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Research Article

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Interaction of Chloride and Bicarbonate Transport across the Basolateral Membrane of Rabbit Proximal Straight Tubule

Evidence for Sodium Coupled Chloride/Bicarbonate Exchange

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Abstract

The existence of chloride/bicarbonate exchange across the basolateral membrane and its physiologic significance were examined in rabbit proximal tubules. S_2 segments of the proximal straight tubule were perfused in vitro and changes in intracellular pH (pHi) and chloride activity (a_{Cl}^i) were monitored by double-barreled microelectrodes. Total peritubular chloride replacement with gluconate increased pHi by 0.8, and this change was inhibited by a pretreatment with an anion transport inhibitor, SITS. Peritubular bicarbonate reduction increased a_{Cl}^i, and most of this increase was lost when ambient sodium was totally removed. The reduction rates of pHi induced by a peritubular bicarbonate reduction or sodium removal were attenuated by 20% by withdrawal of ambient chloride. SITS application to the bath in the control condition quickly increased pHi, but did not change a_{Cl}^i. However, the a_{Cl}^i slightly decreased in response to SITS when the basolateral bicarbonate efflux was increased by reducing peritubular bicarbonate concentration. It is concluded that sodium coupled chloride/bicarbonate exchange is present in parallel with sodium-bicarbonate cotransport in the basolateral membrane of the rabbit proximal tubule, and it contributes to the basolateral bicarbonate and chloride transport.

Introduction

There is growing evidence that most of bicarbonate transport across the basolateral membrane (BLM)[1] of the renal proximal tubule cells is mediated by rheogenic Na-HCO_3 cotransport (1–8). However, it has not been well examined whether there is a chloride dependent bicarbonate flux across BLM of the proximal tubule. It is interesting that a chloride coupled Na-HCO_3 cotransport mechanism (Na-HCO_3/Cl exchange) has been identified in the snail neuron (9), squid axon (10), barnacle muscle fiber (11), and cultured fibroblast (12). Guggino et al. demonstrated the existence of an Na-HCO_3/Cl exchange mechanism in BLM of *Necturus* proximal tubules, and he concluded that most of the basolateral chloride transport is mediated by this mechanism (13). In the previous study (8), we observed that the intracellular pH (pHi) of rabbit proximal straight tubules (PST) increased when chloride of the basolateral fluid was totally removed. This observation was consistent with a chloride/bicarbonate exchange mechanism. If this kind of exchange exists, it is conceivable that the exchange plays some role in both bicarbonate and chloride transport across BLM of the proximal tubule.

The aim of this study was to define whether chloride coupled bicarbonate transport exists in BLM of S_2 segment of rabbit PST. We monitored pHi and intracellular chloride activity (a_{Cl}^i) of PST cells when compositions of ambient fluid were rapidly changed. Our data demonstrated the existence of a sodium coupled chloride/bicarbonate exchange mechanism (Na-HCO_3/Cl exchange) across BLM.

Methods

Isolated segments of rabbit PST were dissected and perfused as previously described (7, 8, 14). Briefly, the proximal portions of PST (S_2 segment) were dissected in cooled (4°C) A solution (Table I) and were then transferred to the bath. To achieve a rapid bath fluid exchange, bath volume was reduced to 0.1 ml, and bath fluid was continuously changed at 5–10 ml/min. This resulted in a complete bath exchange within 5 s. The bath fluid was preheated to 38°C. The composition of artificial solutions used in this study is shown in Table I. These solutions were bubbled with 5% CO_2/95% O_2 gas, and their osmolarities were adjusted to 290 mosmol/kg H_2O by adding principal salts or water.

Basolateral membrane potential (Vbl), pHi, and a_{Cl}^i were measured by double-barreled micro pH and Cl^- electrodes. The method of making double-barreled ion-selective microelectrodes was described elsewhere (7, 8). Double-barreled borosilicate glass tubing of unequal diameter (fiber-containing; Hilgenberg, FRG) was pulled on a horizontal microelectrode puller. The larger barrel (1.5 mm OD, 0.87 mm ID) was used for the ion-selective electrode, and the smaller barrel (0.75 mm OD, 0.35 mm ID) was used for the reference electrode. The inside of the large barrel was made hydrophobic by silane vapor, and the tip portion was backfilled with either H^+ ligand (15), or Cl^- ligand (Corning 477913; Corning Glass Works, Corning, NY). The reference barrel of the pH electrode was filled with 0.5 M KCl and that of the Cl^- electrode with 0.5 M K_2SO_4 + 10 mM KCl solution. Ag-AgCl wires were inserted into the barrels and the electrode was mounted on a micromanipulator (Leitz, FRG).

The calibration method and general characteristics of pH electrodes used in this study were the same as those reported previously (7). General characteristics of the Cl^- electrode were recently reported from this laboratory (16). The electrical resistance of the Cl^--selective barrel ranged from 2 to 5 × 10^{10} ohm, and the response time (95% voltage change) was within 2 s. The slope of the response was 55–60 mV/10-fold change in Cl^- activity. The selectivity coefficients for other anions ($K_{Cl,anion}$) that were determined in the previous study (16) were,

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1. *Abbreviations used in this paper:* a_{Cl}^i, intracellular chloride activity; BLM, basolateral membrane; pHi, intracellular pH; PST, proximal straight tubules; RpHi, pHi reduction rates; SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid; Vbl, basolateral membrane potential.

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Table I. Composition of Artificial Solutions (mM)

	A	B	C	D	E	F	G	H	I	J	K	L
NaCl	125		125			125	145					145
$NaHCO_3$	25	25	5			25	5	25	5			5
Na gluconate		125										
Na isethionate								125	145			
Choline Cl				125	125							
Choline HCO_3				25	5							
Choline gluconate					20							
NMDG Cl										125		
NMDG HCO_3										25	25	
NMDG gluconate			20								125	
Mg SO_4	1	1	1	1	1	1	1	1	1	1	1	1
K_2HPO_4	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$CaCl_2$	1.5		1.5	1.5	1.5					1.5		1.5
Ca gluconate		6.7									6.7	
Ca acetate						1.5	1.5	1.5	1.5			
Glucose	2	2	2	2	2	2	2	2	2	2	2	2
Alanine	2	2	2	2	2	2	2	2	2	2	2	2

All solutions were bubbled with 5% CO_2/95% O_2 gas. NMDG, *N*-methyl-D-glucammonium.

K_{Cl,HCO_3}: 0.11, $K_{Cl,gluconate}$: 0.03, $K_{Cl,isethionate}$: 0.14. The a_{Cl}^i was calculated as

$$a_{Cl}^i = (Clb + K_{Cl,HCO_3} \times HCO_3b) \times 10^{VCl/S} \quad (1)$$

where Clb and HCO_3b are Cl^- and HCO_3^- activities of the bath fluid, respectively. *V*Cl is the differential output between the voltages recorded by the Cl^- selective and reference barrels and *S* is the slope of the voltage response of Cl^- electrode. It is possible that unmeasurable intracellular anions interact with the Cl^- electrode resulting in an overestimation of a_{Cl}^i values. In our previous study (16), we measured a_{Cl}^i of PST in the total absence of ambient Cl^- (gluconate replacement), and found 4.2 mM of unmeasurable anions. We did not substract this value from the measured a_{Cl}^i values in this study. Thus, all a_{Cl}^i values reported in this paper are not the corrected values. The electrical potentials (reference and ion-selective electrodes) were measured with an electrometer (FD223; W-P Instruments, New Haven, CT) and recorded simultaneously on a two-pen chart recorder (R-20, Rika Denki, Tokyo, Japan). A common bath reference electrode was a 3-M KCl flowing electrode in a direct contact with the exit of bath solution to make a liquid junction potential negligibly small.

The data are expressed as means±SE. For intracellular measurements, *n* equals the number of cells. Only one intracellular measurement was performed in each tubule. Student's *t* test was used to determine statistical significance.

Results

Effect of bath Cl^- elimination on pHi. We have previously reported that total bath Cl^- replacement with isethionate depolarized *V*bl by 4.5 mV and alkalinized pHi by 0.06 (8). It is possible that this effect is not due to Cl^- removal, but due to the isethionate addition. To deny this possibility, we performed studies in which bath Cl^- was replaced with another anion, gluconate. In the experiment shown in Fig. 1, total bath Cl^- replacement with gluconate (solutions A and B) depolarized *V*bl by 9.0 mV and alkalinized pHi by 1.0. A summary of eight such studies is given in Table II. These results are essentially the same as those in the previously reported isethionate study, indicating that the effect of bath Cl^- removal is due to the Cl^- elimination rather than to the addition of substituting anions.

Then, we examined whether this bath Cl^- removal related cell alkalinization is inhibitable with the anion transport inhibitor, 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (SITS). Fig. 2 shows one such experiment. SITS (0.5 mM) was added to the bath before a Cl^- removal study, and > 4 min was

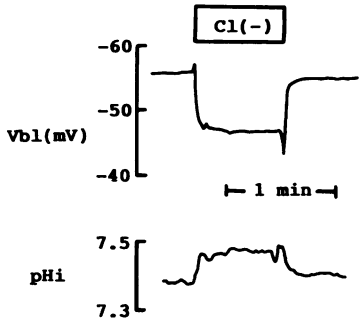

Figure 1. Effect of total bath chloride replacement with gluconate on *V*bl and pHi. A chloride free period is denoted by Cl (−).

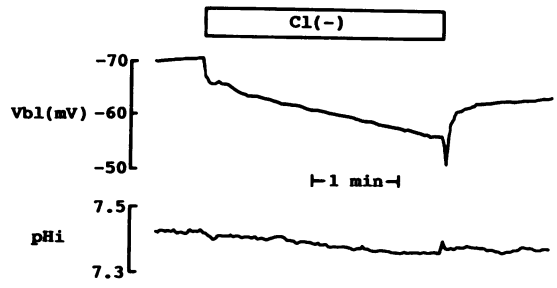

Figure 2. Effect of total bath chloride removal in the presence of SITS. SITS (0.5 mM) was added to the bath prior to the bath chloride removal, and it was present through the study.

allowed as an equilibration period. The addition of SITS to the bath caused a hyperpolarization of Vbl as previously reported by Biagi (2) and an alkalinization of pHi. The bath Cl^- removal performed in this condition caused an initial rapid depolarization of Vbl by 4 mV, which was followed by a gradual depolarization. The initial Vbl depolarization may be due to a circular current flow generated by a diffusion potential at the tight junction as considered in our previous study (8). The mechanism of the later depolarization is not clear at present. Bath Cl^- removal did not cause any change in pHi, demonstrating that the cell alkalinization induced by bath Cl^- removal was prevented with SITS pretreatment. A summary of five studies is also given in Table II. The initial Vbl depolarization was smaller in SITS-treated group than in normal group (1.6 vs. 8.1 mV; Table II). The reason for this difference is not clear, but it may be due to alterations of membrane resistance of the luminal and basolateral membranes, and the shunt pathway, or both.

Effect of reducing bath HCO_3^- on a_{Cl}^i. The results described above indicate the existence of an exchange mechanism of Cl^- and HCO_3^- (or related base) at BLM of S_2 cells. If this exchanger exists, it is anticipated that an alteration of bath HCO_3^- should change a_{Cl}^i. To examine this prediction, a_{Cl}^i was monitored and bath HCO_3^- was reduced from 25 to 5 mM (solutions A and C). Bath HCO_3^- was replaced by gluconate to maintain bath Cl^- concentration constant. As shown in Fig. 3, bath HCO_3^- reduction caused a large and sharp depolarization of Vbl and an increase in a_{Cl}^i. This Vbl depolarization could be due to the rheogenic Na-HCO_3 cotransport. As summarized in Table III, the results of eight studies showed that Vbl instantaneously depolarized by 34 mV and that a_{Cl}^i increased from 25.5 to 47.6 mM (the maximum peak value). These changes were all significant. This increase in a_{Cl}^i is consistent with the presence of Cl/HCO_3 exchange.

We next examined whether this a_{Cl}^i increase in response to bath HCO_3^- reduction can be observed in the nominal absence of ambient Na^+ (solutions D and E). Na^+ of luminal and bath fluids was substituted with choline. In a low HCO_3^- solution, 20 mM of HCO_3^- was replaced with gluconate to keep Cl^- constant. A representative experiment is shown in Fig. 4. In the absence of Na^+, bath HCO_3^- reduction from 25 to 5 mM caused a small depolarization of Vbl and a gradual but small increase in a_{Cl}^i. A summary of five studies (Table III) was that a_{Cl}^i increased from 30.5 to 34.2 mM (peak values, $P < 0.02$).

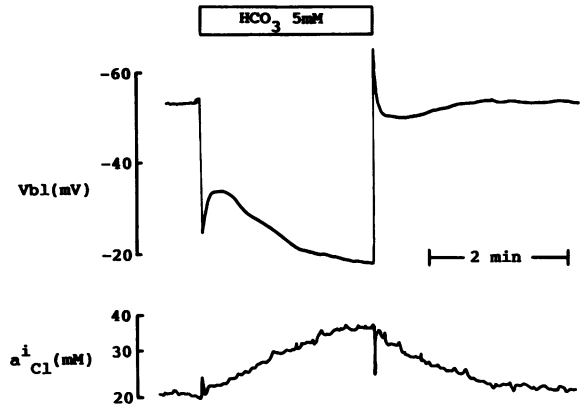

Figure 3. Effect of bath bicarbonate reduction from 25 to 5 mM on Vbl and a_{Cl}^i. Bath bicarbonate was substituted with gluconate to keep chloride constant.

This a_{Cl}^i increase was clearly smaller than that observed in the presence of Na^+ (Table III, and compare Figs. 3 and 4).

Reduction rates of pHi in the presence and absence of Cl^-. To confirm that Cl^- interacts with basolateral HCO_3^- transport, the reduction of pHi induced by either bath HCO_3^- reduction or bath Na^+ removal were compared in the presence and absence of ambient Cl^-. As shown in Fig. 5, bath HCO_3^- reduction from 25 to 5 mM (at constant pCO_2) was repeated twice; first, in the presence of Cl^- and second, in the total absence of Cl^- (solutions F–H, and I). Bath HCO_3^- reduction caused both a large and sharp depolarization of Vbl and a reduction in pHi in the presence and absence of ambient Cl^-. However, an inspection of Fig. 5 shows that pHi reduction induced by lowering bath HCO_3^- was slightly smaller in the absence of Cl^- than in the presence of Cl^-. To quantify this effect, the initial pHi reduction rate (RpHi; pH/min) was measured. RpHi was measured as $(pHi^0 - pHi^{10}) \times (1/6\ min)^{-1}$, where pHi^0 and pHi^{10} were the pHi values at the start and 10 s after the bath HCO_3^- reduction, respectively. Because our pH electrodes possessed some time delay in response, RpHi would be slightly underestimated. This underestimation did not affect our interpretation of the data because the study was a paired study, and the same electrode was used through each pair. The data are summarized in Fig. 6. Removal of ambient Cl^- slowed RpHi by 26% from 1.80±0.13 to 1.33±0.06 pH/

Table II. Effect of Bath Chloride Removal

	Vbl	pHi
	mV	
Control (n = 8)	−46.6±2.4	7.36±0.01
Cl(−)	−38.5±3.1*	7.44±0.02*
SITS (n = 5)	−72.6±0.9	7.45±0.04
Cl(−)	−71.0±1.4	7.45±0.04

* $P < 0.05$.
Cl(−) denotes the studies where total bath Cl^- was replaced with gluconate.
Vbl and pHi values of Cl(−) are the values when Vbl initially depolarized, and pHi reached the maximum after bath Cl^- removal, respectively.

Table III. Effect of Bath Bicarbonate Reduction on a_{Cl}^i

	Vbl	a_{Cl}^i
	mV	*mM*
Control (n = 8)	−46.5±2.6	25.5±2.3
HCO_3^- 5 mM	−12.6±4.8*	47.6±4.1*
Na+ free (n = 5)	−36.8±3.4	30.5±3.9
HCO_3^- 5 mM	−34.8±3.4	34.2±4.0*

* $P < 0.05$.
HCO_3^- 5 mM: bath HCO_3^- was reduced to 5 mM by replacing with 20 mM gluconate to keep bath Cl^- constant.
Vbl and a_{Cl}^i values are the values when Vbl initially depolarized, and a_{Cl}^i reached the maximum after bath HCO_3^- reduction, respectively.

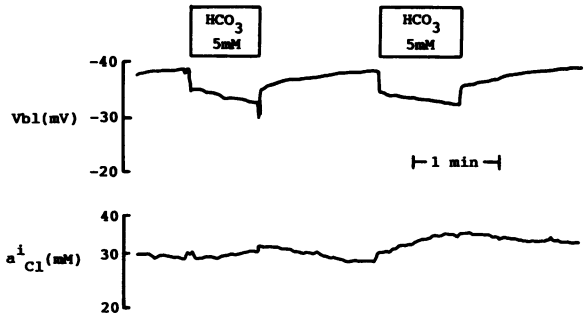

Figure 4. Effect of bath bicarbonate reduction in the absence of sodium. Experiment was performed in the total absence of ambient sodium (choline substitution), and bicarbonate was substituted with gluconate to keep chloride constant.

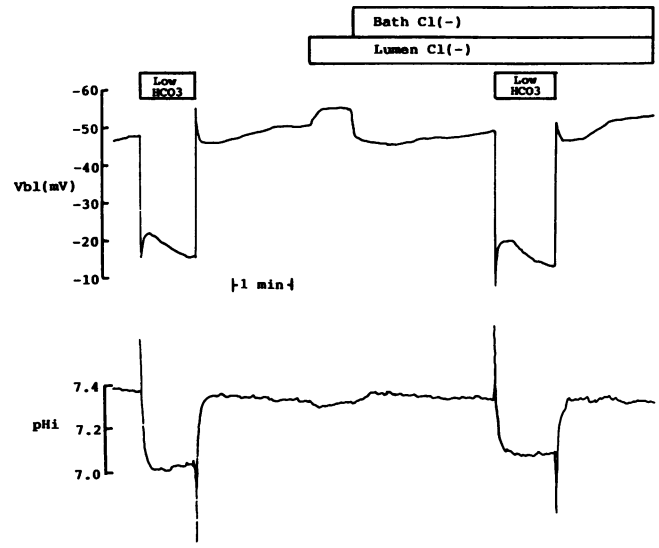

Figure 5. Effect of lowering bath bicarbonate from 25 to 5 mM in the presence and absence of ambient chloride. Bath bicarbonate reduction was performed twice. After the first bicarbonate reduction, both luminal and bath fluids were changed to chloride-free solution, and the second bicarbonate reduction was repeated.

min ($n = 6$, $P < 0.01$). These experiments were performed in the order where the control period always preceded the Cl^- free period. It was possible that the attenuation of the RpHi would be due to a time-dependent process rather than to a specific Cl^- effect. To deny this possibility, we performed the time control study where bath HCO_3^- reduction was performed twice in the presence of ambient Cl^-. Five such studies showed that RpHi did not change in the first and second trials (1.97±0.30 vs. 1.96±0.27 pH/min).

The above result indicated that Cl^- removal affects the HCO_3^- flux, which is induced by bath HCO_3^- reduction. To determine whether this Cl^--sensitive HCO_3^- flux is mediated by a Na^+-dependent process, the RpHi induced by bath Na^+ removal was also compared in the presence and absence of ambient Cl^- (solutions A, B, J, and K). A representative experiment is shown in Fig. 7. The result was almost identical to that observed when bath HCO_3^- was reduced (Fig. 5). The removal of ambient Cl^- slowed RpHi by 19% from 1.78±0.30 to 1.44±0.20 pH/min ($P < 0.05$, $n = 6$). A summary of the data (Fig. 6) demonstrates that ambient Cl^- removal slowed RpHi to a comparable degree when either bath HCO_3^- was reduced or Na^+ was removed indicating that Cl^- sensitive HCO_3^- flux is probably coupled to a Na-HCO_3 cotransport (Na-HCO_3/Cl exchange).[2]

Effect of SITS application to the bath on pHi and a_{Cl}^i. The results thus far indicate the existence of Na-HCO_3/Cl exchange in BLM (see Discussion). In order to determine the roles of this exchanger, SITS was rapidly applied to the bath. SITS has been shown to inhibit both the Na-HCO_3 cotransport and Na-HCO_3/Cl exchange in BLM of renal proximal tubules (1–13, 17). Our prediction was that if Na-HCO_3/Cl exchange contributes to the basolateral HCO_3^- and Cl^- fluxes in the steady-state condition, a sudden stop of the exchange will alter pHi and a_{Cl}^i.

Fig. 8 *A* shows an experiment where the effect on pHi of 0.5 mM SITS applied to the bath was examined. SITS application induced an abrupt increase in *V*bl by 3 mV, which was followed by a gradual hyperpolarization. pHi also quickly increased by 0.9 in response to SITS application, and it further increased gradually in the late phase. A summary of seven such experiments is given in Table IV. The above results indicate that S_2 cells in the control condition continuously transport HCO_3^- across BLM from the cell to the peritubular fluid via SITS inhibitable transport mechanisms, presumably the Na-HCO_3 cotransport. The abrupt *V*bl hyperpolarization is also consistent with the Na-HCO_3 cotransport mechanism because this transporter has been shown to be rheogenic (1–8).

Fig. 8 *B* is an example of the study where the effect of SITS on a_{Cl}^i was examined. As clearly shown in the figure, SITS application induced a quick hyperpolarization of *V*bl (same as in Fig. 8 *A*), but did not change a_{Cl}^i appreciably. A summary of this protocol is shown in Table IV. This result may indicate that basolateral Cl^- flux mediated by the Na-HCO_3/Cl exchange is not significant (see Discussion).

We further examined the possible contribution of a basolateral Na-HCO_3/Cl exchange in the condition where transcel-

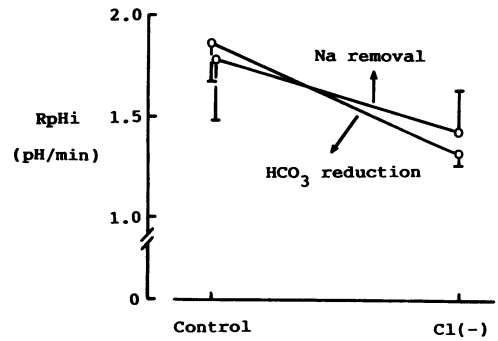

Figure 6. A summary of pHi reduction rates (RpHi) induced by bath bicarbonate reduction (25 to 5 mM) or bath sodium removal in the presence and absence of chloride.

2. We interpreted the effect of Cl^- removal on RpHi as indicating evidence for direct coupling of Cl^- and Na-HCO_3 cotransport. However, we have to note that other secondary effects of Cl^- removal such as alterations in cell functions and luminal H^+ secretion rates might induce the same result. For example, if Cl^- removal reduces metabolic acid production (by inhibiting Na^+ transport), then RpHi induced by HCO_3^- or Na^+ removal may be small in Cl^- free condition because cell acid load is less. At present we cannot neglect these possibilities.

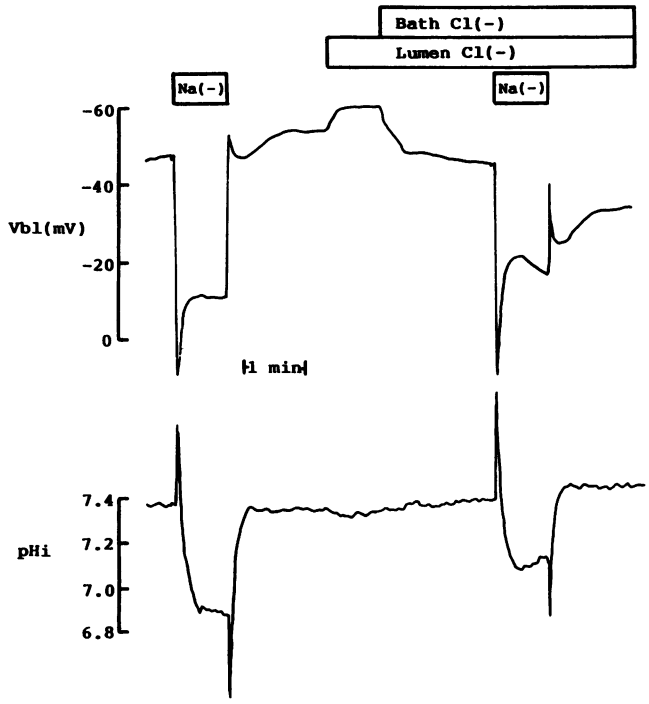

Figure 7. Effect of the total removal of bath sodium in the presence and absence of ambient chloride. Bath sodium removal was performed twice; first, in the presence of chloride, and second, in the total absence of chloride (lumen and bath).

lular Cl^- transport is stimulated. For this purpose, a high Cl^-, low HCO_3^-, and formate containing solution (solution L + formate 1 mM) was used as a luminal perfusate. Recently Schild et al. (18) clearly demonstrated that the addition of formate to a high Cl^-, low HCO_3^- luminal fluid increased transcellular Cl^- transport in the rabbit S_2 segment. As summarized in Table IV, SITS application to the tubule that was perfused with the formate containing luminal perfusate did not change a_{Cl}^i as well.

Finally, we examined the possibility that the Na-HCO_3/Cl exchange contributes as a HCO_3^- exit mechanism when basolateral HCO_3^- transport is stimulated. To stimulate basolateral HCO_3^- transport, low HCO_3^- solution (solution L) was used as a bath fluid. As shown in Fig. 9, an application of SITS in this condition caused a sharp and large hyperpolarization of *V*bl and decreased a_{Cl}^i slightly. A summary of the data (Table IV) shows that a_{Cl}^i decreased significantly by 3 mM in response to SITS application. *V*bl hyperpolarization induced by SITS addition was large in this protocol compared with that observed when the bath fluid was the control solution (15.2 vs. 3.3 mV, Table IV). The difference is in accord with our view that this *V*bl hyperpolarization is due to the rheogenic Na-HCO_3 cotransport. Interestingly, effects of SITS on *V*bl and a_{Cl}^i were reversible, indicating that SITS effects may not be due to a covalent binding of SITS to the transporter. The observed reduction in a_{Cl}^i in response to SITS addition indicates that the Na-HCO_3/Cl exchanger works as a HCO_3^- exit mechanism when basolateral HCO_3^- efflux is stimulated.

Discussion

Mechanisms of the interaction of Cl^- and HCO_3^- transport. Recently, HCO_3^- transport across BLM in renal proximal tubule cells has been shown to be mediated by a Na-HCO_3 cotransport in amphibian (1) and mammals (2–8, 19). This transport is known to be rheogenic due to a stoichiometry of HCO_3/Na > 1, thus, this transport is sensitive to voltage changes. In both previous (8) and this study (Fig. 1), we showed that total elimination of bath chloride alkalinized the cell, indicating the presence of counter-transport of chloride and bicarbonate. Several mechanisms are considered to account for this exchange. Fig. 10 shows five possible mechanisms (mechanisms 1–5). Mechanism 1 is a direct inhibition of the Na-HCO_3 cotransport by substituting anions for chloride. An inhibition of Na-HCO_3 cotransport will increase pHi. Mechanism 2 predicts that bath chloride removal depolarizes *V*bl by a circular current flow generated by a diffusion potential across the tight junction, and this *V*bl depolarization inhibits the Na-HCO_3 cotransport since this transport is voltage sensitive. Mechanism 3 is the existence of a chloride conductance in parallel with the Na-HCO_3 cotransport in BLM. If there is a chloride conductance in BLM, a bath chloride removal depolarizes *V*bl, and this depolarization inhibits Na-HCO_3 cotransport. Mechanism 4 is the parallel existence of the Na-HCO_3 cotransport and Cl/HCO_3 exchange. Mechanism 5 is the parallel existence of the Na-HCO_3 cotransport and Na-HCO_3/Cl exchange. The aim of this study was to dif-

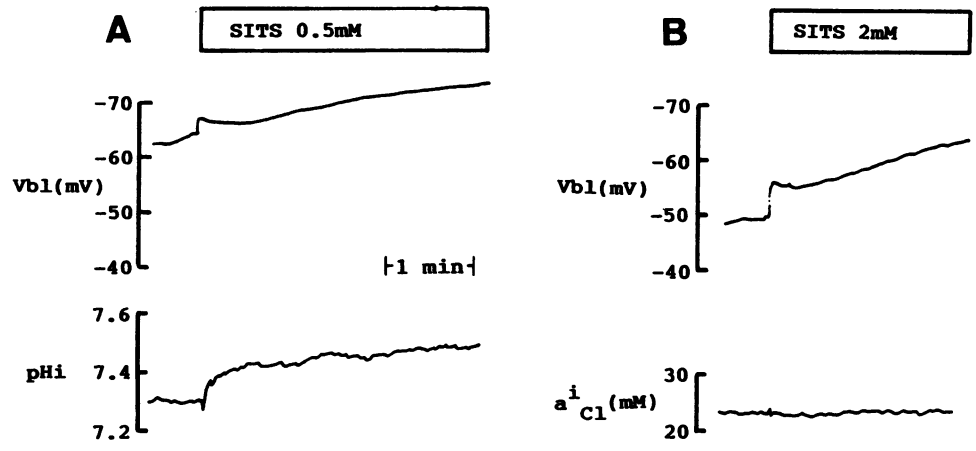

Figure 8. Effect of SITS application to the bath. (*A*) An application of SITS (0.5 mM) to the bath caused a Vbl hyperpolarization and an increase in a_{Cl}^i. (*B*) An application of SITS (2 mM) to the bath increased *V*bl, but did not change a_{Cl}^i.

Table IV. Effect of SITS on pHi and a_{Cl}^i

	Vbl	pHi	a_{Cl}^i
	mV		*mM*
Control (n = 7)	−53.7±3.7	7.29±0.03	
SITS (0.5 mM)	−56.1±3.8*	7.38±0.03*	
Control (n = 8)	−52.3±3.6		23.7±2.1
SITS (0.5 or 2 mM)**	−56.3±3.3*		24.8±2.2
Lumen high Cl^- (n = 4)‡	−49.8±3.3		30.0±1.2
SITS (1 mM)	−56.5±1.7*		29.9±1.2
Bath low HCO_3^- (n = 5)§	−21.0±2.9		44.5±4.8
SITS (1 mM)	−36.2±4.7*		41.6±4.6*

* $P < 0.05$.
Values in SITS period are as follows; Vbl value is when it initially hyperpolarized after bath SITS application, and pHi and a_{Cl}^i values are the values at 30 s after SITS application.
** 0.5 mM SITS was applied in four tubules and in the other four tubules 2 mM SITS was examined. The effects were essentially the same, and the results were gathered.
‡ Luminal perfusate was a low HCO_3^-, high Cl^- and 1 mM formate containing fluid.
§ Bath fluid was a low HCO_3^- and high Cl^- solution.

ferentiate these mechanisms, and to define the role of this exchange.

Mechanism 1 can not explain the results that a_{Cl}^i increased when bath bicarbonate was reduced (Fig. 3 and Table II). This result is in favor of the existence of chloride/bicarbonate exchange. Mechanism 2 also does not explain some of our data. The decreased RpHi by chloride removal is not consistent with this mechanism. This mechanism predicts that RpHi is the same in the presence and absence of ambient chloride. The result that a_{Cl}^i increased in response to bath bicarbonate reduction can not be explained by mechanism 2 as well.

Mechanism 3, on the other hand, can explain all our data. However, we have not been able to obtain the supportive evidence for a significant basolateral chloride conductance. In our recent study (20), *V*bl depolarization or hyperpolarization

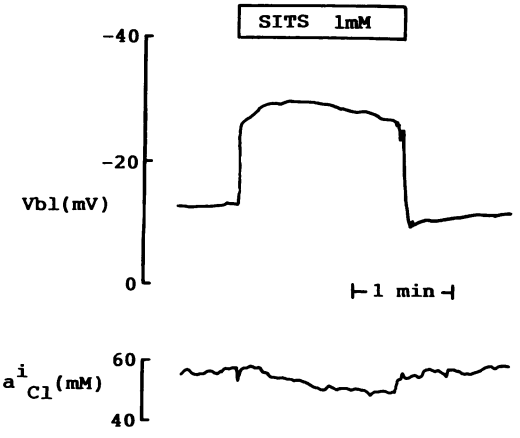

Figure 9. Effect of SITS application on a_{Cl}^i when bath bicarbonate was 5 mM. A low bicarbonate solution was used as a bath fluid and SITS (1 mM) was applied to the bath.

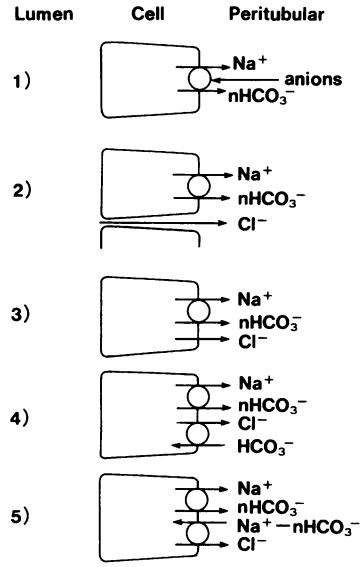

Figure 10. Possible mechanisms that explain the chloride and bicarbonate interaction across the basolateral membrane (see the text for the detail).

caused by a current injection into the tubular lumen through the perfusion pipette changed neither a_{Cl}^i or a_{Cl}^i changes in response to bath chloride removal. This result demonstrated that most of basolateral chloride transport in rabbit S_2 segment is electroneutral. In the present study (Fig. 1) *V*bl depolarized when bath chloride was removed, but the magnitude of the depolarization was small. This small *V*bl depolarization could be easily explained by a circular current flow generated by the chloride diffusion potential across the tight junction as considered previously (8). Thus, a small *V*bl depolarization is in favor of the absence of basolateral chloride conductance, although there is a possibility that Na-HCO_3/Cl exchange is electrogenic, and this electrogenicity obscures a chloride-induced depolarization. Our data do not necessarily neglect the existence of small chloride conductance. As a summary, we conclude that there is not a significant chloride conductance in the basolateral membrane.

Both mechanisms 4 and 5 predict the existence of exchange of chloride and bicarbonate in BLM: one is a pure Cl/HCO_3 exchange (mechanism 4), and the other is a sodium coupled Cl/HCO_3 exchange, possibly Na-HCO_3/Cl exchange (mechanism 5). The chloride/bicarbonate exchange would explain all our data. To differentiate whether the exchange is coupled to sodium, the response of a_{Cl}^i to bath bicarbonate reduction was examined in the absence of sodium (Fig. 4 and Table III). The result clearly demonstrated that the most of Cl/HCO_3 exchange is dependent on sodium (mechanism 5). The small increase in a_{Cl}^i in response to bath bicarbonate reduction in sodium free condition (Fig. 4) may indicate the existence of sodium independent Cl/HCO_3 exchange (mechanism 4), although the possibility exists that a small amount of residual intracellular sodium drives the Na-HCO_3/Cl exchange. The result that total chloride removal reduced the RpHi induced by bath sodium removal is also against mechanism 4. If a pure Cl/HCO_3 exchange exists in parallel to the Na-HCO_3 cotransport, the Cl/HCO_3 exchange will compensate pHi changes caused by a bath sodium withdrawal. Thus, total chloride removal is expected to exaggerate RpHi, which is induced by bath sodium removal (contrast to our observation). As a summary of the above discussion, we conclude that Na-HCO_3/Cl

exchange is a major mechanism for the interaction between chloride and bicarbonate in BLM of rabbit PST.

Chloride/bicarbonate exchange in BLM. We conclude that a sodium coupled Cl/HCO_3 exchange mechanism exists in BLM of rabbit PST (S_2 segment). This kind of transport systems has been examined in other cells. An electroneutral Na-HCO_3/Cl-(H) exchange mechanism has been demonstrated in invertebrate nonepithelial cells such as the snail neuron (9), barnacle muscle (11), and squid giant axon (10). This mechanism has been identified as an acid extrusion mechanism when the cells were acid loaded (Na-HCO_3 influx and Cl-(H) efflux). An apparently similar transport system was also identified in the cultured fibroblasts of hamster (12). In the epithelial cells, Guggino et al. recently identified the Na-HCO_3/Cl exchange in BLM of *Necturus* proximal tubule (13). They concluded that this mechanism is the major route for chloride movement across BLM in their preparation. Alpern reported the possible existence of sodium dependent Cl/HCO_3 exchange mechanism in BLM of rat PCT (17). Vesicle studies in the rat kidney (21), and not in the rabbit kidney (22) have presented evidence for Na-HCO_3/Cl exchange. The demonstration of Na-HCO_3/Cl exchange in the present study strengthens the view that this kind of transport mechanism prevails in many kinds of cells.

Although we identified the Na-HCO_3/Cl exchange mechanism in BLM of rabbit PST, we have to admit that we did not clarify the characteristics of this exchanger. We could not determine even the electrogenicity of this system. This difficulty was clearly due to the coexistence of the Na-HCO_3 cotransporter, which is electrogenic, and possesses a higher transport activity. Any change that is due to the Na-HCO_3/Cl exchange could be easily masked by a dominant Na-HCO_3 cotransporter. Further studies are needed to identify the stoichiometry, and the true substrate of this exchanger.

We observed a small increase in a_{Cl}^i in response to bath bicarbonate reduction in the absence of ambient sodium (Fig. 4) which is consistent with a sodium independent Cl/HCO_3 exchange. The sodium independent Cl/HCO_3 exchange mechanism has been shown in other epithelial preparations (19, 22, 23). In our sodium free condition the possibility exists that a small amount of sodium remains in the cell, and that this sodium drives the Na-HCO_3/Cl exchange. At any rate we can safely conclude that the contribution of sodium independent Cl/HCO_3 exchange is very small in the rabbit S_2 segment.

The role of Na-HCO_3/Cl exchange. If we assume that the stoichiometry of the Na-HCO_3/Cl exchange of rabbit PST is similar to that of cells from invertebrates, i.e., Na-2HCO_3/Cl (electroneutral), we can tell the direction of the exchanger by calculating the driving force. The energy for the electroneutral Na-2HCO_3/Cl exchange across BLM can be given as

$$\frac{\Delta G}{F} = \frac{RT}{F} In \frac{(a_{Na}^i)(a_{HCO_3}^i)^2(a_{Cl}^o)}{(a_{Na}^o)(a_{HCO_3}^o)^2(a_{Cl}^i)}, \quad (2)$$

where ΔG is the free energy change, and F, R, and T are their usual meanings. a_X^i and a_X^o are the ionic activity of X in the cell and bath, respectively. A negative value of G means the influx of sodium and bicarbonate, and efflux of chloride out of the cell. Putting the intracellular ionic activity values in the control condition determined in the previous (8) and present studies to Eq. 2 (a_{Na}^i 47 mM, $a_{HCO_3}^i$ 11 mM, a_{Cl}^i 21 mM), $\Delta G/F$ of -11 mV is obtained. Thus, if this stoichiometry is the case, this transporter is expected to work as a chloride extruding and Na-HCO_3 loading mechanism in the control condition. The latter component may easily be compensated by the dominant Na-HCO_3 cotransporter.

To determine the validity of this speculation, we applied SITS to the bath and immediate changes in pHi and a_{Cl}^i were monitored. Since we demonstrated the parallel existence of two SITS sensitive transporters, namely, Na-HCO_3 cotransport and Na-HCO_3/Cl exchange, if the sum of bicarbonate fluxes mediated by these transporters is bicarbonate efflux out of the cell, SITS application will alkalinize the cell. Similarly, if chloride flux mediated by the Na-HCO_3/Cl exchange is a chloride efflux, SITS application is expected to increase a_{Cl}^i. Our results (Fig. 8) clearly showed that addition of SITS increased pHi, but did not change a_{Cl}^i. This fact demonstrated that the net flux of bicarbonate across BLM is bicarbonate efflux that is mainly mediated by the Na-HCO_3 cotransport, and implied that the basolateral chloride flux mediated by the Na-HCO_3/Cl exchange is very small. The application of SITS did not change a_{Cl}^i even in the condition where the transcellular chloride flux was augmented by perfusing tubular lumen with a high chloride and formate containing solution, indicating that the Na-HCO_3/Cl exchange might not be the main route for basolateral chloride exit. Other mechanisms besides the Na-HCO_3/Cl exchange, such as K-Cl symport (20, 24, 25) may contribute to basolateral chloride transport. However, the lack of short term effect of SITS on a_{Cl}^i does not necessarily indicate the absence of basolateral chloride transport. Our present interpretation depends on the assumption that the apical chloride entry continues after basolateral transport is blocked. If this assumption is not true, then it is impossible to speculate transcellular chloride flux by monitoring the changes in a_{Cl}^i. It might be possible that chloride transport across the luminal and basolateral membrane is functionally coupled, thus, preventing an acute change in a_{Cl}^i. Such functional coupling of solute transport across the two membranes has been considered for sodium (26, 27). Further study is required to examine this possibility.

Our results indicate that Na-HCO_3/Cl exchange contributes to the basolateral bicarbonate efflux when the basolateral bicarbonate transport is stimulated. This was shown by the fact that the RpHi induced by bath bicarbonate reduction was slowed by removal of chloride (Fig. 5), and that a_{Cl}^i decreased when SITS was applied to the low bicarbonate bath solution (Fig. 9, and Table IV).

Speculation regarding the role of the Na-HCO_3/Cl exchange in physiologic conditions may be interesting. As discussed above, this exchange works as a bicarbonate exit mechanism as proximal bicarbonate reabsorption is stimulated. This stimulation of the basolateral bicarbonate efflux increases a_{Cl}^i, which in turn will inhibit the uptake of chloride across the luminal membrane. The net effect is that a stimulation of bicarbonate reabsorption inhibits transcellular chloride reabsorption. Thus, Na-HCO_3/Cl exchange may work to keep the total transport rates of anions (chloride + bicarbonate) across BLM constant in the proximal tubule. For example, hypercapnia has been known to induce chloruresis (28). Micropuncture studies in the rat observed that acute hypercapnia stimulates bicarbonate reabsorption and inhibits chloride reabsorption in PCT (29, 30). Cogan concluded that this inhibition of chloride absorption occurred on transcellular chloride absorption (30). The existence of Na-HCO_3/Cl exchange may fit with this interaction of chloride and bicarbonate. A stimulation of bicarbonate absorption by hypercapnia (exact

mechanisms not known) causes an increase in bicarbonate efflux across BLM. This increased efflux may be handled in part by the Na-HCO_3/Cl exchange, and this causes an increase in a_{Cl}^i. An increase in a_{Cl}^i will inhibit the luminal chloride uptake mechanism leading to the inhibition of the transcellular chloride reabsorption. Thus, it is conceivable that the Na-HCO_3/Cl exchange may play a role when basolateral bicarbonate and chloride fluxes are altered in several physiologic conditions.

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