Basolateral membrane Na+/H+ antiport, Na+/base cotransport, and Na+-independent Cl-/base exchange in the rabbit S3 proximal tubule.

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Research Article

The basolateral membrane Na+ and Cl(-)-dependent acid-base transport processes were studied in the isolated perfused rabbit S3 proximal straight tubule. Intracellular pH (pHi) was measured with 2'7'-biscarboxyethyl-5,6-carboxyfluorescein (BCECF) and a microfluorometer coupled to the tubule perfusion apparatus. Reduction of basolateral HCO3- from 25 to 5 mM caused pHi to decrease at a rate of 0.81 pH/min. Approximately 50% of this rate was Na+-dependent, 30% Cl(-)-dependent and 20% Na+ and Cl(-)-independent. Two basolateral Na+-dependent acid base transport pathways were detected: (a) an amiloride-sensitive Na+/H+ antiporter and (b) a stilbene-sensitive Na+/base cotransporter. No evidence was found for a Na+-dependent Cl-/base exchanger. The Cl(-)-dependent component of basolateral base efflux was mediated by a stilbene-sensitive Na+-independent Cl-/base exchange pathway. The results suggest that the acid base transport pathways of the basolateral membrane of the S3 proximal tubule differ from more proximal nephron segments.

Basolateral Membrane Na^+/H^+ Antiport, Na^+/Base Cotransport, and Na^+-Independent Cl^-/Base Exchange in the Rabbit S_3 Proximal Tubule

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Abstract

The basolateral membrane Na^+ and Cl^--dependent acid-base transport processes were studied in the isolated perfused rabbit S_3 proximal straight tubule. Intracellular pH (pH_i) was measured with 2'7'-biscarboxyethyl-5,6-carboxyfluorescein (BCECF) and a microfluorometer coupled to the tubule perfusion apparatus. Reduction of basolateral HCO_3^- from 25 to 5 mM caused pH_i to decrease at a rate of 0.81 pH/min. Approximately 50% of this rate was Na^+-dependent, 30% Cl^--dependent and 20% Na^+ and Cl^--independent. Two basolateral Na^+-dependent acid base transport pathways were detected: (*a*) an amiloride-sensitive Na^+/H^+ antiporter and (*b*) a stilbene-sensitive Na^+/base cotransporter. No evidence was found for a Na^+-dependent Cl^-/base exchanger. The Cl^--dependent component of basolateral base efflux was mediated by a stilbene-sensitive Na^+-independent Cl^-/base exchange pathway. The results suggest that the acid base transport pathways of the basolateral membrane of the S_3 proximal tubule differ from more proximal nephron segments.

Introduction

Recent studies have demonstrated that the mammalian proximal tubule possesses an apical Na^+/H^+ antiporter that contributes importantly to the absorption of luminal HCO_3^- and to the regulation of intracellular pH (1–3). Previous vesicle and tubule perfusion studies have concluded that the rat and rabbit proximal tubule lack a basolateral Na^+/H^+ antiporter (4–7). Absent Na^+/H^+ antiport activity on the basolateral side of the tubule would increase the efficiency of net Na^+ and HCO_3^- absorption. The preparations in previous studies were derived from the cortex and contained predominantly S_1 and S_2 proximal tubule cells and not S_3 proximal cells which are localized to the outer stripe of the outer medulla. We have recently reported that the rabbit S_3 proximal tubule possesses an apical Na^+/H^+ antiporter and a plasma membrane H^+ATPase that regulates pH_i (3). The basolateral membrane H^+/base transport processes were not characterized in that study. The present study was designed to (*a*) determine whether the S_3 proximal tubule possesses a basolateral Na^+/H^+ antiporter, and (*b*) characterize the basolateral transport processes responsible for H^+/base transport in the S_3 segment.

Methods

S_3 tubules were dissected and perfused as previously described (3). Intracellular pH was monitored using the fluorescent probe 2'7'-biscarboxyethyl-5,6-carboxyfluorescein (BCECF)[1] and a recently described microfluorometer that was coupled to the tubule perfusion apparatus (3). Calibration of intracellular BCECF was performed at the end of each experiment by monitoring the 500/440 nm fluorescence excitation ratio at various values of pH_i. pH_i was set approximately equal to pH_o using high K^+ nigericin standards as previously described (3).

The rate of change of pH_i, dpH_i/dt was measured in the initial 8 s after a solution change.

Solutions. The composition of the perfusate and bathing solutions used in this study are listed in Table I. When Na^+-free Hepes buffered solutions were used, NaCl was replaced with tetramethyl-ammonium chloride (TMACl). In HCO_3^- buffered solutions, $NaHCO_3^-$ was replaced with tetramethylammonium bicarbonate ($TMAHCO_3$). $TMAHCO_3$ was made by bubbling tetramethylammonium hydroxide with 100% CO_2. In Cl^--free experiments, Cl^- was replaced with equimolar gluconate, and the total Ca^{2+} was increased to 3.5 mM as calcium gluconate. In Na^+ and Cl^- free solutions, NaCl was replaced with tetramethylammonium gluconate (TMAgluconate). TMAgluconate was made by reacting tetramethylammonium hydroxide with equimolar D-gluconic acid lactone.

Materials. BCECF-acetoxymethyl ester (BCECF-AM) (Molecular Probes, Inc., Junction City, OR); 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) (Sigma Chemical Co., St. Louis, MO) (a stock solution of 50 mM DIDS [dissolved in DMSO] was made on the day of study and kept in the dark); 4-acetoamido-4-isothiocyanostilbene-2,2'-disulfonate (SITS) (Polysciences, Inc., Warrington, PA); tetramethylammonium chloride (Aldrich Chemical Co., Milwaukee, WI); sodium gluconate; calcium gluconate; magnesium gluconate; nigericin; tetramethylammonium hydroxide; D-gluconic acid lactone (all from Sigma); amiloride hydrochloride dihydrate (Merck, Sharp and Dohme). Amiloride (1 mM) formed a precipitate when added to a solution containing the stilbenes SITS 1 mM or DIDS 1 mM. Therefore studies using amiloride and stilbenes were performed separately.

Statistics. Results are reported as mean±SEM. Unpaired Student's *t* test, paired Student's *t* test, and linear regression analysis were used as required.

Results

Basolateral Na^+/H^+ antiporter. The following experiments were performed to determine whether the S_3 proximal tubule

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1. *Abbreviations used in this paper:* BCECF, 2'7'-bis(carboxyethyl) 5-6-carboxyfluorescein; BCECF-AM, BCECF-acetoxymethyl ester; DIDS, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid; SITS, 4-acetoamido-4-isothiocyanostilbene-2,2'-disulfonate; TMA, tetramethylammonium.

Table I. Solutions

	A	B	C	D	E	F	G	H	I	J	K
Na^+ (*mM*)	140	—	140	140	—	140	—	140	—	140	—
TMA^+ (*mM*)	—	140	—	—	140	—	140	—	140	—	140
K^+ (*mM*)	5	5	5	5	5	5	5	5	5	5	5
Cl^- (*mM*)	144	144	—	119	119	—	—	139	139	—	—
Gluconate (*mM*)	—	—	149	—	—	124	124	—	—	144	144
Ca^{2+} (*mM*)	1	1	3.5	1	1	3.5	3.5	1	1	3.5	3.5
Mg^{2+} (*mM*)	1	1	1	1	1	1	1	1	1	1	1
Phosphate (*mM*)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Glucose (*mM*)	5	5	5	5	5	5	5	5	5	5	5
Alanine (*mM*)	5	5	5	5	5	5	5	5	5	5	5
Hepes (*mM*)	5	5	5	—	—	—	—	—	—	—	—
HCO_3^- (*mM*)	—	—	—	25	25	25	25	5	5	5	5
pH	7.4	7.4	7.4	7.4	7.4	7.4	7.4	6.7	6.7	6.7	6.7

possesses a basolateral Na^+/H^+ antiporter. The tubules were perfused and bathed in 140 mM Na^+, Hepes-buffered solutions pH 7.4, in the absence of organic anions and sulfate (solution A). Steady state pH_i was 7.18±0.06 (n = 16). When basolateral Na^+ was decreased from 140 mM to zero, intracellular pH decreased by 0.27±0.04 pH units (n = 7), $P < 0.001$ (Fig. 1 *a*). When 140 mM Na^+ was added to the basolateral side of the tubules, pH_i recovered at a rate of 0.58±0.03 pH/min (n = 7) (Fig. 1 *a*, Table II). In the presence of 10^{-3} M basolateral amiloride (Fig. 1 *b*, Table II), the rate of recovery of pH_i upon returning 140 mM Na^+ to the basolateral side decreased to 0.09±0.03 pH/min (n = 6), $P < 0.001$. When amiloride was removed, intracellular pH recovered at a more rapid rate towards the baseline value (Fig. 1 *b*). In the absence of luminal and basolateral chloride or in the presence of basolateral SITS (1 mM), the rate of recovery of intracellular pH upon the readdition of 140 mM Na^+ to the basolateral side of the tubule was not significantly different from control (Fig. 1, *c* and *d*, Table II). These results demonstrate that the S_3 proximal tubule possesses a basolateral Na^+/H^+ antiporter.

To determine whether the basolateral Na^+/H^+ antiporter functions under steady state conditions to mediate basolateral H^+ efflux, 1 mM amiloride was added to the basolateral side of the tubule in the presence of 140 mM Na^+ (solution A lumen, bath). As demonstrated in Fig. 2, the addition of 1 mM amiloride resulted in a slow decrease in pH_i. The mean decrease in pH_i was 0.07±0.01 pH units (n = 3), $P < 0.05$. These results suggest the S_3 tubule basolateral Na^+/H^+ antiporter mediates cellular H^+ efflux at resting pH_i.

The kinetics of the basolateral Na^+/H^+ antiporter were determined by measuring the rate of increase in pH_i upon the readdition of varying concentrations of Na^+ to the basolateral side of the tubule following basolateral Na^+ removal (Fig. 3 *a*). A Hanes-Woolf analysis of the data indicates that the K_m for basolateral Na^+ is 53 mM with a V_{max} of 0.75 pH/min (Fig. 3 *b*).

Basolateral HCO_3^- (OH^-) transport. Earlier it was demonstrated that after basolateral Na^+ removal in Hepes-buffered solutions, the rate of increase of pH_i upon readdition of baso-

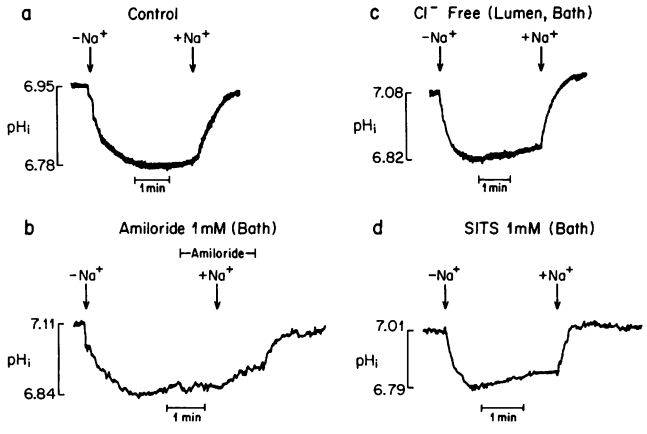

Figure 1. Effect of basolateral sodium removal and readdition on pH_i (Hepes-buffered solutions). (*a*) Control: tubules were perfused with 140 mM Na^+, pH 7.4 (solution A, Table I). When basolateral Na^+ was removed, pH_i decreased. When 140 mM Na^+ was added to the basolateral side, pH_i increased at a rate of 0.58±0.03 pH/min to the control value. (*b*) Amiloride 1 mM (bath) significantly decreased the recovery of pH_i following the addition of 140 mM Na^+ to the bath to 0.09±0.03 pH/min. In tubules perfused and bathed in Cl^--free solutions for ~ 30 min (*c*) or exposed to SITS 1 mM (bath) beginning ~ 5 min before the study and throughout the experiment (*d*), the recovery of pH_i following the addition of 140 mM Na^+ to the basolateral side of the tubule was not different from the control rate. (Refer to Table II for summary of above results.)

Table II. Rate of Increase in pH_i after Basolateral Sodium Addition in S_3 Tubules

	Control	Amiloride (bath)	SITS (bath)	Chloride free (lumen, bath)
		1 mM	*1 mM*	
Starting pH_i	6.79±0.01	6.86±0.05	6.89±0.02	6.87±0.02
dpH_i/dt (*pH/min*)	0.58±0.03	0.09±0.03*	0.57±0.06	0.66±0.14
n	7	6	5	5

Starting pH_i represents the pH_i measured immediately before basolateral Na^+ addition. All studies were performed in Hepes (5 mM)-buffered solutions. dpH_i/dt was measured during the initial 8 s following the basolateral addition of 140 mM Na^+.

* $P < 0.001$ vs. control.

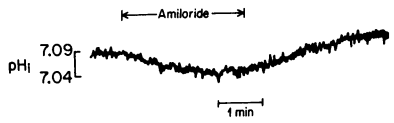

Figure 2. Effect of basolateral amiloride on steady state pH_i. Tubules were perfused and bathed in solution A. When amiloride (1 mM) was added to the basolateral side, pH_i decreased by 0.07±0.01 pH units (n = 3) $P < 0.05$. Removal of amiloride resulted in the recovery of pH_i.

lateral Na^+ was not significantly different from the rate of increase of pH_i in tubules perfused and bathed in Cl^--free solutions or exposed to SITS (1 mM) (bath). These findings suggested that in Hepes-buffered solutions, the basolateral Na^+-coupled pH_i regulatory pathway was not a Na^+-dependent Cl^-/base exchanger or a Na^+/base cotransporter. These latter transport processes may require a greater concentration of HCO_3^- to function than was present in the Hepes solutions. Therefore, further experiments were performed in HCO_3^- buffered solutions to determine whether S_3 tubules possess basolateral Na^+ and/or Cl^--coupled base transport pathways that are HCO_3^--dependent and stilbene-sensitive. S_3 tubules were perfused and bathed in 25 mM HCO_3^-, pH 7.4 (solution D, Table I). Steady state pH_i of tubules bathed in solution D was 6.92±0.06, n = 6. When basolateral HCO_3^- was decreased to 5 mM, pH_i decreased at a rate of 0.81±0.04 pH/min, n = 6 (Fig. 4 *a*, Table III). When similar studies were performed in the absence of Na^+ (lumen, bath), the rate of decrease in pH_i was significantly less 0.33±0.05 pH/min, n = 8, $P < 0.001$ (Fig. 4 *b*, Table III). When basolateral HCO_3^- was decreased to 5 mM in tubules bathed and perfused in the absence of chloride, pH_i decreased at a rate of 0.57±0.04 pH/min, n = 7 (Fig. 4 *c*, Table III), which was also significantly less than the control rate. In the absence of sodium and chloride (lumen, bath), pH_i decreased at a rate of 0.16±0.02 pH/min, n = 5 (Fig. 4 *d*, Table III) following a decrease in basolateral HCO_3^- to 5 mM. These results suggest that ~ 50% of the rate of decrease in pH_i following a decrease in bath HCO_3^- is Na^+-dependent, 30% is Cl^--dependent and 20% is Na^+ and Cl^--independent.

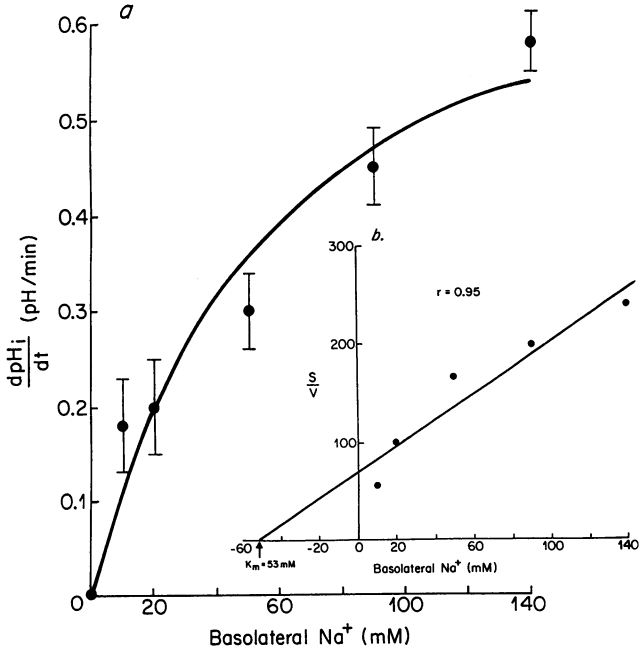

Figure 3. Kinetics of the basolateral Na^+/H^+ antiporter. (*a*) After the removal of basolateral Na^+, varying concentrations of Na^+ (0–140 mM) were added to the basolateral side of the tubule and the rate of increase in pH_i was measured (initial 8 s). Each point represents the mean of at least three determinations. (*b*) A Hanes-Woolf analysis of the data indicates that the K_m for basolateral Na^+ is 53 mM; V_{max} 0.75 pH/min.

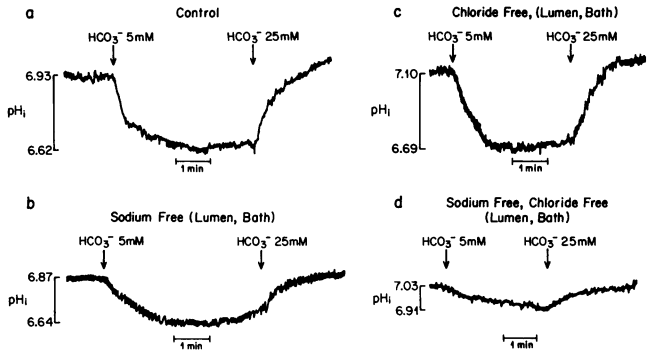

Figure 4. Effect of a decrease in basolateral HCO_3^- from 25 mM to 5 mM on pH_i. (*a*) Control: tubules were perfused and bathed in solution D. Following the decrease in basolateral HCO_3^- to 5 mM, pH_i decreased at a rate of 0.81±0.04 pH/min. (*b*) Sodium-free: In the absence of luminal and basolateral Na^+ (~ 30 min), when basolateral HCO_3^- was decreased from 25 mM to 5 mM, the rate of decrease in pH_i was significantly less than the control rate, 0.33±0.05 pH/min. (*c*) Chloride-free: when basolateral HCO_3^- was decreased to 5 mM in tubules perfused and bathed in the absence of Cl^- for ~ 30 min, the rate of decrease in pH_i was less than the control rate, 0.57±0.04 pH/min. (*d*) Sodium, chloride-free: In the absence of luminal and basolateral Na^+ and Cl^-, (~ 30 min) the rate of decrease in pH_i was only 0.16±0.02 pH/min. (Refer to Table III for summary of above results.)

Additional experiments were performed to characterize the Na^+-coupled base efflux pathway in more detail. S_3 tubules were perfused and bathed in 25 mM HCO_3^- (solution D). As demonstrated in Fig. 5 *a*, Table IV, after the removal of basolateral Na^+, pH_i decreased at a rate of 0.24±0.03 pH/min, n = 4. When similar experiments were performed in the absence of chloride (lumen, bath), the rate of decrease in pH_i was not significantly different; 0.31±0.08 pH/min, n = 4 (Fig. 5 *c*, Table IV). These results suggest that the basolateral Na^+-coupled transport process is Cl^--independent and is therefore not a Na^+-dependent Cl^-/base exchanger. Since it had been dem-

Table III. Rate of Decrease in pH_i after Decrease in Basolateral HCO_3^- (25 mM to 5 mM)

	Control	Sodium free (lumen, bath)	Chloride free (lumen, bath)	Sodium free, Chloride free (lumen, bath)
Steady state pH_i	6.92±0.06	6.87±0.05*	7.10±0.07	7.11±0.06
dpH_i/dt (*pH/min*)	0.81±0.04	0.33±0.05‡	0.57±0.04§	0.16±0.02‡
n	6	8	7	5

dpH_i/dt was measured in the initial 8 s after the decrease in the basolateral HCO_3^- concentration.
* n = 13.
‡ $P < 0.001$ vs. control.
§ $P < 0.01$ vs. control.

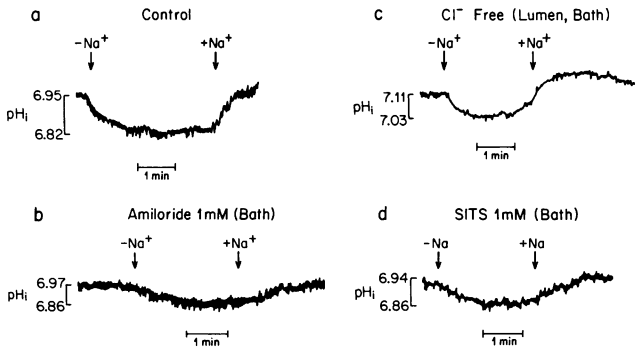

Figure 5. Effect of basolateral sodium removal and readdition on pH_i (HCO_3^- buffered solutions). (*a*) Control: tubules were perfused and bathed in solution D. When basolateral Na^+ was removed, pH_i decreased at a rate of 0.24±0.03 pH/min. (*b*) Amiloride (1 mM, bath, exposure time ~ 5 min before the study and throughout the experiment) significantly decreased dpH_i/dt following basolateral Na^+ removal; 0.11±0.02 pH/min. (*c*) Chloride-free: in the absence of luminal and basolateral Cl^-, (~ 30 min) the rate of decrease in pH_i following basolateral Na^+ removal was not significantly different from control. (*d*) SITS (1 mM, bath, exposure time ~ 5 min before the study and throughout the experiment) significantly decreased dpH_i/dt after basolateral Na^+ removal to 0.13±0.02 pH/min. (Refer to Table IV for summary of above results.)

onstrated that a Na^+/H^+ antiporter is present on the basolateral membrane of the S_3 tubule, and since part of the decrease in pH_i after basolateral Na^+ removal could have been due to inhibition or reversal of the basolateral Na^+/H^+ antiporter, basolateral Na^+ removal studies were performed in HCO_3^- buffered solutions (25 mM) in tubules exposed to basolateral amiloride (1 mM, bath) for ~ 5 min before basolateral Na^+ removal and throughout the experiment (Fig. 5 *b*, Table IV). Amiloride significantly decreased the fall in pH_i to 0.11±0.02 pH/min, $n = 5$, $P < 0.01$. This result indicates that ~ 50% of the sodium-dependent decrease in pH_i in these studies can be accounted for by basolateral Na^+/H^+ exchange. To determine whether the remaining 50% of the Na^+-dependent decrease in pH_i was due to Na^+/base cotransport, Na^+ was removed from the basolateral side of the tubule in HCO_3^- buffered solutions (25 mM) in the presence of 1 mM SITS (bath). Tubules were exposed to SITS for ~ 5 min before the onset of the study and thereafter throughout the remainder of the experiment. 1 mM SITS (Fig. 5 *d*, Table IV) inhibited the rate of sodium-dependent decrease in pH_i by ~ 50%. The Na^+ and HCO_3^- dependent SITS-inhibitable Cl^--independent base efflux pathway is likely a Na^+/base cotransporter that has previously been described in the basolateral membrane of the salamander, rat, and rabbit proximal tubule (1, 6–10). Therefore, the S_3 tubule possesses two Na^+-dependent H^+/base transport pathways: a basolateral Na^+/H^+ antiporter and a Cl^--independent Na^+/base cotransporter, each of which accounts for ~ 50% of the total basolateral Na^+-coupled acid-base transport in the basolateral Na^+ removal experiments.

The previous results indicated that in tubules perfused and bathed in the absence of Cl^-, the change in pH_i induced by basolateral Na^+ removal was not different from the result obtained in the presence of luminal and basolateral Cl^-, indicating that this Na^+-coupled process is not a Na^+-dependent Cl^-/base exchanger. However, in tubules perfused and bathed in Cl^- free solutions, the rate of decrease in pH_i following a decrease in basolateral HCO_3^- to 5 mM was ~ 70% of the control rate suggesting that basolateral base efflux is in part Cl^--dependent in the S_3 segment. Further experiments were performed to determine whether S_3 tubules possesses a basolateral Na^+-independent Cl^-/base exchanger. S_3 tubules were perfused and bathed in Na^+-free solutions containing 119 mM Cl^-, 25 mM HCO_3^- (solution E lumen, bath). When basolateral Cl^- was decreased from 119 mM to zero, pH_i increased at a rate of 0.40±0.02 pH/min, $n = 5$ (Fig. 6, upper trace, Table V). Returning Cl^- to the basolateral side resulted in the recovery of pH_i. When basolateral Cl^- was decreased to zero in the presence of 50 μM DIDS (exposure time 5 min before Cl^- removal and throughout the experiment, bath), pH_i did not increase (Fig. 6, lower trace, Table V). These results suggest that the S_3 tubule possesses a Na^+-independent Cl^-/base exchanger. The results of the basolateral Cl^- removal studies complement the earlier finding that in the absence of Cl^- (lumen, bath) after a decrease in bath HCO_3^- from 25 to 5 mM, the rate of decrease in pH_i was 30% less than control. The latter effect was most likely a result of the inhibition of a basolateral Cl^- base exchanger.

Further experiments were performed to examine the HCO_3^- dependence of the Cl^- base exchanger. The tubules were perfused and bathed in Hepes-buffered solutions, pH 7.4,

Table IV. Rate of Decrease in pH_i after Basolateral Sodium Removal

	Control	Chloride free (lumen, bath)	Amiloride bath	SITS bath
			1 mM	*1 mM*
Steady state pH_i	6.92±0.06*	7.13±0.03	6.94±0.05	6.94±0.06
dpH_i/dt (*pH/min*)	0.24±0.03	0.31±0.08	0.11±0.02‡	0.13±0.02§
n	4	4	5	5

All studies were performed in HCO_3^- (25 mM) buffered solutions.
dpH_i/dt was measured in the initial 8 s after basolateral sodium removal.
* $n = 6$.
‡ $P < 0.01$ vs. control.
§ $P < 0.05$ vs. control.

Table V. Rate of Increase in pH_i after Basolateral Chloride Removal

	Control*	DIDS* bath	Hepes
		50 μM	
Steady state pH_i	6.87±0.05‡	7.26±0.01§	6.98±0.06
dpH_i/dt (*pH/min*)	0.40±0.02	0±0‖	0.45±0.01
n	5	4	3

All studies were performed in the absence of Na^+ (lumen, bath).
dpH_i/dt was measured in the initial 8 s after basolateral chloride removal.
* These studies were performed in HCO_3^- (25 mM) buffered solutions.
‡ $n = 13$.
§ $P < 0.01$ vs. control.
‖ $P < 0.001$ vs. control.

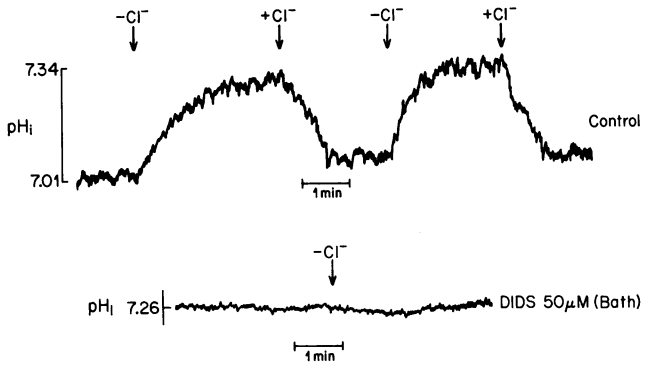

Figure 6. Effect of basolateral Cl^- removal and readdition on pH_i (HCO_3^- buffered solutions). *Upper trace,* Control: Tubules were perfused and bathed in Na^+ free solutions (solution E) for ~ 30 min. When basolateral Cl^- was decreased from 119 mM to zero, pH_i increased at a rate of 0.40±0.02 pH/min. Returning 119 mM Cl^- to the basolateral side resulted in the recovery of pH_i. The experiment could be repeated more than once on the same tubule. *Lower trace:* The tubules were exposed to 50 μM DIDS (bath) for 5 min; 50 μM DIDS (bath) prevented the elevation of pH_i induced by basolateral Cl^- removal. (Refer to Table V for summary of above results.)

in the absence of Na^+ (solution B, lumen, bath). When basolateral Cl^- was decreased from 144 mM to zero, pH_i increased at a rate of 0.45±0.01 pH/min, $n = 3$ (Fig. 7, Table V), which was not significantly different from the rate measured in the presence of HCO_3^- (25 mM). Since the cell buffer capacity is greater in the presence of HCO_3^-, the finding that the rate of change of pH_i induced by Cl^- removal was similar in HCO_3^- and Hepes-buffered solutions suggests that the flux of base equivalents on this exchanger is greater in HCO_3^--buffered solutions.

Discussion

The results of this study demonstrate that the basolateral membrane of the rabbit S_3 proximal tubule possesses a Na^+/H^+ antiporter, a Na^+/base cotransporter, and a Na^+-independent Cl^-/base exchanger. The results provide the first direct evidence for a basolateral Na^+/H^+ antiporter in a mammalian proximal tubule. In a previous study of the salamander proximal tubule, Boron and Boulpaep demonstrated the presence of both an apical and basolateral Na^+/H^+ antiporter (11).

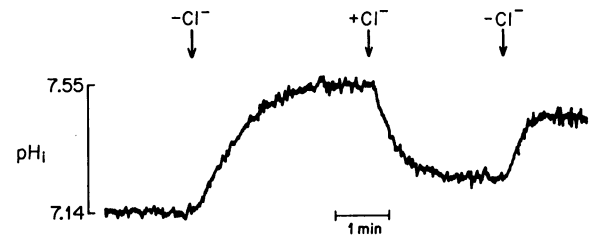

Figure 7. Effect of basolateral Cl^- removal and readdition on pH_i (Hepes-buffered solutions). Tubules were perfused and bathed in Na^+ free solutions (solution B) for ~ 30 min. After the removal of basolateral Cl^-, pH_i increased at a rate of 0.45±0.01 pH/min. Addition of 144 mM Cl^- to the basolateral side of the tubule caused pH_i to recover.

Therefore, the rabbit S_3 proximal tubule resembles this segment in that it too possesses an apical and basolateral Na^+/H^+ antiporter. The kinetics of the apical antiporter and basolateral antiporter in the S_3 segment were found to differ. The previously reported K_m for luminal Na^+ was 29 with a V_{max} of 0.47 pH/min (3). The K_m for basolateral Na^+ was 53 mM with a V_{max} of 0.75 pH/min. Although the exact steady state ion gradients are not known in vivo, it seems likely that both apical and basolateral antiporters would mediate Na^+ influx and H^+ efflux in vivo. Influx of Na^+ on the basolateral Na^+/H^+ antiporter would decrease the efficiency of net Na^+ and HCO_3^- absorption in this segment. Recent studies have demonstrated that the rate of HCO_3^- absorption in the S_3 segment is significantly less than the S_2 segment (12). The decreased rate of HCO_3^- absorption in the S_3 segment compared to more proximal nephron segments could be due in part to an increased rate of basolateral Na^+/H^+ exchange and/or a decreased rate of apical Na^+/H^+ exchange. Further studies are required to distinguish between these possibilities.

Previous basolateral vesicle studies (presumed to be derived primarily from cortical proximal straight tubules) and direct in vitro perfusion studies of rat proximal tubules and rabbit cortical proximal straight tubules have concluded that the mammalian proximal tubule lacks a basolateral Na^+/H^+ antiporter (4–7, 13). This finding is compatible with the requirement of the proximal tubule to absorb filtered bicarbonate efficiently. For vectorial transport of HCO_3^- to occur in the S_3 segment, base absorbed via the luminal Na^+/H^+ antiporter must exit the basolateral cell membrane. This would require a separate basolateral base efflux pathway or basolateral H^+ influx pathway. In the rat proximal convoluted tubule and the rabbit early proximal straight proximal tubule, most basolateral base efflux is mediated by a Na^+-coupled, Cl^--independent electrogenic SITS-sensitive base efflux mechanism, with less base efflux coupled to basolateral Cl^- influx (13, 14). Most of the Cl^--dependent base efflux in these tubule segments is mediated by Na^+-dependent Cl^-/base exchange with a small component of Na^+-independent Cl^-/base exchange. In the present study approximately 50% of the total rate of decrease in pH_i following a decrease in bath HCO_3^- in the S_3 tubule was Na^+ dependent. However, none of the Na^+-dependent change in pH_i was Cl^- dependent, suggesting that unlike the rat proximal convoluted tubule and the rabbit early proximal straight tubule, the S_3 tubule lacks a basolateral Na^+-dependent Cl^-/base exchanger. The basolateral Na^+-dependent induced change in intracellular pH had an amiloride-sensitive component (~ 50%) that most likely represents the basolateral Na^+/H^+ antiporter, and a stilbene-sensitive component (~ 50%) that most likely represents a basolateral Na^+/base cotransporter.

30% of the rate of change of pH_i following a decrease in bath HCO_3^- was found to be Cl^--dependent. Further experiments revealed that the basolateral membrane Cl^--dependent base transport pathway was stilbene inhibitable and Na^+-independent and therefore most likely represents a basolateral Na^+-independent Cl^- base exchanger. This exchanger evidently plays a more important role in basolateral base efflux in the S_3 segment than more proximal nephron segments. Since under in vivo conditions, it is likely that $[Cl^-]_o/[Cl^-]_i > [Base^-]_o/[Base^-]_i$, where $[Cl^-]_o$ and $[Base^-]_o$ refer to the basolateral concentration of these ions, the exchanger would

function not only as a base efflux pathway but would also provide a mechanism for basolateral Cl^- influx. The plasma membrane H^+ATPase in the S_3 segment has recently been shown to require intracellular Cl^- (3). Of interest, basolateral Cl^- induced changes in pH_i were observed in tubules bathed and perfused in Hepes-buffered solutions. Since the influx of base equivalents across the basolateral membrane induced by chloride removal was greater in the presence of HCO_3^- (25 mM), the results suggest that either the internal K_m of HCO_3^- for the basolateral Cl^-/base exchanger is low, or the Cl^-/base exchanger can transport OH^- at appreciable rates. Unlike the Cl^-/base exchanger, stilbene-sensitive Na^+-coupled base efflux was not observed in Hepes-buffered solutions. In a recent study of the rabbit proximal convoluted tubule (15), the Na^+/base cotransporter was found to function in Hepes-buffered solutions. A previous study in the salamander proximal tubule however failed to demonstrate an effect of SITS on basolateral Na^+-coupled base efflux in the nominal absence of external HCO_3^- (11). The difference between these studies can be explained if the rate of basolateral Na^+/base cotransport in the rabbit proximal convoluted tubule exceeds the rate of Na^+/base cotransport in the rabbit S_3 segment and the salamander proximal tubule. It is also possible that the metabolic CO_2 production rate is lower in the S_3 segment and the salamander proximal tubule than the rabbit proximal convoluted tubule.

In the present study, the basolateral H^+/base transport pathways were studied at resting intracellular pH ($\sim$ 6.9–7.1). It has previously been demonstrated that both the Na^+/H^+ antiporter and the Na^+-independent Cl^-/base exchanger are sensitive to changes in pH_i. The Na^+/H^+ antiporter is stimulated at low values of pH_i and inhibited when pH_i is elevated, whereas the Na^+-independent Cl^-/base exchanger is stimulated when pH_i is elevated and inhibited when pH_i is acidified (16–18). The pH sensitivity of the Na^+/base cotransporter has not been determined. It might be expected that the proportion of basolateral H^+/base transport via the different pathways described herein would be pH-sensitive and differ after acute acid and alkaline loads. Further studies addressing this issue are in progress.

Although the luminal Na^+/H^+ antiporter has been shown to contribute to HCO_3^- absorption and pH_i regulation in the proximal tubule, in brush border vesicles, Kinsella and Aronson et al. have demonstrated that the Na^+/H^+ antiporter can function in the Na^+/NH_4^+ exchange mode (19). It has previously been suggested that the luminal secretion of ammonia by the proximal straight tubule (S_2 and S_3 segments) would contribute to the countercurrent transport of ammonia in vivo (12, 20). Ammonia secretion has been demonstrated in vitro in both these segments and was shown to be dependent on an acidic luminal pH suggesting that NH_3 is secreted into the lumen rather than NH_4^+. The mechanism by which ammonia is transported into the cell across the basolateral side of the proximal straight tubule is less well understood. Given the high renal plasma membrane permeability to NH_3 (21) it is likely that a large component of basolateral ammonia flux results from NH_3 influx. However, NH_4^+ transport across the basolateral membrane may also contribute to basolateral ammonia influx. Previous studies have demonstrated that NH_4^+ can replace K^+ in supporting transepithelial transport mediated by basolateral Na^+/K^+-ATPase (22), the hydrolytic activity of the basolateral Na^+-K^+-ATPase (23), and that NH_4^+ can be transported intracellularly by this enzyme instead of K^+ (23). It remains to be determined whether the basolateral Na^+/H^+ antiporter in the S_3 tubule can function in the Na^+/NH_4^+ exchange mode. For NH_4^+ to enter the cells via the basolateral Na^+/H^+ antiporter, $[NH_4^+]_0/[NH_4^+]_i$ would have to be greater than $[Na^+]_0/[Na^+]_i$, where $[NH_4^+]_0$ and $[Na^+]_0$ refer to the basolateral concentration of these ions. The peritubular concentration of NH_4^+ in the outer medulla is likely higher than in the cortex as a result of lower peritubular blood flow. Interstitial NH_4^+ having entered the cell on the basolateral antiporter would be partially converted to NH_3 and H^+ providing NH_3 for luminal NH_3 secretion, and a source of protons for maintaining the luminal disequilibrium pH in the S_3 tubule (12). In addition, basolateral Na^+/NH_4^+ exchange (Na^+ efflux, NH_4^+ influx) would increase the efficiency of net Na^+ and HCO_3^- absorption in the S_3 segment. Further studies are required to determine whether NH_4^+ can be transported on the basolateral Na^+/H^+ antiporter in this tubule segment.

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