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Electrical Effects of Potassium and Bicarbonate on Proximal Tubule Cells of *Necturus*

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Summary. The effects of stepwise concentration changes of K^+ and HCO_3^- in the basolateral solution on the basolateral membrane potential (V_{bl}) of proximal tubule cells of the doubly-perfused *Necturus* kidney were examined using conventional microelectrodes. Apparent transference numbers were calculated from changes in V_{bl} after alterations in external K^+ concentration from 1.0 to 2.5 mM ($t_{K,\,1.0-2.5}$), 2.5 to 10, and in external HCO_3^- concentration (at constant pH) from 5 to 10 mM ($t_{HCO_3,\,5-10}$), 10 to 20, or 10 to 50. $t_{K,\,2.5-10}$ was 0.38 ± 0.02 under control conditions but was sharply reduced to 0.08 ± 0.03 ($P > 0.001$) by 4 mM Ba^{++}. This concentration of Ba^{++} reduced V_{bl} by 9 ± 1 mV (at 2.5 external K^+). Perfusion with SITS (5×10^{-4} M) for 1 hr hyperpolarized V_{bl} by 10 ± 3 mV and increased $t_{K,\,2.5-10}$ significantly to 0.52 ± 0.01 ($P < 0.001$). Ba^{++} application in the presence of SITS depolarized V_{bl} by 22 ± 3 mV. In control conditions $t_{HCO_3,\,10-50}$ was 0.63 ± 0.05 and was increased to 0.89 ± 0.07 ($P < 0.01$) by Ba^{++} but was decreased to 0.14 ± 0.02 ($P < 0.001$) by SITS. In the absence of apical and basolateral chloride, the response of V_{bl} to bicarbonate was diminished but still present ($t_{HCO_3,\,10-20}$ was 0.35 ± 0.03). Intracellular pH, measured with liquid ion-exchange microelectrodes, increased from 7.42 ± 0.19 to 7.57 ± 0.17 ($P < 0.02$) when basolateral bicarbonate was increased from 10 to 20 mM at constant pH. These data show that the effects of bicarbonate on V_{bl} are largely independent of effects on the K^+ conductance and that there is a significant current-carrying bicarbonate pathway in the basolateral membrane. Hence, both K^+ and HCO_3^- gradients are important in the generation of V_{bl}, and their relative effects vary reciprocally.

Key Words bicarbonate · *Necturus* · proximal tubule · basolateral membrane · electrical properties · transference numbers

Introduction

The key transport processes involved in renal acid secretion are thought to be luminal H^+ extrusion and basolateral HCO_3^- exit from the cell. The exact nature of both processes is still controversial, and with respect to HCO_3^- exit, both conductive and nonconductive pathways have been postulated (Burckhardt & Frömter, 1981; Bello-Reuss, 1982; Boron & Boulpaep, 1983).

The electrical effects of bicarbonate gradients *per se* must be distinguished from the effects of pH on other pathways, particularly the K^+ conductance (Steels & Boulpaep, 1976). The effects of bicarbonate changes on peritubular potential difference (PD) in the rat proximal tubule were demonstrated by Frömter and Sato (1976), and more recently Burckhardt and Frömter (1981) showed that these effects persist after application of Ba^{++}, though the electrical response to K^+ changes had been sharply blunted. They concluded that the HCO_3^- responses were not due to effects on the K^+ conductance and that they reflected the presence of a current-carrying bicarbonate pathway. In contrast, Biagi, Kubota, Sohtell and Giebisch (1981) and Bello-Reuss (1982), both working in a different preparation, the isolated rabbit proximal tubule, found the bicarbonate responses almost eliminated by Ba^{++}. They concluded that there was no evidence for an important current-carrying bicarbonate pathway.

Similar changes in basolateral membrane potential with changes in basolateral bicarbonate concentration have been reported in the *Necturus* by Kubota, Biagi and Giebisch (1983*a*) and in *Ambystoma* by Boron and Boulpaep (1983). The latter authors felt that bicarbonate movement was directly sodium linked and, although not electrodiffusive, was thought to be current carrying. The possible role of K^+ conductance changes in the PD responses to bicarbonate changes was not explored. The purpose of this study was to explore further the interrelationship between the basolateral electrical responses to potassium and bicarbonate concentration steps in the *Necturus* proximal tubule.

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Materials and Methods

Kidney Preparation

Adult male and female *Necturus maculosus* (Connecticut Valley Biological Supply Co., Southampton, MA) were kept in an aquarium at 8–12°C for at least one month prior to use and fed live goldfish. The doubly-perfused preparation was used as previously described (Boulpaep, 1978). The left caudal branch of the ventral abdominal vein was used for the portal perfusion (the second technique for portal perfusion described by Boulpaep, 1978). The flow rate of the peritubular perfusion fluid was 2.6 ml/min rather than the usual 1.0 ml/min, while the aortic perfusion rate was the standard 1.5 ml/min. Distribution of perfusate throughout the kidney was checked by injection of small amounts of FD&C green dye (H. Kohnstamm & Co., New York).

Solutions

The composition of the control Ringer was 100.5 mM Na^+, 2.5 mM K^+, 1.8 mM Ca^{++}, 1.0 mM Mg^{++}, 98.1 mM Cl^-, 10.0 mM HCO_3^-, 0.5 mM $H_2PO_4^-$, 15 g/liter polyvinylpyrrolidone (PVP), 0.4 g/liter glucose and 2000 units/liter heparin. pH was maintained at 7.6 by bubbling with a 99% O_2/1% CO_2 mixture. The 1.0-K solution was made by deleting the appropriate amount of KCl and adding 1.5 mM mannitol as partial osmotic compensation. The 10-K solution was made using an equimolar substitution of KCl for NaCl. In the 2, 5, 20 and 50 HCO_3^- solutions, equimolar substitutions of $NaHCO_3$ for NaCl or vice-versa were made. The 5, 20 and 50 HCO_3^- solutions were bubbled with 0.5, 2 and 5% CO_2 (remainder O_2) to keep pH at 7.6, while the CO_2 was kept at 1% in the 2 HCO_3^- solution (pH 6.8). The zero-chloride solutions were made using 94 mM Na gluconate (84 mM in the 20-HCO_3^- solution), 1.25 mM K_2SO_4, 7.6 mM $CaSO_4$ (to maintain free Ca^{++} concentration at control levels), 1.0 mM $MgSO_4$, 0.5 mM NaH_2PO_4, and 10 mM $NaHCO_3$ (20 mM $NaHCO_3$ in the 20-HCO_3 solution). Glucose, PVP and heparin were added as in the other solutions and 1% CO_2 and 2% CO_2 were used in the 10-HCO_3 and 20-HCO_3^- solutions, respectively.

Barium chloride from a 150-mM stock solution (adjusted to pH 7.6) was added to peritubular perfusion solutions to give a final concentration of 4 mM. In those experiments in which SITS (4-acetamido-4′-isothiocyano-stilbene-2,2′-disulfonic acid; Pierce Chemical Co., Rockford, IL) was used, PVP was deleted from the solution to prevent binding.

Changes in peritubular perfusion solution were made by way of a four-way stopcock placed near the site of caudal vein cannulation. All experiments were performed at room temperature (19–22°C).

Electrical Potential Measurements

Basolateral membrane potentials were measured using Ling-Gerard microelectrodes pulled on a horizontal microelectrode puller (Model PD5, Narishige Scientific Instruments, Tokyo, Japan) from 1.2 mm OD and 0.5 mm ID fiber containing glass capillaries (Frederick Haer & Co., Brunswick, ME). Electrodes were filled with 0.5 M KCl and had resistances of 50–150 MΩ and tip potentials less than 5 mV.

Potentials were measured by a high input impedance (~10^{15} Ω) electrometer (F223A, W.P. Instruments, Hamden, CT) and printed out by a brush pen recorder (Gould Model 220, Cleveland, OH). A 3-M KCl agar bridge placed on the surface of the kidney served as reference. Both the microelectrode and bridge were connected to the electrometer via Ag/AgCl half cells. Criteria for accepting impalements have been described previously (Guggino, London, Boulpaep & Giebisch, 1983). Only early segments of proximal tubules were used.

Calculation of Transference Numbers

Apparent transference numbers were calculated as:

$$t_{C,\,C_1-C_2} = \frac{\Delta V_{bl}}{58 \log\left(\frac{C_2}{C_1}\right)}$$

where ΔV_{bl} is the peak change in basolateral potential caused by the concentration change and C_1 and C_2 the two concentrations of ion C used. Under certain conditions this will equal G_C/G_T, that is, the fraction of total membrane conductance (G_T) represented by the conductance of ion C (G_C) (Boulpaep, 1976; Helman & Thompson, 1982). However, the presence of nonlinear ionic pathways and electrogenic pumps complicate the theoretical interpretation of transference numbers as defined this way, yet, as Helman & Thompson (1982) have pointed out, they may still be useful in a much wider range of circumstances as approximate measures of the relative contribution of various ionic pathways to the electrical properties of a given membrane. Care must still be taken though to account for the possible effects of intraepithelial current loops, transmitted through the shunt pathway, on the measured responses. Thus, the measured basolateral potential change (ΔV_{bl}) due to an alteration in an ionic pathway in the basolateral membrane may be less than the true change in electromotive force at this membrane (Δemf). Using a typical epithelial equivalent circuit (Boulpaep, 1976) it can be shown that the ratio of ΔV_{bl} to Δemf will be given by:

$$\frac{\Delta V_{bl}}{\Delta emf} = \frac{R_a + R_s}{R_a + R_b + R_s}$$

where R_a, R_b and R_s are the apical, basolateral and shunt resistances, respectively. Using the resistance data of Guggino, Boulpaep and Giebisch (1982), this will equal approximately 0.75 in this preparation. Thus the attentuation in electrical response due to current loops will be relatively small. It is unlikely that a change in this attenuation factor has produced the alterations in the apparent transference numbers reported here. The experimental maneuvers in all cases produced reciprocal changes in the two pathways studied. This assures that the changes in apparent transference numbers were due to specific changes in basolateral ionic pathways rather than to changes in the degree of attenuation by current loops, as the latter mechanism would change both transference numbers in the same direction.

Intracellular pH Measurements

Liquid ion-exchange electrodes for measuring intracellular pH were constructed using the neutral-carrier based ligand recently reported by Ammann, Lanter, Steiner, Schulthess, Shijo and Simon (1981). The resin was a kind gift from Dr. Simon. Some measurements were made using two separate single-barreled

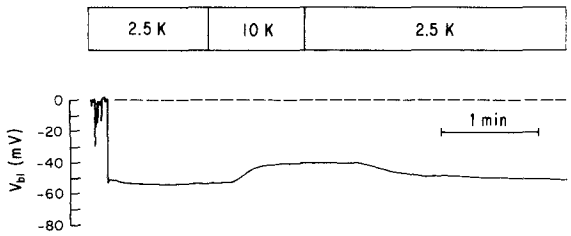

Fig. 1. Recording of basolateral membrane potential during switch from 2.5 mM K basolateral solution to one containing 10 mM K. Some of the delay in the response from the time of the solution change is due to the transit time of the solution from stopcock to the animal. Impalement is made at far left

electrodes maintained simultaneously in separate cells as described previously in this laboratory (Guggino et al., 1983). Liquid ion exchange electrodes were pulled as described above and dipped for 30 sec in a 1.5% (vol/vol) solution of silanizing agent A137 (Pharmacia Chem., NJ) in 1-chloronaphthalene. They were then heated on a heating plate set at 300°C for 1 hr. Resin was introduced into the tip by backfilling with a syringe with polyethylene tubing (pulled to a fine tip) attached. The pH electrode was then backfilled with buffer solution described by Ammann et al. (1981). 0.5 M KCl was used to fill the reference electrode as in the other experiments reported here. Electrodes were beveled as described previously (Guggino et al., 1983). In some experiments double-barreled electrodes were used. Construction of these was similar to construction of double-barreled potassium electrodes described in the following paper (Matsumura, Cohen, Guggino & Giebisch, 1984). pH electrodes used in this study had an average slope of 57 when tested in 90 mM KCl solutions at pH 6.8 (20 mM PIPES buffer) and 7.8 (20 mM HEPES buffer). The desired pH of the test solutions was achieved by titration with sodium hydroxide.

Before undertaking these studies the properties of the resin were studied carefully. Electrodes had near Nernstian slopes in both 90 mM NaCl and 90 mM KCl solutions (with PIPES or HEPES buffer) indicating very low selectivity coefficients for these ions and eliminating the possibility of interference from either of these ions at concentrations seen in physiologic solutions. Slopes were similar in solutions containing bicarbonate and CO_2, and no offset was seen when switching from a bicarbonate/CO_2 solution to a HEPES or PIPES buffered solution at the same pH. Slope was maintained in a standard amphibian Ringer containing mM concentrations of calcium, magnesium, and phosphate as well as PVP, bicarbonate, and CO_2.

Electrode resistance was $\sim 10^{11}\ \Omega$. The electrodes were connected by way of chlorided silver wires to the two channels of the high-input impedance dual-differential electrometer (F223A, W.P. Instruments, New Haven, CT). The voltages registered by the ion-sensitive (V_H) and reference electrodes (V_{bl}) as well as the difference between the two were recorded by two Gould 220 recorders.

From the equation $E_{LIX} = E_o + S \log [H^+]$, where E_{LIX} is the liquid ion-exchange electrode potential and S the slope of that electrode, E_o was calculated from *in vitro* calibration. This was then used to calculate intracellular pH from the equation:

$$pH = \frac{E_o - E_{a-b}}{S}$$

where E_{a-b} equals $V_H - V_{bl}$.

Table 1. Apparent transference numbers for potassium

t_K	1.0–2.5	2.5–10
Control	0.38 ± 0.06 (5)	0.38 ± 0.02 (28)
4 mM Ba^{++}	—	0.08 ± 0.03[a] (13)
SITS	0.65 ± 0.06[b] (6)	0.52 ± 0.01[a] (8)

[a] Significantly different from corresponding control, $P < 0.001$.
[b] Significantly different from corresponding control, $P < 0.02$.
Results are expressed ± SEM. Number of observations is in parenthesis.

Statistics

All data are reported as mean ± SEM. Significance was determined by unpaired t test unless otherwise stated.

Results

Control Conditions

Potassium

The typical response to a step change in potassium concentration is shown in Fig. 1. Response was always monophasic and reached its peak in less than 2 min. It has been shown (Kubota, Biagi & Giebisch, 1983*b*) that the change in intracellular K^+ activity is less than 2 mM in this time period for changes in external K^+ up to 10 mM. Results for step changes of 1.0 to 2.5 and 2.5 to 10 mM K^+ are shown in Table 1. Control V_{bl} was 64 ± 1 mV (n = 46).

Bicarbonate

The typical biphasic response to an increase in bicarbonate (a fast hyperpolarization followed by small slow repolarization), and to restoration of the original concentration is shown in Fig. 2. This biphasic response has been observed to varying degrees by others (Burckhardt & Frömter, 1981; Boron & Boulpaep, 1983). For calculation of t_{HCO_3}, the initial peak electrical response was used. This usually occurred in less than 30 sec. Apparent transference numbers calculated from HCO_3^- concentration changes from 5 to 10, 10 to 20, and 10 to 50 mM are summarized in Table 2. To determine whether these electrical responses were due to the recently described Na^+-HCO_3^-/Cl^- chloride exchange pathway present in this membrane (Guggino et al., 1983) or

whether a chloride-independent bicarbonate pathway also exists, $t_{HCO_3,\ 10-20}$ in the absence of basolateral and apical chloride was measured. Results are also shown in Table 2.

The Effect of Barium

Barium (in mM concentrations) is known to block potassium conductances in a variety of epithelial preparations including proximal tubules (Biagi et al., 1981; Nagel, 1979). When the peritubular perfusion fluid was switched to a solution containing 4 mM Ba^{++} the basolateral PD depolarized by 9 ± 1 mV (from 64 ± 1 mV to 55 ± 1 mV, $P < 0.001$, $n = 46$). A similar depolarization in this preparation has been reported by others (Planelles, Teulon & Anagnostopoulos, 1981). Apparent t_K's were calculated from the electrical responses to K^+ concentration changes from 2.5 to 10 and 2.5 to 30 with barium present continuously. As seen in Table 1, barium sharply reduced the electrical response to K^+, consistent with a block in K^+ conductance. A typical recording is shown in Fig. 3. On the other hand, as shown in Table 2, $t_{HCO_3,\ 5-10}$, $t_{HCO_3,\ 10-20}$ and $t_{HCO_3,\ 10-50}$ were all increased significantly when barium was applied (*see* Fig. 4 for recording with and without barium). This observation argues strongly against the idea that the electrical response to bicarbonate is due to any significant degree to changes in the K^+ conductance. It is, however, consistent with the presence of a current-carrying bicarbonate pathway whose relative importance in determining the peritubular membrane PD increases when the potassium conductance is diminished.

The Effect of SITS

SITS is a stilbene derivative known to block chloride-bicarbonate exchange in red cells (Cabantchik & Rothstein, 1972) and in other systems including proximal tubules (Guggino et al., 1983). It has also been shown to block nonchloride-dependent bicarbonate exit in some epithelia including amphibian proximal tubule (Cohen, Mueller & Steinmetz, 1978; Boron & Boulpaep, 1983). SITS was perfused bilaterally for one hour before experiments were carried out. During that perfusion, V_{bl} hyperpolarized by 10 ± 3 mV (from 58 ± 6 to 68 ± 6, $P < 0.01$, $n = 4$) and remained constant for at least another hour, during which time experimental maneuvers were performed. As shown in Tables 1 and 2, SITS almost eliminated the electrical response to bicarbonate concentration changes. In contrast, $t_{K,\ 1-2.5}$ and $t_{K,\ 2.5-10}$ were both significantly increased. This is analogous to the barium experiment in which blocking the K^+ conductance increased the response to bicarbonate. Thus, the electrical responses of the peritubular cell membrane to the two ions vary reciprocally as one would expect if each had a separate current-carrying transport pathway. Typical tracings are shown in Figs. 5 and 6.

Also consistent with this idea is the response of V_{bl} to barium in the presence of SITS. The mean depolarization was 22 ± 3 mV (from 85 ± 5 to 63 ± 7 mV, $P < 0.01$, $n = 4$), compared to the 9 ± 1 mV change caused by barium alone (the difference between the two barium groups was significant at $P < 0.002$).

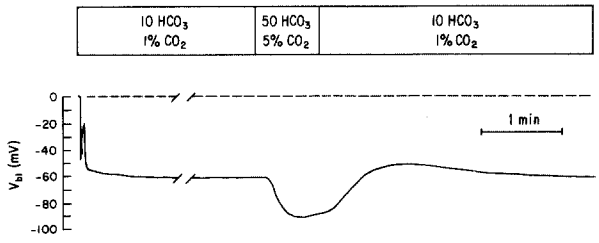

Fig. 2. Recording of V_{bl} during switch from control Ringer to one containing 50 mM HCO_3 at pH 7.6. Peak response was used to calculate transference number

Table 2. Apparent transference numbers for bicarbonate

t_{HCO_3}	5–10	10–20	10–50
Control	0.41 ± 0.04 (21)	0.60 ± 0.03 (33)	0.63 ± 0.05 (12)
4 mM Ba^{++}	0.75 ± 0.05[a] (15)	0.67 ± 0.02[c] (34)	0.89 ± 0.07[b] (6)
SITS	—	—	0.14 ± 0.02[a] (13)
Zero chloride	—	0.35 ± 0.03[a] (10)	—

[a] Significantly different from corresponding control, $P < 0.001$.
[b] Significantly different from corresponding control, $P < 0.01$.
[c] Significantly different from corresponding control, $P < 0.05$.

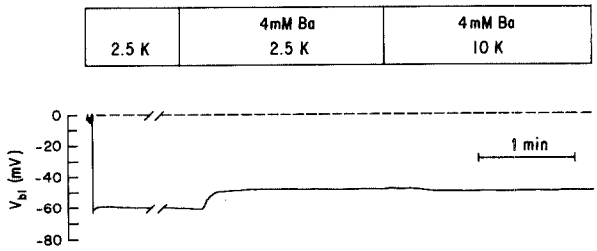

Fig. 3. Recording showing the depolarizing effect of barium on V_{bl} and the blunting of the electrical response to a step increase in peritubular potassium concentration (*compare* Fig. 1). In this particular example a slight hyperpolarization is noted when peritubular K^+ concentration is increased. This may be due to stimulation of the (presumably electrogenic) Na-K pump at a time when membrane resistance was increased

Intracellular pH

In order to validate our intracellular pH measurements with the new liquid ion-exchange resin, we used the resin in repeating a set of experiments done previously in this preparation with recessed-tip glass pH electrodes (Guggino et al., 1983). Single-barreled electrodes were used as described above. Intracellular pH was measured as peritubular bicarbonate concentration was lowered from 10 to 2 mM with CO_2 constant at 1%. Intracellular pH was found to be 7.39 ± 0.09 in control and 7.13 ± 0.08 after the switch to low bicarbonate solution (NS, $n = 3$). These are similar to the values obtained previously. A typical recording is shown in Fig. 7. V_{bl} in this group fell from 53 ± 9 in control to 30 ± 11 mV ($P < 0.02$ paired).

In a second group intracellular pH was measured using double-barreled electrodes while peritubular bicarbonate was raised from 10 to 20 mM at constant pH as in the transference number experiments reported here. Intracellular pH rose from 7.42 ± 0.19 in control to 7.57 ± 0.17 ($P < 0.02$ paired t test, $n = 5$) in the high bicarbonate situation while V_{bl} changed from 59 ± 4 to 68 ± 5 mV ($P < 0.001$ paired). As CO_2 concentration inside the cell could only have increased, the pH change must represent an increase in cell bicarbonate concentration. Thus the electrical response to bicarbonate is also associated with bicarbonate movement.

Discussion

Bicarbonate exit across the basolateral membrane is widely held to be a fundamental step in acid secretion in a variety of urinary epithelia (Warnock & Rector, 1979), but the exact mechanism is still debated and may actually differ from tissue to tissue. Two issues are of interest. The first concerns the mechanism of bicarbonate-induced potential changes. A second issue is the mechanism of bicarbonate transfer across the peritubular cell membrane.

Though a striking, if sometimes transient, electrical response to a step change in peritubular bicarbonate concentration is seen in many tubule preparations (Burckhardt & Frömter, 1981; Biagi et al., 1981; Bello-Reuss, 1982; Boron & Boulpaep, 1983), care must be taken in distinguishing between a direct effect of bicarbonate as a current-carrying ion species and an indirect effect of nonbicarbonate pathways as the origin of the electrical response. In particular, basolateral potassium conductances have been thought to be pH sensitive (Steels & Boulpaep, 1976; Kubota et al., 1983*a*). One ap-

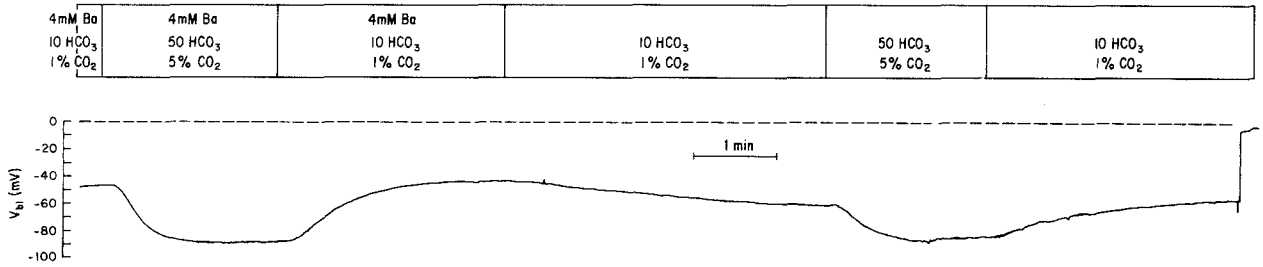

Fig. 4. The sequence at far left shows the response to a peritubular bicarbonate concentration step increase in the presence of barium. The hyperpolarization of V_{bl} when barium is removed is seen in the middle of the recording. The response to the subsequent bicarbonate step (in the absence of barium) is smaller than the first

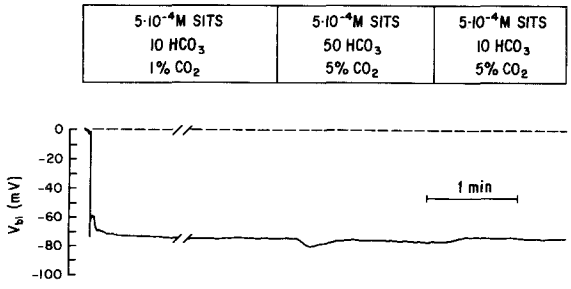

Fig. 5. Recording showing the dramatic blunting of the electrical response to bicarbonate in the presence of SITS (*compare* Fig. 2)

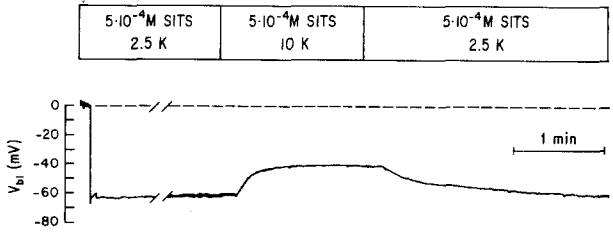

Fig. 6. Response of V_{bl} to increase in peritubular K concentration in the presence of SITS

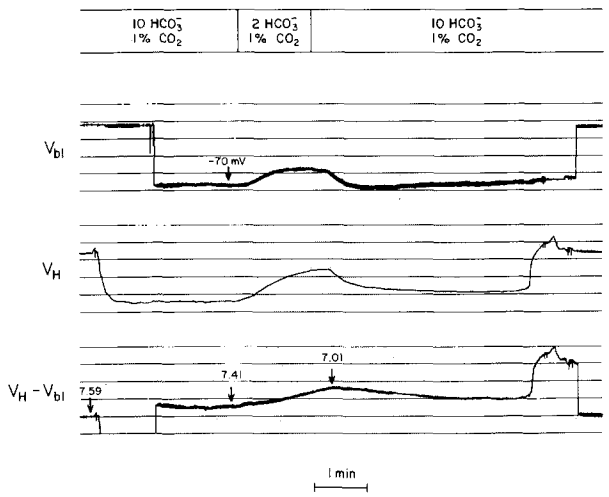

Fig. 7. Recording of intracellular pH during change of peritubular solution from control to a 2 mM HCO_3 solution at constant CO_2. The middle tracing is the voltage recorded by the pH electrode. In the bottom tracing the basolateral membrane potential (V_{bl}) is subtracted from V_H giving a signal that can be compared with the *in vitro* calibration of the pH electrode to calculate the pH. Each division represents 20 mV

proach that has been used to differentiate between direct and indirect bicarbonate effects has been to measure the response to bicarbonate changes in the presence of a blocker of potassium conductances such as barium. In the *in vivo* rat preparation (Burckhardt & Frömter, 1981) the bicarbonate response is still present in the presence of barium. In sharp contrast, it is virtually eliminated in *in vitro* rabbit tubular perfusion studies (Biagi et al., 1981; Bello-Reuss, 1982).

We have carried out similar studies in *Necturus* (early) proximal tubule. In general, the electrical response to a step change in one ion reflects its contribution to membrane potential relative to the contribution of the other ion pathways. Therefore a maneuver that inhibits only one ion pathway should enhance the response to the other ions if transport occurs via conductive transport mechanisms. Thus, if potassium and bicarbonate have separate current-carrying pathways, inhibition of either should enhance the response to the other ion. This is exactly what we found in this study. Barium blockade of the potassium conductance (as evidenced by the greatly decreased apparent transference numbers) resulted in an enhanced electrical response to bicarbonate and vice versa. This establishes the existence of a separate current-carrying bicarbonate pathway.

Previous work from this laboratory has demonstrated the presence of sodium-linked chloride-bicarbonate exchange in the basolateral membrane of *Necturus* (Guggino et al., 1983), responsible for most chloride exit from the cell. The exact stoichiometry of this transport mechanism is not fully resolved, but some evidence suggested that it could be associated with charge movement. Since chloride exit (coupled to sodium and bicarbonate entry) through this pathway appeared to hyperpolarize V_{bl}, stimulation of this chloride exit by raising peritubular bicarbonate concentration would cause potential changes similar to those predicted for a membrane with an electrodiffusive bicarbonate pathway. To examine the role of this pathway in our electrical responses to bicarbonate we repeated the bicarbonate steps in chloride-free solutions. Though the bicarbonate transference number was diminished significantly in the absence of chloride, there was still a substantial electrical response to an increase in bicarbonate concentration at constant external pH. The potential response to changes in bicarbonate that remained in the absence of chloride does demonstrate that there is a chloride-independent current-carrying bicarbonate pathway in the basolateral membrane. The results of intracellular pH measurements confirm that the electrical responses are associated with bicarbonate movement and that a finite bicarbonate permeability exists. Recent work from this laboratory (O'Regan, Malnic & Giebisch, 1982) has demonstrated the participation of the cellular pathway in net bicarbonate reabsorption in this preparation. Thus the current-carrying pathway is probably the basolateral exit step for bicarbonate.

Boron and Boulpaep (1983) have proposed that bicarbonate ions exit via a mechanism that carries two bicarbonates and one sodium in the same direction. They have shown that this process is energetically feasible in their tubules. In their preparation, the isolated perfused *Ambystoma* proximal tubule, intracellular sodium activity was found to be approximately 24 mM. Given the intracellular sodium measurements (mean of 7.4 mM) in the *Necturus* proximal tubule from this laboratory (Cemerikic & Giebisch, 1980), and the control pH values reported in this paper, such a 2:1 HCO_3^-/Na^+ cotransport stoichiometry, proposed for *Ambystoma*, would, however, result in bicarbonate entry, not bicarbonate exit, across the basolateral membrane in *Necturus*. This conclusion is based on the following considerations. The free energy change for bicarbonate and sodium leaving the cell with this coupling ratio would be:

$$\frac{\Delta G}{F} = -(V - 2E_{HCO_3} - E_{Na})$$
$$= -(64 - 24 - 59) = +19 \text{ mV}$$

where V (=64), E_{HCO_3} (=12) and E_{Na} (=59) are the

absolute values of the membrane PD and the Nernst potentials for bicarbonate and sodium, respectively, and F is the Faraday constant. As the ΔG is positive, net exit of bicarbonate cannot occur by this mechanism.

As Boron and Boulpaep (1983) chose the simplest stoichiometry that was energetically feasible in their system, it is possible that the stoichiometry is other than 2 : 1. A bicarbonate-to-sodium ratio of 3 : 1, for example, would result in HCO_3^- exit under control conditions in *Necturus* ($\Delta G/F = -33$ mV). Alternatively, the HCO_3^- exit mechanism is different in our tubules, possibly an electrodiffusion pathway or a carrier not linked to sodium. We cannot distinguish among these possibilities at the present time, but we conclude that a significant part of the bicarbonate pathway is charge carrying in *Necturus* proximal tubule.

It is somewhat surprising, given the large apparent transference numbers for bicarbonate and the low bicarbonate Nernst potential (−12 mV), that the membrane depolarization caused by barium was not larger than that found. Since the potassium transference number was so low in the presence of barium, one might have expected a membrane potential closer to the bicarbonate Nernst potential under these conditions. There are several possible explanations for this. First, there may be pathways other than those for potassium and bicarbonate that influence membrane potential. The $Na^+ - HCO_3^-/Cl^-$ appears to carry charge and the $Na^+ - K^+$ pump may be electrogenic. In particular, the electrogenicity of the latter may become more manifest when basolateral membrane resistance is increased by barium. This could also lead to an underestimate of the residual potassium transference number. Second, as discussed above, potential changes due to alterations in ionic pathways in a given barrier will be attenuated because of the presence of the shunt. Third, if bicarbonate exit is carrier-mediated, its contribution to membrane potential may not be simply related to the bicarbonate Nernst potential. Of interest though is the larger effect of barium of V_{bl} when SITS was also present. This suggests that SITS changed basolateral electromotive force and/or resistance, presumably by direct inhibition of the bicarbonate pathway. This is analogous to the difference in the effects of barium on skeletal muscle seen in the presence and absence of chloride (Sperelakis, Schneider & Harris, 1967). The hyperpolarization caused by SITS alone is also consistent with this interpretation.

In conclusion, the evidence presented here demonstrates that potassium and bicarbonate have separate current-carrying pathways that play a major role in determining the membrane potential in the basolateral membrane of the *Necturus* proximal tubule. While a current-carrying $Na^+ - HCO_3^-/Cl^-$ exchanger has been found in this membrane (Guggino et al., 1983), there also appears to be present an additional current-carrying bicarbonate pathway. This latter mechanism is most likely mediating bicarbonate exit across the basolateral membrane and is thus an important component of the process of proximal tubular bicarbonate reabsorption.

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