early proximal tubule segments

pH	CO_2 (vol%)	$[HCO_3^-]$ (meq/liter)	E_m (mV)	a_K^i (meq/liter)	ε_K (mV)
7.61 ($n=24$)	1%	10	-58.8 ± 0.9	66.7 ± 1.4	-89.6 ± 0.5
7.56 ($n=20$)	0.1%	2	$-25.3^a \pm 0.8$	$56.5^a \pm 1.5$	$-85.5^a \pm 0.7$
7.59 ($n=9$)	2%	20	$-65.0^b \pm 1.6$	70.3 ± 2.7	-91.0 ± 1.0

Values are mean ± SEM. ε_K was calculated assuming $a_K^e = 1.8$ meq/liter.
P values represent comparisons to pH = 7.61.
[a] $P<0.001$. [b] $P<0.005$

from 67.2 to 19.9 mV is consistent with the decrease in a_K^i, the magnitude of the E_m change is much larger than what would be predicted even if the basolateral membrane behaved as a pure potassium electrode. Whereas the Nernst equilibrium potential for potassium (ε_K) decreased by only 8.0 mV, the E_m depolarized by 47.3 mV.

The discrepancy between changes in the ε_K and E_m suggests that other mechanisms are involved in the change in E_m as the pH_e is lowered. However, since in these experiments pH_e was decreased by lowering HCO_3^- at constant pCO_2 (Table 1), a possible role of lowered extracellular bicarbonate cannot be ruled out. To examine a possible bicarbonate effect, kidneys were perfused for 1 hr with solutions of different $[HCO_3^-]$ keeping pH *constant* by adjusting pCO_2. The results of these steady-state measurements are summarized in Table 2 and show that similar changes in both E_m and a_K^i were observed when $[HCO_3^-]$ was varied from 2–20 meq/liter. Again, the change in the ε_K (5.5 mV) was significantly smaller than the depolarization of E_m (39.7 mV).

Rapid Perfusion of the Peritubular Solution

In order to examine in more detail the effects of lowered pH_e and $[HCO_3^-]$ on E_m, experiments were done in which the peritubular perfusion solution was rapidly changed and E_m and a_K^i were continuously monitored. A representative example of these experiments is presented in Fig. 2. As shown, peritubular $[HCO_3^-]$ was lowered from 10 to 2 meq/liter at constant pH or with decreased pH_e. In both cases large depolarizations of E_m were associated with little or no consistent change in a_K^i as reflected by the E_K (mV) tracing. When this observation is compared with the results in Tables 1 and 2, it is clear that extended periods of perfusion (1 hr) with low pH_e or low $[HCO_3^-]$ solutions are required to produce changes in a_K^i. In sharp contrast, the depolarization of E_m occurs in less than one minute.

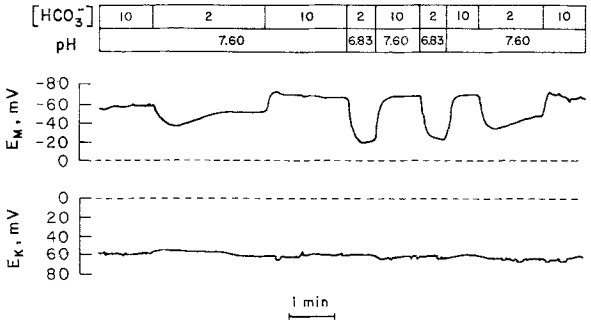

Fig. 2. Effect of acute changes in pH (constant pCO_2) and $[HCO_3^-]$ (constant pH) of peritubular solutions on the basolateral membrane potential (E_m), and the intracellular K^+ activity (represented here as the differential voltage response between the ion-selective and reference barrels of the electrode, E_K)

Differences in the transient behavior of E_m can also be seen in Fig. 2, depending upon whether or not pH_e was allowed to fall when the bicarbonate concentration was lowered. At constant pH_e, E_m first depolarized and then partially recovered toward the initial control value. The new steady state E_m remained, however, less negative than the control value. In contrast, peritubular perfusion with low $[HCO_3^-]$/low pH solution produced a larger and more rapid depolarization of E_m. In addition, the depolarization with low pH_e was also maintained and showed no trend to recover toward the control value.

Apparent Bicarbonate Transference Number in the Basolateral Membrane

The transient pattern of E_m seen in Fig. 2 is similar, although of slower time course, to that observed in the rat proximal tubule *in vivo* (Frömter & Sato, 1976; Burckhardt & Frömter, 1980). In the rat tubule, the peak depolarization has been interpreted as a conductive HCO_3^- (or CO_2/OH^-) efflux from the cell in response to lowered external bicarbonate concentration. The slower recovery phase could then reflect the reduction in cellular HCO_3^- and possible secondary rearrangement of intracellular ion content. In order to evaluate the bicarbonate conductance of the basolateral membrane of *Necturus* early proximal tubule, extracellular bicarbonate was varied at constant pH and the peak depolarization of E_m used to calculate the apparent bicarbonate transference number as:

$$t_{HCO_3^-} = \frac{\Delta E_m}{\Delta E_{HCO_3^-}}$$

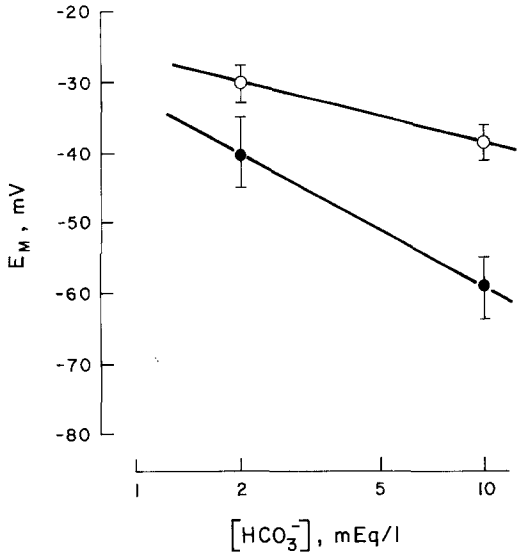

Fig. 3. Relationship between the basolateral membrane potential (E_m) and the $[HCO_3^-]$ of the peritubular fluid. In the presence of 2.5 mM K^+ (closed circles) $t_{HCO_3^-}=0.5$. In the presence of 20.5 mM K^+ (open circles) $t_{NCO_3^-}=0.2$

where ΔE_m is the peak depolarization and $\Delta E_{HCO_3^-}$ is the predicted change in the bicarbonate equilibrium potential (58 mV), both values estimated per 10-fold change in concentration. These results are presented in Fig. 3. Lowering $[HCO_3^-]$ from 10 to 2 mM when external potassium was at the control value (2.5 mM) gave a line which had a slope equal to 27 mV/decade change in $[HCO_3^-]$. This is equivalent to an apparent $t_{HCO_3^-}=0.5$. Thus, in these experiments the bicarbonate transference is on the same order as that for potassium across the basolateral membrane. Recent studies in our laboratory have confirmed this conclusion (Matsumura, Guggino & Giebisch, 1982).

Apparent Chloride and Potassium Transference Numbers in the Basolateral Membrane

The previous results indicate a relatively high $t_{HCO_3^-}$ in the proximal tubule of *Necturus*. Since the sum of all membrane transference numbers must, by definition, be equal to 1.0, the value of $t_{HCO_3^-}$ was compared with similarly measured values of t_{Cl^-} and t_{K^+}. In order to evaluate t_{Cl} in our preparation, $[Cl^-]$ was lowered from 90 to 36 meq/liter by substitution of cyclamate for Cl^- during quick perfusion of the peritubular solution. As illustrated in Fig. 4, lowering Cl^- produced only a small hyperpolarization of E_m. This is in marked contrast to the large depolarization of E_m resulting from lowered $[HCO_3^-]$ and pH. Thus it seemed likely that the Cl^- conductance of the basolateral membrane is exceedingly small. Other studies from this

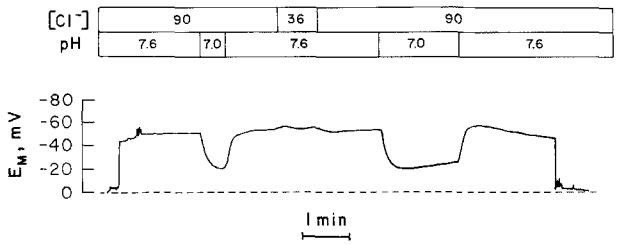

Fig. 4. Effect of reducing extracellular pH (pCO_2 constant) and extracellular $[Cl^-]$ on the basolateral membrane potential. Reducing extracellular $[Cl^-]$ from 90 to 36 mM had no appreciable effect on E_m

laboratory also support this conclusion (Guggino et al., 1982*a*).

The Effect of High External K^+ on E_m and a_K^i Responses to Acidosis

The relatively large depolarization of E_m when both the $[HCO_3^-]$ and pH are lowered suggests that there are secondary effects associated with this response. It has been suggested that changes in potassium permeability may be involved (Steels & Boulpaep, 1976; Biagi et al., 1981*b*). Since metabolic acidosis is associated with an increase in plasma $[K^+]$, we examined, under conditions of high external potassium concentration, both the transient responses to low $[HCO_3^-]$ at constant pH, and the steady-state response to low $[HCO_3^-]$, low pH perfusion.

The *transient* response of E_m to lowered peritubular bicarbonate at constant pH is summarized in Fig. 3. As seen in this figure, increasing peritubular potassium from 2.5 to 20.5 depolarizes E_m by approximately 20 mV. Perfusion with a low bicarbonate solution at constant pH produces a further depolarization of E_m. When peritubular $[K^+]=20.5$ meq/liter, reducing $[HCO_3^-]$ from 10 to 2 meq/liter resulted in a 12-mV depolarization of E_m, a value equivalent to $t_{HCO_3^-}=0.2$. This is different from the same response at normal external $[K^+]$ where $\Delta E_m=27$ mV and $t_{HCO_3^-}=0.5$. Thus raising external potassium results in a decrease in the apparent bicarbonate conductance of the basolateral membrane.

The *steady-state* response of both E_m and a_K^i to low $[HCO_3^-]$/low pH perfusion at increased external $[K^+]$ is summarized in Fig. 5. As shown previously (see Fig. 1), reducing pH to 6.8 resulted in a decrease in intracellular potassium (Δa_K^i) and a depolarization of E_m(ΔE_m). Again, however, it is clear that the magnitude of the change in E_m is considerably larger than that predicted on the basis of the change in a_K^i or the ε_K for potassium across the basolateral membrane. This is true for

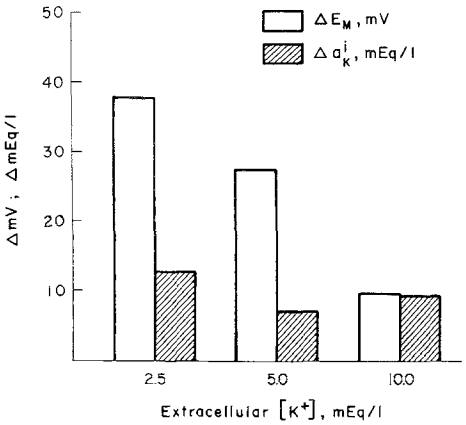

Fig. 5. Summary of the effect of reducing extracellular pH (constant pCO_2) at varying extracellular $[K^+]$ on the steady values of membrane potential and intracellular K^+ activity. In each case a depolarization in the potential (ΔE_m) and a fall in intracellular K^+ activity (Δa_K^i) was observed

all values of extracellular $[K^+]$ (2.5, 5.0, and 10.0 meq/liter). Thus the decrease in a_K^i is in the range of 7 to 13 meq/liter corresponding to a decrease in ε_K of only 3–5 mV. E_m depolarizes, however, by 10 to 38 mV, and the magnitude of the change is clearly dependent upon the external $[K^+]$. At higher external $[K^+]$, the depolarization of E_m due to extracellular acidosis is smaller. Thus raising extracellular potassium modifies both the transient and steady-state responses to lowering $[HCO_3^-]$ and pH of the peritubular fluid.

Discussion

We have shown in this and the companion report (Kubota et al., 1983) that K^+ is actively accumulated within the cells of the *Necturus* proximal tubule.

The active accumulation of intracellular potassium has now been observed in a number of renal tissues using ion-specific microelectrodes, including proximal tubule cells of *Ambystoma* (Sackin & Boulpaep, 1981), rat (Edelman, Curci, Samarzija & Frömter, 1978; Cemerikić et al., 1982), and rabbit (Biagi et al., 1981*b*). In all these tissues, the activity of basolateral Na^+-K^+-ATPase is considered as the primary mechanism for potassium accumulation. With regard to the present experiments in which extracellular bicarbonate and pH were varied, significant changes in both cellular K^+ activity and electrical potential were observed, yet in all experimental situations potassium was maintained above its equilibrium value. Thus, while the effects of changes of external pH and bicarbonate could certainly involve a component of direct action upon the Na^+-K^+-ATPase (Brown, Cohen & Noble, 1980), it is clear that Na^+-K^+-ATPase activity continues to function during widely differing extracellular acid-base conditions.

Steady-state perfusions with solutions of varying pH_e and $[HCO_3^-]$ have been shown in the present study to have pronounced effects on the basolateral membrane potential and intracellular potassium activity (Fig. 1 and Table 1). Under acidotic conditions with lowered $[HCO_3^-]$ and pH, there is a decrease in intracellular potassium activity and basolateral membrane potential. Opposite changes were observed when the extracellular fluid was alkalotic. The directional changes in intracellular potassium activity are consistent with the observed changes in plasma potassium concentrations occurring during acid-base disturbances (Simmons & Avedon, 1959; Malnic et al., 1971). It appears that the proximal tubule cells share with other body cells the response commonly associated with acid-base disturbances (Miller et al., 1963; Struyvenberg et al., 1968; Adler & Farley, 1977).

The relationships between E_m, a_K^i, pH_e, and $[HCO_3^-]$ illustrated in Fig. 1 show that E_m is affected to a greater degree than a_K^i. The shape of the E_m *vs.* pH_e curve shows the greatest sensitivity between pH 6.8 and 7.6 with smaller effects observed at pH_e values below and above these extremes. In all experiments, however, the measured ΔE_m are greater than the measured changes in ε_K. In an ideally selective potassium membrane, the change in ε_K would represent the maximum voltage ΔE_m if the shifts in intracellular potassium activity were the sole determinant of ΔE_m. This is clearly not the case in the proximal tubule, and the observation that $\Delta E_m > \Delta \varepsilon_K$ suggests that additional mechanisms are involved in the ΔE_m response to external $[HCO_3^-]$ and pH. The fact that the experiments presented in Table 1 were all done at constant pCO_2 would suggest that external HCO_3^- and/or pH and not pCO_2 were the principle variables of interest.

In an attempt to distinguish between the effects of lowering $[HCO_3^-]$ and the effect of changing pH_e, the extracellular fluid was perfused with solutions of constant pH at different HCO_3^- concentrations. The results were qualitatively the same as the experiments in which both $[HCO_3^-]$ and pH_e were lowered. At constant pH_e, reduced $[HCO_3^-]$ (2.0 meq/liter) resulted in a marked decline in E_m and a decrease in a_K^i. Raising $[HCO_3^-]$ to 20 meq/liter resulted in a small hyperpolarization of E_m with no significant change in a_K^i. Again, it was observed that extracellular acidosis produced larger

changes in E_m than could be the result of Δa_K^i alone.

Taken at face value, these results would indicate a specific effect of extracellular bicarbonate concentration on both E_m and a_K^i since very similar results could be obtained when either pH_e or pCO_2 were maintained constant. Such a conclusion would imply, then, that increasing pCO_2 at constant $[HCO_3^-]$ would be expected to have little effect on either E_m or a_K^i. This experiment was not performed in the present series of experiments in *Necturus,* but has been reported in the rat by Cemerikić et al. (1982). In their experiments extracellular acidification produced either by lowering $[HCO_3^-]$ or by increasing pCO_2 resulted in a sharp reduction in E_m. At the same time intracellular potassium activity was reduced to lower levels in metabolic (low $[HCO_3^-]$) than respiratory (increased pCO_2) acidosis. These results complicate the hypothesis presented above, i.e., that extracellular $[HCO_3^-]$ is the primary variable influencing E_m and a_K^i. Taken together, our results can only be interpreted as involving additional changes in the intracellular environment which have as yet not been elucidated. Clearly, intracellular pH measurements would be of primary interest since it has been shown in a variety of species that lowering extracellular pH can reduce basolateral membrane potassium selectivity (Anagnostopoulos, 1972; Biagi et al., 1981*a*, *b*; Cemerikić et al., 1982; Steels & Boulpaep, 1976). Guggino et al. (1982*b*) have shown that during perfusion with low HCO_3^--low pH solution the intracellular pH falls with a rise in intracellular Cl^-. Thus, while it is clearly demonsrated in our experiments that extracellular HCO_3^- and pH can influence both the basolateral membrane potential and intracellular potassium activity the elucidation of the precise mechanism requires a more complete understanding of concomitant changes in the cellular acid-base environment.

Bicarbonate and Chloride Transference in the Basolateral Membrane

The basolateral membrane of the rat proximal tubule has been shown to have a high bicarbonate transference number (Frömter & Sato, 1976; Frömter, Sato & Gessner, 1975; Burckhardt & Frömter, 1980). This conclusion is based upon the large transient depolarization of E_m seen when the peritubular capillaries are perfused with a low $[HCO_3^-]$ solution at constant pCO_2. The results in Table 1 suggested a similar pattern in the *Necturus*. Using rapid exchanges from control to low $[HCO_3^-]$ at constant pH in the external solutions, we observed an apparent $t_{HCO_3^-} = 0.5$, a value which is approximately equal to $t_{K^+} = 0.4$ measured by rapid perfusion with 20 mM potassium solution. The apparently large transference for potassium and bicarbonate suggest a low transference for chloride, the principal anion in the perfusion solutions. This was confirmed in experiments in which cyclamate was substituted for chloride. As illustrated in Fig. 4, lowering extracellular chloride produced negligible changes in E_m, suggesting a low chloride conductance of the basolateral membrane. The finding of a low chloride conductance in the basolateral membrane of *Necturus* proximal tubules is in agreement with a number of recent reports (Edelman, Bouthier & Anagnostopoulos 1981; Guggino et al., 1982*a*; Shindo & Spring, 1981; Spring & Kimura, 1978). However, it should be noted that the studies reported here were confined to early segments of the proximal tubule. In late segments the basolateral membrane appears to have a measurable Cl^- conductance (Kubota et al., 1983).

Two points deserve mention concerning the analysis presented above. First, the present approach is not a complete analysis of individual ion permeabilities since more extensive substitution in both luminal and peritubular compartments would be required in order to evaluate the contribution of intraepithelial current flow on observed changes in membrane potential (Boulpaep & Giebisch, 1978; Boulpaep & Sackin, 1980). The results do suggest, however, that the basolateral membrane has a high conductance for K^+ and HCO_3^- and a relatively low Cl^- conductance.

Secondly, caution must again be exercised with regard to possible changes in the intracellular environment which might occur in addition to the measured variables of electrical potential and potassium activity. The estimation of an apparent bicarbonate transference number was done with rapid perfusions in an attempt to minimize possible intracellular activity changes of HCO_3^- and pH. Although it was shown that no Δa_K^i occurred with the marked ΔE_m the constancy of $[HCO_3]_i$, pH_i and their effects on membrane parameters remain to be tested.

Increased Extracellular K^+ Affects the Cellular Response to Acidosis

Our results have demonstrated a decreased a_K^i following extracellular acidosis. The fall in cell potassium in acidosis is frequently associated with extracellular hyperkalemia. In order to examine whether

increased extracellular K^+ levels influence the cellular response following acid-base changes, peritubular $[HCO_3^-]$ was lowered at constant pCO_2. Increased extracellular K^+ reduced both the transient (Fig. 3) and steady-state (Fig. 5) responses of E_m and a_K^i. Thus increased extracellular potassium serves to reduce the depolarizing effect of acidosis, a response tending to maintain the cellular environment, with regard to E_m and a_K^i, at values closer to the control condition.

Figure 3 suggests two additional points. First, when extracellular K^+ is increased from 2.5 to 20.5 meq/liter the apparent bicarbonate response decreases. Secondly, although potassium was not increased in rapid perfusions at low $[HCO_3^-]$, the smaller ΔE_m seen with step changes in $[K^+]$ at $[HCO_3^-] = 2.0$ meq/liter, suggests also that the transference for potassium is also reduced in acidosis. Both effects may reflect a decreased basolateral conductance in acidosis.

In conclusion, our experiments have demonstrated a tight relationship between acid-base conditions of the extracellular environment and both intracellular electrical potential and potassium activity. E_m and a_K^i decrease in acidosis as well as after decreasing $[HCO_3^-]$ at constant pH. The peritubular cell membrane has a high K^+ and HCO_3^- conductance, and the membrane changes in acidosis involve both a fall in K^+ conductance as well as a decline in a_K^i. The depolarizing effect of metabolic acidosis is attenuated by increasing extracellular K^+.

We are greatly indebted to Dr. B. Koeppen for constructive help and critical reading of the manuscript. This work was done by T. Kubota in partial fulfillment of the requirements for the Ph.D. degree. This work was supported by NIH Grant AM 17433-07.

A preliminary account of some data was published in: Current Topics in Membranes and Transport (E. Boulpaep, editor) 1980, Vol. 13, pp. 63–72. Academic Press, New York.

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Received 18 June 1982; revised 12 November 1982