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Microelectrode Characterization of the Basolateral Membrane of Rabbit S3 Proximal Tubule

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Summary. The purpose of this study was to characterize the basolateral membrane of the S3 segment of the rabbit proximal tubule using conventional and ion-selective microelectrodes. When compared with results from S1 and S2 segments, S3 cells under control conditions have a more negative basolateral membrane potential (V_{bl} = −69 mV), a higher relative potassium conductance (t_K = 0.6), lower intracellular Na^+ activity (A_{Na} = 18.4 mM), and higher intracellular K^+ activity (A_K = 67.8 mM). No evidence for a conductive sodium-dependent or sodium-independent HCO_3^- pathway could be demonstrated. The basolateral Na-K pump is inhibited by 10^{-4} M ouabain and bath perfusion with a potassium-free (0-K) solution. 0-K perfusion results in A_{Na} = 64.8 mM, A_K = 18.5 mM, and V_{bl} = −28 mV. Basolateral potassium channels are blocked by barium and by acidification of the bathing medium. The relative K^+ conductance, as evaluated by increasing bath K^+ to 17 mM, is dependent upon the resting V_{bl} in both S2 and S3 cells. In summary, the basolateral membrane of S3 cells contains a pump-leak system with similar properties to S1 and S2 proximal tubule cells. The absence of conductive bicarbonate pathways results in a hyperpolarized cell and larger Na^+ and K^+ gradients across the cell borders, which will influence the transport properties and intracellular ion activities in this tubule segment.

Key Words basolateral membrane potential · S3 segment · proximal renal tubule · potassium conductance · Na/K pump · pH · barium

Introduction

There is now ample evidence for functional heterogeneity along the length of the proximal renal tubule. Morphological and electrophysiological differences can be demonstrated in both amphibian [22] and mammalian proximal tubules [3, 15–17, 32], which correlate with the heterogeneous transport functions present. These characteristics have led to the identification of three segments in the mammalian proximal tubule referred to as the S1, S2 and S3 segments [26, 32, 38]. The S3 segment, or late proximal tubule, connects with the thin descending limb of Henle and is located in the deep cortex and outer strip of the outer medulla [15].

Recent evidence has demonstrated that the S3 segment is distinct from the S1 and S2 segments in at least two areas. The regulation of intracellular pH and the associated pathways for bicarbonate and hydrogen ion equivalents across the cell borders may be unique [12, 19, 20, 29]. In contrast to early proximal tubules, Kurtz et al. [20] demonstrated an acid disequilibrium pH in the luminal fluid of perfused S3 segments, which resulted from acid secretion in the absence of "functional" luminal carbonic anhydrase. Nakhoul and Boron [29] have reported a HCO_3^- dependent acid extrusion mechanism in the basolateral membrane of S3 tubules, which is independent of Na^+ and Cl^- and is insensitive to the anion transport inhibitor SITS. These findings are in sharp contrast to the demonstration of a SITS-sensitive, electrogenic Na-HCO_3 cotransport in the basolateral membrane of amphibian and mammalian (S1 and S2) proximal tubules [1, 6, 9, 14]. Our preliminary report of S3 cell characteristics [10] and a subsequent report by Kondo and Fromter [17] regarding axial heterogeneity of sodium-bicarbonate cotransport indicate that electrogenic bicarbonate pathways are not present in this segment.

The examination of ischemic damage and repair in rat proximal tubule segments has also established that the S3 tubule is exceptionally vulnerable to ischemic injury [34]. The extent of cellular injury can be exacerbated by stimulation of active ion transport [33] while reduced extracellular pH provides a protective effect from hypoxic injury [37]. The susceptibility of S3 tubules to cellular injury and the unique transport characteristics in these cells provides the rationale for our examination of the electrophysiological properties of S3 tubule cells in the rabbit kidney.

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In previous studies, we have characterized the electrophysiology of rabbit proximal tubules perfused in vitro using the simple anatomical distinction of proximal "convoluted" and proximal "straight" tubules dissected from the superficial cortex [4, 6–8]. Because of the dissection criteria used in these studies, the results correspond to the S1 and S2 segments of the proximal tubule, respectively. The purpose of the present experiments was to characterize the electrophysiological properties of the S3 segment of the rabbit proximal tubule perfused in vitro. Emphasis was placed upon the pump-leak characteristics of the basolateral membrane and upon the pathways for bicarbonate transport across this membrane border. The results demonstrate a relatively high potassium permeability in the basolateral membrane, which is sensitive to both barium and pH in the bathing solution. In addition, ouabain-sensitive transport of sodium and potassium was demonstrated, which could also be inhibited by the removal of bath potassium. These properties are similar to previous results in S1 and S2 cells. In marked contrast to S1 and S2 cells, there is an absence of either sodium-dependent or sodium-independent bicarbonate conductive pathways across the basolateral membrane. These unique characteristics generate a more negative resting membrane voltage in S3 cells and influence the steady-state ion activities within the intracellular compartment.

Materials and Methods

The techniques for isolation and perfusion of nephrons from rabbit kidney were identical to those used previously for the measurement of basolateral membrane potentials and potassium activities in the proximal straight and convoluted segments [4, 6, 8]. In this study, proximal straight tubules were dissected from the superficial cortex down to the thin limb of Henle and were cut to include a length of proximal tubule cells of approximately 800 μm. Cut tubules included the initial portion of the thin limb, which allowed cannulation and perfused from the proximal to distal end. While cells could be punctured along the entire length of the tubule, the majority of punctures were obtained close to the proximal, or perfusion, end of each segment.

The basic "control" solution contained (in mM) NaCl 118, KCl 5, $NaHCO_3$ 30, $MgSO_4$ 1.0, $CaCl_2$ 1.8, NaH_2PO_4 2.0, Na-acetate 2.0, glucose 8.3 and alanine 5.0. The solution pH was 7.3–7.4 when equilibrated with 95% O_2/5% CO_2. The osmolality was in the range of 290 to 295 mOsm and all experimental solutions were adjusted to be equal to the measured value of the control solution.

Ion substitutions were made in the control solution in order to decrease bicarbonate (L-HCO_3), increase potassium (HK), and reduce sodium (0-Na) and potassium concentrations (0-K). Bicarbonate concentration was reduced to 6.6 mM by replacement with Cl^- and the pH of this solution was adjusted to 7.3, 6.8, and 6.1 with appropriate CO_2/O_2 mixtures. The increase (16.7 mM) or decrease (0 mM) in potassium concentration was accomplished by exchanging with sodium.

For single-salt dilution potential measurements, 50 mM NaCl was replaced isosmotically with sucrose. Total sodium replacement was made by replacing sodium with N-methyl-D-glucamine (NMDG) and a component of choline. The NMDG stock solution was titrated with HCL to a pH of 7.4 before mixing the experimental solution. The zero-sodium solution contained, in mM: NMDG-Cl 118, KCl 1, choline HCO_3 30, $MgSO_4$ 1, KH_2PO_4 2, $CaCl_2$ 1.8, K acetate 2, alanine 5 and glucose 8.3.

The luminal perfusate consisted of a low bicarbonate solution containing 6.6 mM HCO_3^- and, in addition, glucose and alanine were replaced with 13.3 mM mannitol. This solution resembles that found in the late proximal tubule in vivo.

Ouabain (Sigma Chemical, St. Louis, MO) and SITS (Pierce Chemical, Rockford, IL) were added to the control solution at a concentration of 10^{-4} M and perfused through the bath. Barium chloride, 2 mM, was also added to the control solution without further adjustments.

The basolateral membrane potential, V_{bl}, was measured using single-barreled microelectrodes pulled from thick wall capillary tubing containing an internal fiber (Frederick Haer, o.d. = 1.2 mm). Electrodes were filled with 1 M KCl at the time of use and had a resistance of 100–150 Ω. To reduce liquid junction potentials to < 2 mV, the bath reference electrode consisted of a flowing 3 M KCl junction placed in the outflow of the perfusion chamber. Consequently, V_{bl} values were not corrected for these potentials. Intracellular activities of sodium and potassium were measured using double-barreled electrodes. The construction and calibration techniques were identical to those used previously for the measurement of intracellular potassium activity in S2 proximal cells [8]. For intracellular sodium measurements, the ion-selective resin was Sodium-Cocktail I obtained from Fluka Chemical, Hauppauge, NY. Construction of sodium selective electrodes was similar to potassium except that the reference barrel was filled with 1 M KCl and the ion-selective barrel was filled with 1 M NaCl. For the double-barreled potassium electrodes, the average slope was 59.1 ± 0.93 (12) mV/decade K^+ and the selectivity coefficient relative to Na^+ was 0.0700 ± 0.0100 (12). For the double-barreled sodium electrodes, the slope was 55.0 ± 1.9 (10) mV/decade Na^+ and the selectivity relative to potassium was 0.0490 ± 0.0120 (10).

The results are expressed as means ± SE with the number of cells in parentheses. Statistical significance was determined using the Student's t test and accepted at $P < 0.05$.

Results

Identification of S3 Segments

In general, S3 rabbit tubule segments were identified by dissecting proximal straight tubules from the superficial cortex to the thin descending limb of Henle. These segments were 2–3 mm in length and for perfusion were cut to a length of approximately 800 μm as measured from the descending thin limb.

In one series of experiments, to ensure that superficial S3 segments were selected, both dissected and perfused lengths were quantitated. The average dissected length was 3274 ± 145 (17) μm indicating

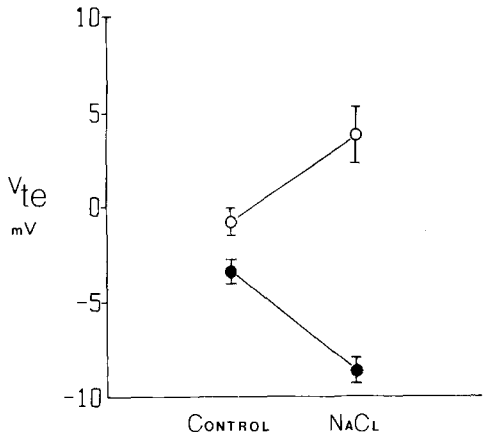

Fig. 1. Transepithelial dilution potentials when 50 mM of bath solution NaCl replaced with sucrose. Open circles are tubules, which were chloride selective (n = 9), and closed circles are tubules, which were sodium selective (n = 8). V_{te} is the transepithelial potential difference

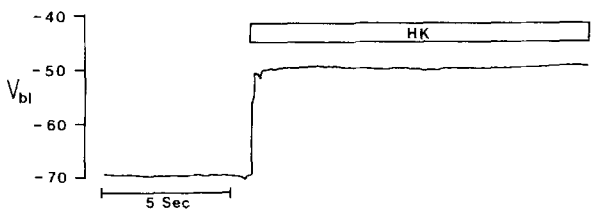

Fig. 2. Response of the basolateral membrane potential, V_{bl}, to a step change in bath potassium concentration from 5 to 16.7 mM (HK). Tracings in Figs. 2–4 are redrawn from digitized recordings

that these segments were indeed superficial proximal tubules, since the total length of juxtamedullary *pars recta* is approximately 800 μm [15, 32, 38]. The average perfused length was 845 ± 69 (17) μm. Single-salt dilution potentials indicated two populations of S3 segments when responses were grouped according to the direction of V_{te} change, either positive or negative, when bath NaCl was reduced. In the first, the average V_{te} was −0.8 ± 0.7 (9) mV, and when 50 mM of NaCl was replaced with sucrose the change in V_{te} was +4.6 ± 0.96 (9) mV, becoming lumen positive. In the second population of S3 segments, the average V_{te} was −3.4 ± 0.46 (8) mV. Removal of 50 mM NaCl gave a change in V_{te} of −5.2 ± 0.76 (8) mV, becoming more lumen negative. Figure 1 shows the mean and SE for the two groups of superficial S3 segments. Intracellular voltage, V_{bl}, was not different between the two groups of tubules. The combined average V_{bl} of 10 cells from 10 tubules was −78 ± 3.1 (10) mV.

V_{bl} of S3 Segments

The basolateral membrane potential (V_{bl}) was measured in 34 superficial S3 segments dissected from 22 rabbits. The average V_{bl} cell was −69.0 ± 0.93 (83) mV. This value is more negative than the V_{bl} in S1 and S2 segments reported previously by our laboratory [4–7] and by others [2, 11, 21, 23, 24, 30, 31]. For comparison, we combined the V_{bl} values in S2 segments obtained in the year preceding these studies and obtained a mean V_{bl} = −46.7 ± 0.69 (334) mV. These values are significantly different with $P < 0.001$.

Basolateral Potassium Conductance

To evaluate the relative potassium conductance of the basolateral membrane, bath potassium was raised from 5 to 16.7 mM. A typical response is given in Fig. 2 and additional responses are also illustrated in Fig. 4. HK solution produces a rapid and sustained depolarization, which is completely reversible on return to normal bath potassium concentration. In 37 S3 cells, the response to high potassium bath solution was a depolarization of 18.3 ± 0.72 mV. The theoretical Nernstian change for a purely K^+-selective membrane in the absence of a paracellular conductance would be 32 mV. As discussed by others, the HK response in S3 cells in the presence of an attenuating shunt conductance allows the calculation of a minimal transference number of 0.57 for potassium [2, 35].

Ba^{2+}-sensitive K^+ channels have been identified in the basolateral membranes of a variety of epithelia [25]. When 2 mM $BaCl_2$ was added to the bath, a rapid and reversible depolarization of 28 ± 3.1 (8) mV was induced. This is clearly consistent with the presence of Ba^{2+}-sensitive potassium channels.

We have previously demonstrated a marked pH dependence of the basolateral membrane potential in both S1 and S2 proximal tubule cells, which results in the depolarization of V_{bl} by approximately 35 mV/pH unit [7]. As summarized in Table 1, when bath pH was reduced by lowering bicarbonate concentration and increasing CO_2, a pH sensitivity of 29 mV/pH unit could be demonstrated. As in S2 proximal cells, the pH sensitivity in S3 cells is reduced in the presence of 2 mM Ba^{2+}. A representative tracing of this interaction is illustrated in Fig. 3.

Basolateral Sodium/Potassium Pump

To test for the presence of a Na^+/K^+ pump in the basolateral membrane, ouabain (10^{-4} M) was added to the bath perfusion solution and V_{bl} was measured. The S3 cells depolarized by 39.9 ± 3.79 mV

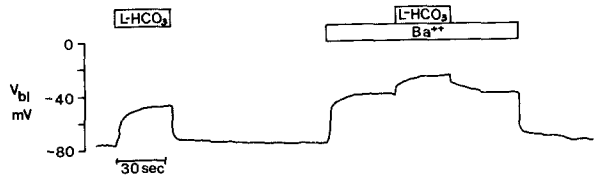

Fig. 3. Response of V_{bl} to bath perfusion with 6.6 mM bicarbonate solution (pH = 6.1), L-HCO_3^-, in the absence and presence of 2 mM barium

Table 1. pH sensitivity of S3 cells: control V_{bl}'s with HCO_3^- = 30 mM and pH = 7.4[a]

Control V_{bl}	Experimental	
	Bath pH	ΔV_{bl}
−68.0 ± 1.7(21)[b]	7.3	1.2 ± 0.5(21)
−67.5 ± 1.9(19)	6.8	6.9 ± 0.8(19)
−66.1 ± 2.0(16)	6.1	35.4 ± 2.3(16)

[a] Experimental V_{bl} responses (ΔV_{bl}) with HCO_3^- = 6.6 mM and CO_2 adjusted to give the indicated pH.
[b] Values are mean ± SE (number of cells).

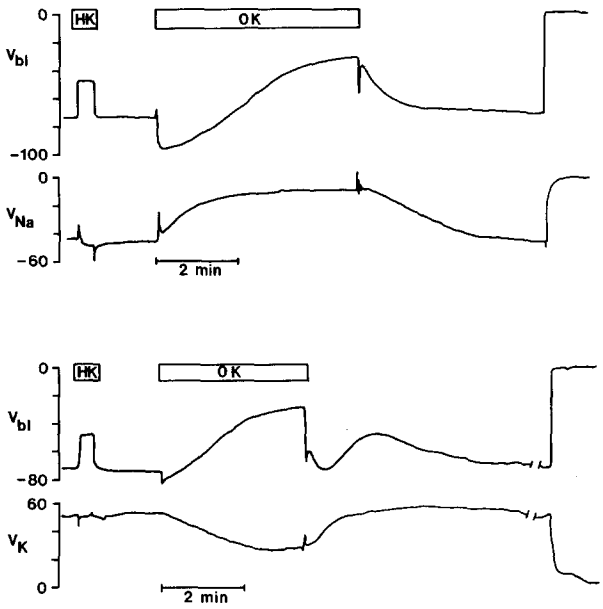

Fig. 4. Tracings from two cells illustrating the response of V_{bl}, intracellular sodium activity, V_{Na}, and intracellular potassium activity, V_K, to increasing (HK) or decreasing (0-K) bath potassium concentration. The V_{Na} and V_K signals (mV) are the differential signals between the ion-selective barrel and the conventional barrel of the double-barreled electrode

(8) after 3–5 min of ouabain exposure. To further support the presence of a Na^+/K^+ pump, A^i_{Na} and A^i_K were measured with doubled-barreled ion-selective microelectrodes. Resting A^i_{Na} was 18.4 ± 5.5 (10) mM and A^i_K was 67.8 ± 4.80 (14) mM.

The effects of extracellular potassium concentration on V_{bl} and intracellular ion activities were examined. Representative tracings taken during bath perfusion with control, HK and 0-K solutions are illustrated in Fig. 4. HK bath solution resulted in a typical V_{bl} depolarization while intracellular sodium and potassium activities were not significantly different. During 0-K bath perfusion, V_{bl} underwent an initial rapid hyperpolarization, which was followed by a slower depolarization to a new steady-state value. As illustrated in Fig. 4 and summarized in Fig. 5 and Table 2, the slow depolarization of V_{bl} was associated with a decrease in A^i_K to 18.5 ± 4.0 (10), mM and an increase in A^i_{Na} to 64.8 ± 9.0 (9) mM.

Basolateral HCO_3^- Pathways?

To test for the presence of a conductive bicarbonate pathway in the basolateral membrane, bath bicarbonate was lowered from 30 mM to 6.6 mM at constant pH (pH = 7.4). There was no transient or steady-state change in V_{bl}, which could be associated with a conductive bicarbonate pathway. When Na^+ was replaced with NMDG in the bath solution, a small depolarization of V_{bl} was seen. Addition of SITS (10^{-4} M) to the bath had no effect on V_{bl} or on the responses to L-HCO_3 or 0-Na^+ bath perfusion. Mean values from these ion substitution experiments are summarized in Table 3. These data indicate the lack of a conductive Na-independent or Na-dependent pathway for bicarbonate exit across the basolateral membrane of the S3 segment.

Discussion

We have attempted to characterize the basolateral membrane of the rabbit S3 proximal tubule using microelectrode and ion-substitution experiments. Care was taken to ensure that only S3 cell types were studied by perfusing the terminal portions of superficial proximal straight tubules located in the deep cortex or outer stripe of the outer medulla [15, 18, 38].

Single-salt dilution potential experiments were done to determine relative sodium-to-chloride permeabilities in this population of nephrons. Both dissected and perfused lengths were quantitated to ensure that the tubule was from the superficial nephron population while the perfused length of 845 μm ensured that the cell type would be S3. The

Table 2. Intracellular ion activities and the responses to increasing bath potassium to 16.7 mM (HK) and reducing bath potassium to 0 (0-K) measured with double-barreled ion-selective microelectrodes

	Control	HK	Post-control	0-K	Post-control
A_{Na} (mM)	18.4 ± 5.5(10)[b]	11.7 ± 3.1(9)	18.2 ± 5.1(9)	64.8 ± 9.0(9)	13.4 ± 4.1(9)
V_{bl} (mV)	−61.2 ± 3.9	−40.3 ± 3.7	−62.0 ± 3.9	−26.7 ± 2.8	−60.1 ± 3.9
A_K (mM)	67.8 ± 4.8(14)	72.6 ± 5.2(14)	70.9 ± 5.2(13)	18.5 ± 4.0(10)	79.0 ± 6.7(9)
V_{bl} (mV)	−59.5 ± 2.0	−41.0 ± 1.5	−60.8 ± 2.3	−29.9 ± 2.2	−59.2 ± 3.8

[a] Steady-state values were obtained after approximately 30 sec of HK and 5 min of 0-K perfusion.
[b] Values are mean ± SE (number of cells).

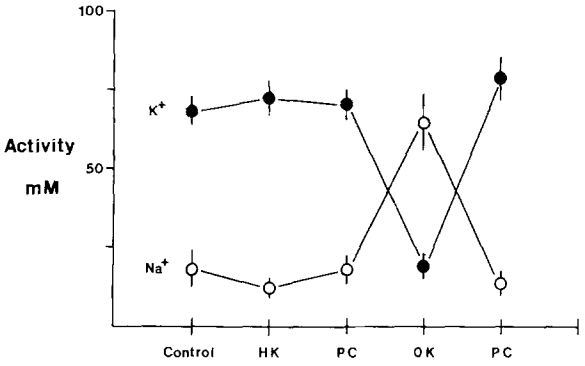

Fig. 5. Mean changes in intracellular sodium and potassium activities when bath potassium is increased (HK) or decreased (0-K) relative to the control and post-control (PC) values

Table 3. Steady-state values of V_{bl} and the V_{bl} responses (ΔV_{bl}) to ion substitutions under control conditions and following addition of 0.1 mM SITS to the bathing solution

	Control	SITS
V_{bl}	−66 ± 4.1(5)[a]	−72 ± 2.6(5)
ΔV_{bl} (mV)		
L-HCO_3^-	0.0 ± 1.4	2.0 ± 2.4
0-Na^+	9.0 ± 2.3	8.2 ± 2.0
HK	17.2 ± 3.4	19.4 ± 2.3

[a] Values are mean ± SE (number of cells).

single-salt dilution experiments revealed two populations of tubules. One group had a V_{te} close to zero and responded with a depolarization of V_{te}. The calculated P_{Na}/P_{Cl} was 0.81. This value is close to the free solution mobility ratio for these ions [36], and thus indicates little discrimination between sodium and chloride in these tubules. The second population of tubules, however, had a more negative V_{te} and responded with a hyperpolarization of V_{te}. The calculated P_{Na}/P_{Cl} was 3.58 and indicates a marked selectivity for sodium relative to chloride. These results demonstrate that the selectivity properties of the paracellular pathway can be widely different in a well-defined nephron population and that the results of single-salt dilution potentials cannot be used as a criteria to define the S3 nephron segment. We did not see such differences in the properties of the basolateral membranes, however, as evidenced by identical intracellular voltages in the cells taken from the two populations of tubules and uniform responses to the various ion-substitution experiments.

S3 cells show many of the same properties as those described for S1 and S2 segments of the rabbit proximal tubule. The results demonstrate that the basolateral membrane contains the same pump-leak system as in the more proximal segments. The most obvious difference, however, is the more negative intracellular voltage in S3 cells, which results from the higher intracellular potassium activity and the higher relative potassium permeability of the basolateral membrane under control conditions. The high relative K^+ conductance, in turn, is a consequence of the absence of the conductive pathway(s) for bicarbonate, either as the isolated ion or as a sodium-dependent system, which are present in S1 and S2 segments.

Intracellular sodium and potassium activities are clearly maintained by a ouabain- and potassium-sensitive pump in the basolateral membrane. Based upon the resting intracellular sodium (18.4 mM) and potassium (72.6 mM) activities, the equilibrium voltages for sodium and potassium are +48 and −78 mV, respectively. When compared with the resting V_{bl} of −69 mV, it is clear that both ions are maintained away from their equilibrium value by this active transport process. A similar displacement from equilibrium distribution is present in the S2 cell, but, in contrast, the intracellular K^+ activity is 49 mM [8] and intracellular Na^+ activity is 46.8 mM [31]. Reported values for V_{bl} in rabbit S1 and S2

segments generally range from approximately −45 to −55 mV [5, 11, 21, 23, 30, 31].

Inhibition of the Na-KATPase by perfusion with a potassium-free solution resulted in equivalent but opposite changes in potassium and sodium activities; which were accompanied by a depolarization of the cell (Fig. 5). As previously shown in S2 cells under similar conditions [8], the return of potassium results in a pump current, which produces a marked hyperpolarization of V_{bl}. This is opposite to the change in the potassium equilibrium potential and occurs before intracellular potassium begins to increase. Thus, under conditions of dissipated ion gradients, the transient in V_{bl} when potassium is returned to the bathing solution suggests a Na/K stoichiometry greater than one. While the pattern of this 0-K response to similar to that in S2 cells, the magnitudes of the transient responses are greater and more clearly defined in the S3 cells. This includes an initial hyperpolarization, in agreement with the immediate change in the potassium equilibrium potential, which occurs when bath potassium is removed but before intracellular potassium decreases substantially (Fig. 4).

The characteristics of the basolateral potassium conductance in S3 cells are also very similar to those described for S1 and S2 proximal tubule segments. The close agreement between the intracellular voltage (−69 mV) and the potassium equilibrium potential (−78 mV) and the large V_{bl} response to increasing bath potassium concentration both indicate that potassium is the major conductive pathway in the basolateral membrane. Even in the presence of a paracellular shunt conductance, which would tend to attenuate a HK response, the change in V_{bl} indicates a minimum transference number of 0.6 for potassium [2, 35]. This basolateral potassium conductance is also sensitive to bath barium and thus is similar to barium-sensitive potassium channels found in proximal tubule segments and other epithelia [2, 5, 22, 25].

The pH sensitivity of S3 cells is also similar to that described for S1 and S2 tubule segments [7]. While the absolute magnitude of the depolarization seen in S3 cells is less than in S1 of S2 cells, the slope of the relationship between the change in V_{bl} and bath pH (29 mV/pH unit) is similar to that seen in the more proximal segments. The fact that barium blocks the pH response suggests that a common pathway is involved and that it is most likely the basolateral potassium conductance, which is sensitive to extracellular pH. An alternative possibility would be a H^+/OH^- conductance, which can be eliminated only on the assumption that barium is specific for potassium channels and has no effect on a H^+/OH^- conductance. This assumption remains to be tested.

We cannot distinguish between effects of intracellular pH and extracellular pH changes on potassium conductance. Clearly, intracellular pH will change when bath pH is reduced in our experiments. S3 cells have the ability to regulate intracellular pH [19, 29] and such regulation would be expected to reduce the magnitude of intracellular pH changes. It is clear, however, that a strong relationship exists between the extracellular or bath pH (over the range of 6.1 to 7.4) and the intracellular voltage in S3 as well as S1 and S2 cell types [7]. In summary, the barium and pH sensitivity of V_{bl} indicate that the properties of the basolateral potassium conductance are similar in all proximal tubule segments and, therefore, reflect a common potassium channel, which is present in each segment of the rabbit proximal tubule.

The similar properties of S2 and S3 cell's potassium channels can also be demonstrated with the V_{bl} response to increasing bath potassium concentration. We have previously noted a dependence of the HK response on the resting membrane potential in S2 cells [7]. Voltage-sensitive potassium conductance has been demonstrated in the frog [27, 28] and mouse [35] proximal tubules. Similarly, a voltage-dependent channel, which does not discriminate between Na^+ and K^+, has been described in late proximal tubules from rabbit kidneys [13]. A similar voltage dependence of the HK response in S2 and S3 rabbit proximal tubules is shown in Fig. 6. The differences in V_{bl} result from widely different conditions including differences in S2 and S3 resting V_{bl}, depolarization with ouabain or bath pH, and hyperpolarization with SITS. A simple linear relationship, however, appears to hold for all conditions. The common variable is the V_{bl} in each condition, since intracellular ion activities do not show a consistent relationship. For example, depolarization of V_{bl} following ouabain results in large shifts in intracellular potassium, while depolarization with an acid bath produces no change in intracellular K^+ [8]. The relationship in Fig. 6 suggests a voltage-dependent relative potassium conductance, which is present in both S2 and S3 basolateral membranes.

Finally, the clearest difference in transport properties between S3 and S1 or S2 cells is the absence of conductive bicarbonate pathways in the S3 segment. The failure to observe a transient depolarization when bath bicarbonate is reduced at constant pH, a small depolarization when bath Na^+ is reduced to zero, and the lack of a significant SITS effect in S3 cells all are in marked contrast to results in more proximal segments [4, 6, 21, 30, 31]. Similar responses combined with direct measurement of intracellular pH have been used to identify Na^+/HCO_3^- symport in amphibian and mammalian proximal tubules [1, 9, 31]. Our results are in agreement

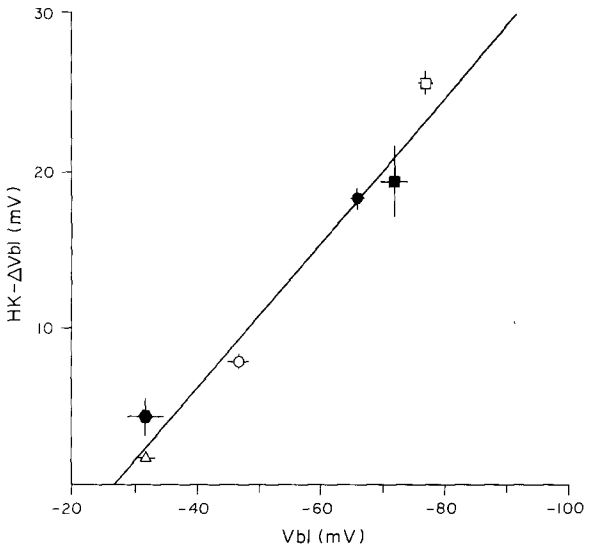

Fig. 6. The presence of V_{bl} to increasing bath potassium in S3 (filled symbols) and S2 (open symbols) cells. ○, ● = control; □, ■ = 10^{-4} M SITS; ⬢ = 10^{-4} M ouabain; and △ = bath pH 6.1

with the preliminary report of Nakhoul and Boron [29] who demonstrated Na^+ and SITS-insensitive system for intracellular pH regulation in the basolateral membrane of rabbit S3 tubules. Our results confirm the absence of conductive bicarbonate pathways in S3 cells but do not further define the mechanisms for intracellular pH regulation or net bicarbonate transport in this segment.

In conclusion, we have attempted to define some of the properties of the S3 proximal tubule, which distinguish this segment from more proximal segments. The electrical and chemical driving forces for Na^+ and K^+ across the apical and basolateral membranes are clearly different from S1 and S2 tubule cells as are the mechanisms for intracellular pH regulation and bicarbonate transport. It is possible that these differences are related to the susceptibility of the S3 cell to hypoxic or ischemic injury and the examination of the S3 cell's transport properties and their relationship to intracellular ion homeostasis and cell viability are areas for further study.

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