KCl Co-Transport across the Basolateral Membrane of Rabbit Renal Proximal Straight Tubules

Sei Sasaki, Kenichi Ishibashi, Naoki Yoshiyama, and Tatsuo Shiigai
Department of Internal Medicine, Tokyo Medical and Dental University, Tokyo 113, Japan

Abstract

Mammalian renal proximal tubules reabsorb large amounts of chloride. Mechanisms of the transcellular chloride transport are poorly understood. To determine whether KCl co-transport exists in the basolateral membrane of mammalian renal proximal tubule, isolated rabbit proximal straight tubules (S_2 segment) were perfused in vitro, and intracellular activities of potassium and chloride (a_K^i, a_{Cl}^i) were measured by double-barreled ion-selective microelectrodes. a_{Cl}^i did not change when basolateral membrane voltage was altered by application of a direct current through perfusion pipette. a_{Cl}^i changes in response to bath chloride elimination were not affected by current application as well, indicating that the basolateral chloride transport is electroneutral. An increase in potassium concentration of the bath fluid from 5 to 20 mM reversibly increased a_{Cl}^i by 10 mM. This response of a_{Cl}^i to a change in the bath potassium concentration was also observed when luminal chloride was removed, or ambient sodium was totally removed. a_K^i significantly decreased by 5 mM when chloride was removed from the bath. These data demonstrate the existence of an electroneutral Na^+-independent KCl co-transport in the basolateral membrane of the rabbit proximal tubule. Calculated electrochemical driving force was favorable for the movement of KCl from the cell to the peritubular fluid.

Introduction

Solutes reabsorption in the proximal tubule of the mammalian kidney is divided into two phases. In the early proximal tubule, bicarbonate and organic solutes are preferentially reabsorbed with sodium leaving chloride concentration in the luminal fluid high. In the late proximal tubule, the principle mode of solutes transport is NaCl reabsorption (1). Although a substantial portion of chloride reabsorption occurs through the paracellular shunt pathway (2, 3), accumulating evidence has suggested the existence of active transcellular chloride transport in the proximal tubule. Chloride reabsorption was demonstrated in the absence of favorable electrochemical gradient (4–7), and chloride reabsorption was inhibited by application of anion transport inhibitors (6–8). In addition, intracellular chloride activity $(a_{Cl}^i)^1$ has been shown to be higher than the value predicted from a passive equilibrium (9, 10). These results are consistent with the presence of transcellular chloride transport.

Little is known about the mechanisms of chloride transport across the basolateral membrane (BLM) of proximal tubule cells. Electrophysiological studies (11–14) demonstrated that chloride conductance in BLM of proximal tubules was unmeasurably low. These results suggest that chloride is electroneutrally transported by coupled transport mechanisms such as Cl/HCO_3 exchange, $NaHCO_3/Cl$ (H) exchange, and KCl co-transport. Among these, KCl co-transport is promising because the sum of electrochemical driving forces of potassium and chloride seems to be favorable for a KCl exit. Recently KCl co-transport was demonstrated in another NaCl-reabsorbing epithelium, Necturus gallbladder (15).

This study was designed to determine whether KCl co-transport exists in BLM of rabbit proximal straight tubules (PST). Intracellular chloride and potassium activities were measured with ion-selective microelectrodes. The results demonstrated the presence of basolateral KCl symport.

Methods

Isolated segments of rabbit PST were dissected and perfused as previously described (14, 16, 17). Briefly, the proximal portions of PST (S_2 segment) were dissected in cooled (4°C) control 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.

BLM potential (Vbl) and a_{Cl}^i were measured by a double-barreled Cl^- electrode and intracellular potassium activity (a_K^i) by a K^+-selective electrode. The method of making double-barreled ion-selective microelectrodes was described elsewhere (14, 17). A double-barreled borosilicate glass tubing of unequal diameter (fiber containing, Hilgenberg GmbH, Malsfeld, FRG) was pulled on a horizontal microelectrode puller. The larger barrel was used for the ion-selective electrode, and the smaller barrel was used for the reference electrode. The inside of the large barrel was made hydrophobic by exposure to silane vapor for 90 s and baking at 150°C for 6 h, then the tip portion was backfilled with either Cl^- or K^+ ligands (Corning Medical, Medfield, MA). The rest of the barrel was filled with 0.5 M KCl. The reference barrel was filled with either 0.5 M K_2SO_4 (containing 10 mM KCl) or 1 M NaCl in Cl^-- and K^+-selective electrodes, respectively. Ag/AgCl wires were in-

Address correspondence and reprint requests to Dr. Sasaki, Dept. of Internal Medicine, Tokyo Medical and Dental University, Bunkyo-ku, Yushima, Tokyo 113, Japan.

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1. *Abbreviations used in this paper:* a_{Cl}^i, intracellular chloride activity; a_K^i, intracellular potassium activity; BLM, basolateral membrane; NMDG, *N*-methyl-D-glucammonium; PCT, proximal convoluted tubules; PST, proximal straight tubules; Vbl, basolateral membrane potential.

Table I. Composition of Artificial Solutions (Millimolars)

	Control	ONa	OCl	ONaCl	20 mM K	105 mM Na	105 mM Na-25 mM K	ONa-20 mM K
NaCl	125				110	105	105	
$NaHCO_3$	25		25		25	25	25	
$MgSO_4$	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
N-Methyl-D-glucammonium (NMDG) Cl		125						110
NMDG HCO_3		25		25				25
NMDG gluconate				125				
Na gluconate			125					
Choline Cl						20		
KCl					15		20	15
$CaCl_2$	1.5	1.5			1.5	1.5	1.5	1.5
Ca gluconate			6.7	6.7				
Glucose	2	2	2	2	2	2	2	2
Alanine	2	2	2	2	2	2	2	2

All solutions were bubbled with 5% CO_2/95% O_2 gas. When Cl^- was replaced with gluconate, extra calcium was added to keep ionized Ca^{2+} concentration constant.

serted into the barrels and the electrode was mounted on a micromanipulator (E. Leitz, Wetzlar, FRG).

After each intracellular activity measurement, ion-selective electrodes were calibrated at 38°C. Calibration solutions for Cl^- electrode were: 10 mM KCl + 90 mM K gluconate, 50 mM KCl + 50 mM K gluconate, and 100 mM KCl, and the mean slope of the responses was 55.7±0.7 mV/10-fold change in Cl^- activity (n = 20). The selectivity coefficients for other anions ($K_{Cl,anion}$) determined by using equimolar (100 mM) pure solutions (18) were: K_{Cl,HCO_3}, 0.11±0.01, and $K_{Cl,gluconate}$, 0.03±0.002. The a_{Cl}^i was calculated as $a_{Cl}^i = (Clb + K_{Cl,HCO_3} \times HCO_3b) \times 10\ V/S$, where Clb and HCO_3b are Cl^- and HCO_3^- activities of the bath fluid, and V is the differential output between the voltages recorded by the Cl^--selective and reference barrels, and S is the slope of the response of the Cl^- electrode. Calibration solutions for the K^+ electrode were: 160 mM KCl, 80 mM KCl + 80 mM NaCl, 40 mM KCl + 120 mM NaCl, and 5 mM KCl + 155 mM NaCl. These solutions had the same ionic strength (0.16 M) and approximated those of control solution and possibly of the cell cytoplasma, assuming that an increase in intracellular K^+ concentration is accompanied by a fall in intracellular Na^+ concentration. a_K^i was determined from the relationship of the voltage (an ion-selective minus a reference electrode) and K^+ activities. Electrical resistances of the K^+ and Cl^- electrodes were 0.1–1.0 and 0.5–2.0 × 10^{11} ohm, respectively, and the response time (95% voltage change) was < 2 s in both electrodes. Criteria for an acceptable cell impalement was the same as previously reported (14, 17). The electrical potentials were measured with an electrometer (FD223; W-P Instruments, Inc., New Haven, CT) and recorded on a two-pen chart recorder (R-20; Rikadenki Co., Ltd., Tokyo). A common bath reference electrode was a 3-M KCl flowing electrode in direct contact with the exit of bath solution to make a liquid junction potential negligibly small. In two series of experiments, a current was injected into the tubular lumen through the perfusion pipette via an Ag/AgCl wire. The current was sent by a constant current generator (IP-600; Physio-Tech Co., Ltd., Tokyo), and the current loop was closed by putting another Ag/AgCl wire in the bath.

The data are expressed as means±SE. Unless otherwise stated, n equals the number of cells. Only one intracellular measurement was performed in one tubule. Therefore the number of cells was equal to the number of tubules. Student's t test was used to determine statistical significance.

Results

Effect of current application on a_{Cl}^i. If Cl^- transport across the basolateral and/or luminal membrane is electrogenic, then an application of electrical currents that pass through the cell (from the lumen to the bath or vice versa) will alter a_{Cl}^i. A very rare exception to this prediction may be that Cl^- transports of both luminal and basolateral membranes are electrogenic, and the amounts of transported Cl^- are equal at both membranes when the current is applied. In this case, current application after removing Cl^- from one side (i.e., luminal or bath fluid) will alter a_{Cl}^i. After these predictions, direct electrical currents were injected from the perfusion pipette into the lumen. Fig. 1 is redrawings of representative experiments. As shown in Fig. 1 *A*, applications of +1,300- and −1,300-nA currents caused 12-mV depolarization and 9-mV hyperpolarization of Vbl, respectively, but they did not alter a_{Cl}^i appreciably. Current application of ±1,300 nA in the absence of luminal Cl^- (Fig. 1 *B*) caused large depolarization and hyperpolarization of Vbl, but did not change a_{Cl}^i appreciably. A summary of such studies is given in Table II, confirming that the application of electrical currents caused little or no effect on a_{Cl}^i. Applications of positive currents in normal condition decreased a_{Cl}^i very slightly from 29.8±2.1 to 29.0±2.0 mM ($P < 0.05$, Table II). The orientation of this small change in a_{Cl}^i, however, is contradictory to the movement of Cl^- predicted from the Vbl voltage change.

Results thus far indicate that Cl^- transport across the BLM as well as the luminal membrane is not electrogenic. However, it is possible that current application activates electroneutral Cl^- transport mechanisms, and that this newly activated Cl^- transport compensates electrogenic Cl^- transport. To examine this possibility, studies as shown in Fig. 2 were performed. Elimination and addition of bath Cl^- were performed twice in each tubule in the presence and absence of current application. If current application activated new Cl^- transporting mecha-

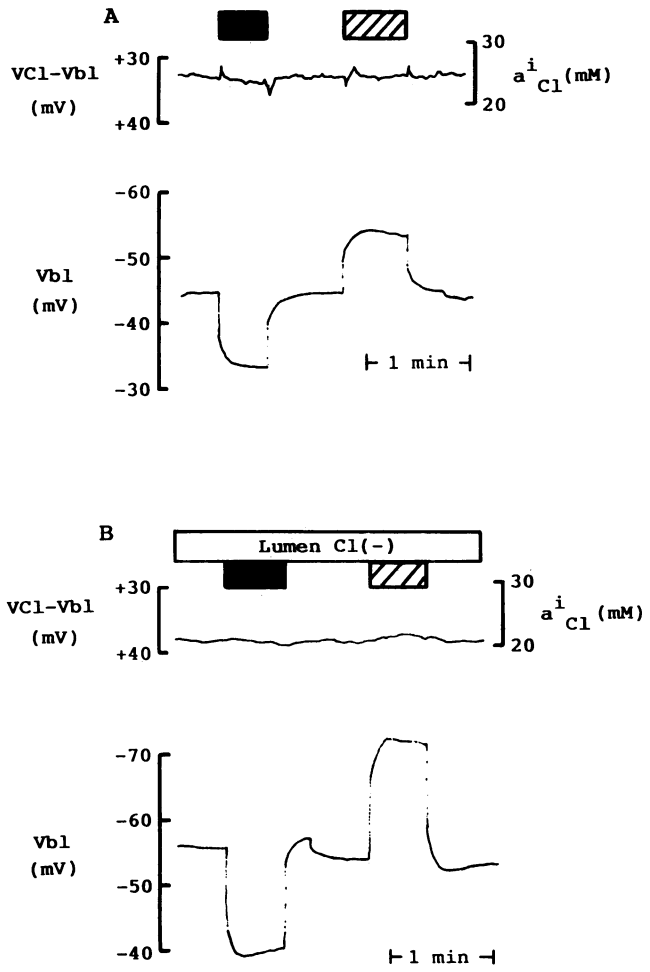

Figure 1. Effect of electrical current application on a_{Cl}^i. Electrical current was injected into the tubular lumen through perfusion pipette. Black and hatched bars denote applications of +1,300- and −1,300-nA currents, respectively. In *B*, currents were applied after luminal chloride was totally removed.

Table II. Effect of Current Application on a_{Cl}^i

	Vbl	a_{Cl}^i
	mV	*mM*
Normal		
Control (n = 10 in 10 cells)	−44.0±2.3	29.8±2.1
Current (+1,020±44 nA)	−30.7±2.2*	29.0±2.0*
Control (n = 9 in nine cells)	−45.6±2.7	29.5±2.3
Current (−1,022±49 nA)	−54.1±3.3*	30.1±2.5
Lumen Cl free		
Control (n = 6 in three cells)	−54.0±1.8	21.7±1.4
Current (+1,167±56 nA)	−37.7±1.7*	21.4±1.2
Control (n = 7 in three cells)	−51.0±2.0	21.9±2.5
Current (−1,186±51 nA)	−65.0±4.7*	22.3±2.6

Current was applied into the tubular lumen through perfusion pipette.
* $P < 0.05$ between control and current applied conditions.

nisms, a time course of a_{Cl}^i change would be modified. Bath Cl^- elimination (Fig. 2) decreased a_{Cl}^i, indicating that the BLM possessed Cl^- transport ability. Removal of bath Cl^- caused a depolarization of Vbl by 5 mV. No spike shape depolarization was observed, confirming previous results (11–14) that Cl^- conductance across BLM is small. As we considered previously (14), Vbl depolarization induced by elimination of bath Cl^- could be due to a circular current generated by biionic diffusion potential across the paracellular pathway.

Fig. 2 shows that a_{Cl}^i changes in response to bath Cl^- elimination were quite comparable in the presence and absence of current. To quantify these data, recovery phase of a_{Cl}^i after bath Cl^- re-addition was analyzed. As shown in Fig. 3, final a_{Cl}^i–a_{Cl}^i (time) are plotted against time in semilog scale, where final a_{Cl}^i and a_{Cl}^i (time) are a_{Cl}^i values when a_{Cl}^i recovered completely and those at time second after bath Cl^- re-addition, respectively. Data points usually lined along the straight line until 60 s, and the slope of a linear regression line (k) was calculated. Such paired study was performed in five tubules, and the mean k was −0.0138±0.0019, and −0.0132±0.0011 in the absence and presence of current, respectively (Fig. 3). These two values were not significantly different. This result indicates (*a*) current application does not activate new basolateral Cl^- transport, and (*b*) basolateral Cl^- transport is not modified by electrical current (i.e., electroneutral).

Effect of bath K^+ on a_{Cl}^i. If KCl co-transport exists at BLM, then alteration of bath K^+ will change a_{Cl}^i. This prediction was examined in the studies shown in Fig. 4. An increase in bath K^+ (Na^+ substitution) from 5 to 20 mM depolarized Vbl by 16 mV and increased a_{Cl}^i from 20.5 to 29.2 mM. These effects were almost identical in the absence of luminal Cl^-. Summary of five studies are given in Table III. These results indicate that a_{Cl}^i changes induced by bath K^+ elevation may be due to an inhibition of basolateral Cl^- transport, and not due to luminal Cl^- transport. Importantly, although K^+ elevation in the bath fluid caused Vbl depolarization, this depolarization may not account for a_{Cl}^i changes, because Vbl depolarization induced by current application did not alter a_{Cl}^i (Fig. 1, Table II).

In the above studies bath K^+ was elevated in exchange for Na^+ (Na^+ reduced from 150 to 135 mM). The results would possibly be explained by bath Na^+ reduction instead of bath K^+ elevation, since a $NaHCO_3$/Cl exchange mechanism has been demonstrated in the BLM of the Necturus proximal tubule (19). To rule out this possibility six experiments were performed where bath Na^+ was maintained constant through the study and bath K^+ was elevated to 25 mM (solution, 105 mM Na to 105 mM Na/25 mM K). a_{Cl}^i again increased in response to bath K^+ elevation in this experimental condition (Table III). These results clearly indicate that the basolateral Cl^- transport is modulated by bath K^+. Effects of increasing bath K^+ on a_{Cl}^i were also examined in the total absence of ambient Na^+. An increase in a_{Cl}^i in response to bath K^+ elevation was also observed in this situation (Table III), indicating that KCl transport was independent of Na^+. a_{Cl}^i values in the Na^+-free condition were slightly higher than that observed in control condition (Table III). The reason for this higher a_{Cl}^i value in a Na^+-free situation is not clear at present.

Effect of bath Cl^- on a_K^i. If there is KCl co-transport at BLM, one may predict that a_K^i will be altered when bath Cl^- is changed. Fig. 5 shows a study which examined this issue. a_K^i slightly decreased in response to removal of bath Cl^- (gluconate replacement). This change was reversible. Six studies re-

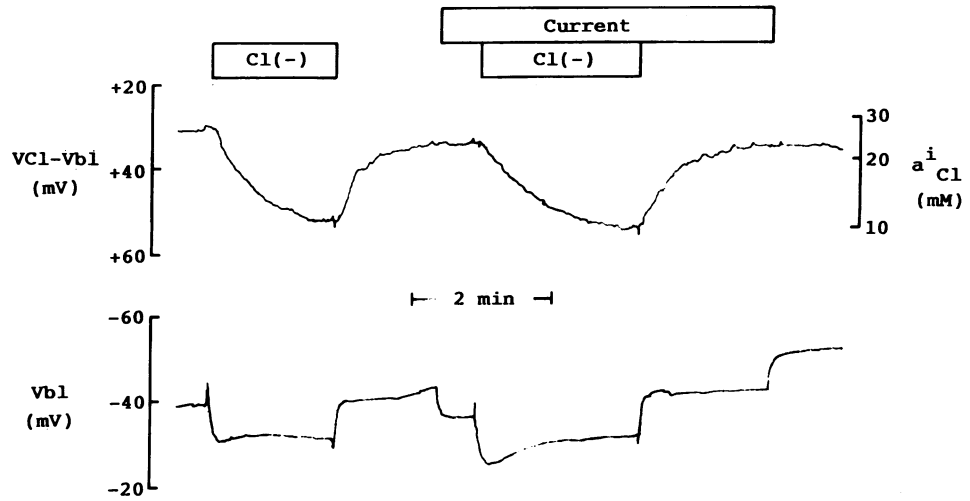

Figure 2. Effect of currents application on the response of a_{Cl}^i to bath Cl^- removal. Bath Cl^- removal (gluconate substitution) was performed twice, in the absence and presence of current application.

vealed that elimination of bath Cl^- decreased a_K^i from 60.0±3.2 to 54.6±3.4 mM ($P < 0.05$), and decreased Vbl slightly from −60.6±3.6 to −58.9±2.4 mV (although the difference was not significant).

Discussion

NaCl is the main salt reabsorbed along the late proximal tubule of mammalian kidney. The mechanisms by which chloride is absorbed from high chloride and low bicarbonate luminal fluid in late proximal tubule are still poorly understood (1). Recent evidence suggests that a fraction of chloride absorbed passes through the cell in proximal convoluted tubules (PCT) and PST. In the rat and rabbit PCT, chloride reabsorption was demonstrated in the absence of a favorable electrochemical gradient (4–6), and it was inhibited by anion transport inhibitors such as furosemide and 4-acetamido-4′-isothyocyanostilbene-2,2′-disulfonate (6, 8). In the rabbit S_2 segment, which is comprised of late PCT and early PST, Schild et al. (7) showed that addition of formate in the ambient fluid increased NaCl reabsorption without changing transepithelial voltage. NaCl reabsorption stimulated by formate was inhibited by ouabain and luminal addition of 4,4′-diisothiocyanostilbene-2,2′-disulfonate. They concluded that chloride enters the cell across the luminal membrane by electroneutral Cl/formate exchange mechanism (7). Demonstration of a_{Cl}^i value higher than that predicted from a passive equilibrium in rat PCT (9) also supports the existence of transcellular chloride transport. We have recently reported that a_{Cl}^i is above an equilibrium value in the rabbit S_2 segment as well (10).

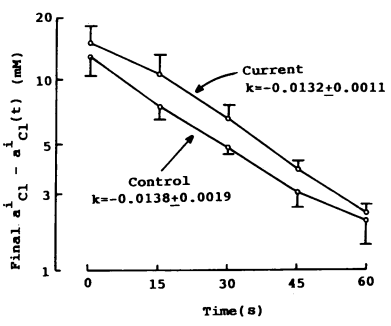

Figure 3. Effect of currents application on the recovery of a_{Cl}^i after re-addition of Cl^-. Final a_{Cl}^i and a_{Cl}^i (time) are a_{Cl}^i values observed when a_{Cl}^i completely recovered and reached a steady state, and those observed at time second after bath Cl^- re-addition, respectively.

It is clear that transcellular Cl absorption requires an exit of intracellular chloride across BLM. Intracellular potential measurements consistently demonstrated little change in Vbl in response to alterations in peritubular chloride, suggesting that there is little or no chloride conductance (11–14). Present results (Fig. 2) also confirmed these observations. The electroneutral chloride transport mechanisms, therefore, are needed to maintain transcellular chloride reabsorption.

As a candidate for such an electroneutral transport mechanism, KCl co-transport was examined in this study. Three lines of evidence demonstrated the electroneutral KCl cotransport. First, voltage changes in luminal and basolateral membranes induced by current application did not alter a_{Cl}^i (Fig. 1 *A*). This independence of a_{Cl}^i from voltages changes strongly suggests that chloride transports at both luminal and basolateral membrane are mediated by electroneutral mechanisms, although a rare possibility that current-induced chloride fluxes at both membranes are counterbalanced could not be excluded. To rule out this possibility current application was performed in the absence of luminal chloride. Independency of a_{Cl}^i was still observed in the absence of luminal chloride, neglecting this rare possibility (Fig. 1 *B*). These results are also important in interpreting the study where bath K^+ was increased (Fig. 4 and Table III). An increase in bath K^+ depolarized Vbl and increased a_{Cl}^i. Because Vbl depolarization did not alter a_{Cl}^i, this result can be taken as strong evidence for KCl symport. Second, increasing bath K^+ reversibly increased a_{Cl}^i (Fig. 4 and Table III). This response of a_{Cl}^i was still present in the experimental condition where bath Na^+ was kept constant or ambient Na^+ was totally eliminated (Table III). These results preclude the possibilities that the response of a_{Cl}^i is mediated by $NaHCO_3$/Cl exchange (19) or Na-K-2 Cl cotransport. Third, the elimination of bath Cl^- reversibly decreased a_K^i by 5.4 mM (Fig. 5). The magnitude of a_K^i change was small compared with the change in a_{Cl}^i observed when bath potassium was increased. One possible explanation may be that there are many potassium transporting mechanisms beside KCl co-transport, and a_K^i change induced by bath chloride elimination is compensated by other potassium transporting mechanisms. Taken together these three kinds of results

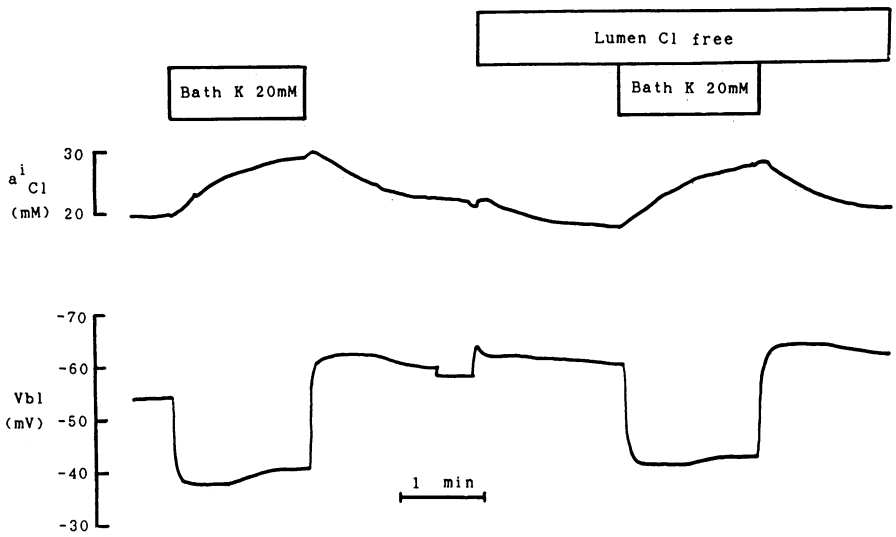

Figure 4. Effect of increasing bath K^+ on a_{Cl}^i. Bath K^+ was increased from 5 to 20 mM (Na^+ substitution). Bath K^+ was increased twice, in the presence and absence of luminal Cl^-.

demonstrate the existence of Na^+-independent KCl symport in BLM of rabbit S_2 segment.

Electroneutral KCl transport has been demonstrated in red blood cells of sheep, duck, and Amphiuma when they are imposed to osmotic cell swelling (20–22). KCl co-transport was also demonstrated in sheep red blood cells after treatment with *N*-ethylmaleimide (23). Reuss clearly demonstrated this transport mechanism in BLM of Necturus gallbladder (15). In renal epithelial cells, KCl co-transport has been proposed as a mechanism of chloride exit across BLM of rabbit cortical thick ascending limb (24) and early distal tubule of Amphiuma (25). A preliminary report by Eveloff and Warnock (26) indicated the existence of this transport mechanism in BLM vesicles obtained from the rabbit kidney cortex.

Because we measured a_{Cl}^i and a_K^i values in this study, it is possible to calculate an electrochemical driving force of the KCl symport. Sum of chemical driving forces (millivolts) of Cl^- and K^+ are 61 log 18/90 + 61 log 60/3.8 = +28.8 mV (a_{Cl}^i is corrected for undetermined interfering anions of 4.2 mM [10]). This calculation clearly indicates that KCl co-transport can serve as an exit mechanism of Cl^- and K^+ in normal condition. At present it is not known whether this KCl symport is the sole mechanism for basolateral chloride transport, or whether other mechanisms such as Cl/HCO_3 exchange, $NaHCO_3/Cl$ exchange, and small chloride conductance also exist in this membrane. Future studies are also needed to clarify the exact nature of this KCl co-transport. The possibilities that this co-transport is mediated by parallel K/H and Cl/HCO_3 exchangers (22) can not be eliminated by the experiments presented here.

Evaluation of the physiological significance of this co-transport also needs future studies, because it is not certain how much chloride passes through the cell when NaCl is reabsorbed in the rabbit S_2 segment. Previous studies including ours (3, 10) indicate that only a small fraction of transepithelial

Table III. Effects of Increasing Bath K on Vbl and a_{Cl}^i

	Vbl	a_{Cl}^i
	mV	*mM*
1 Control	−49.9±1.4	21.2±1.3
20 mM K (n = 5)	−32.6±1.8*	32.9±1.8*
2 Lumen Cl free	−51.4±2.5	19.5±1.8
20 mM K (n = 5)	−34.6±2.3*	29.7±2.7*
3 105 mM Na	−45.0±2.9	27.7±2.1
25 mM K (n = 5)	−31.0±2.3*	35.7±2.4*
4 Na free	−33.8±4.3	33.8±1.9
20 mM K (n = 4)	−24.0±2.6*	42.3±1.6*

In groups 1 and 2, bath K^+ was elevated in exchange for Na^+. In group 3 bath Na^+ was kept constant at 130 mM through the study and bath K^+ was increased to 25 mM. In group 4 total Na^+ was replaced with NMDG in luminal and bath fluids, and bath K^+ was increased to 20 mM.

* $P < 0.05$ between normal K and high K conditions.

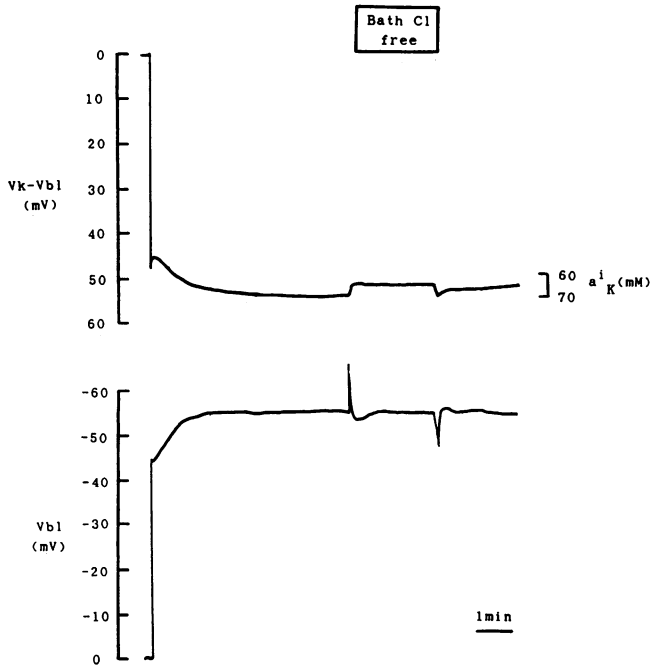

Figure 5. Effect of eliminating bath Cl^- on a_K^i. Bath Cl^- was replaced with gluconate.

chloride flux passes through the cell in the rabbit PST. However, these studies were performed in the absence of formate, therefore it is quite possible that a fraction of transcellular chloride flux increases when formate is included in the ambient fluid. At any rate, we can safely conclude that electroneutral KCl co-transport exists in BLM of the rabbit proximal tubule (S_2 segment).

Acknowledgments

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