Mechanism of Apical and Basolateral Na^+-independent Cl^-/Base Exchange in the Rabbit Superficial Proximal Straight Tubule

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Abstract

The present study was undertaken to determine the magnitude and mechanism of base transport via the apical and basolateral Na^+-independent Cl^-/base exchangers in rabbit isolated perfused superficial S_2 proximal tubules. The results demonstrate that there is an apical Na^+-independent Cl^-/base exchanger on both membranes. HCO_3^- fails to stimulate apical Cl^-/base exchange in contrast to the basolateral exchanger. Inhibition of endogenous HCO_3^- production does not alter the rate of apical Cl^-/base exchange in Hepes-buffered solutions. Both exchangers are inhibited by H_2DIDS and furosemide; however, the basolateral anion exchanger is more sensitive to these inhibitors. The results indicate that the apical and basolateral Cl^-/base exchangers differ in their transport properties and are able to transport base equivalents in the absence of formate. The formate concentration in rabbit arterial serum is ~ 6 μM and in vitro tubule formate production is < 0.6 pmol/min per mm. Formate in the micromolar range stimulates J_v in a dose-dependent manner in the absence of a transepithelial Na^+ and Cl^- gradient and without a measurable effect on Cl^--induced equivalent base flux. Apical formic acid recycling cannot be an important component of any cell model, which accounts for formic acid stimulation of transcellular NaCl transport in the rabbit superficial S_2 proximal tubule. We propose that transcellular NaCl transport in this nephron segment is mediated by an apical Na^+/H^+ exchanger in parallel with a Cl^-/OH^- exchanger and that the secreted H^+ and OH^- ions form H_2O in the tubule lumen. (*J. Clin. Invest.* 1994. 94:173–183.) Key words: Na^+/H^+ exchange • Cl^-/base exchange • pH • fluorescence • formate

Introduction

In the mammalian proximal tubule, coupled apical Na^+/H^+ and Na^+-independent Cl^-/base exchange are believed to mediate transcellular NaCl transport (1). Although the role of apical Na^+/H^+ exchange in mediating transcellular $NaHCO_3$ and NaCl absorption is well established, conflicting results regarding the presence or absence of apical Cl^-/base exchange have been obtained. Warnock and Yee (2) first demonstrated an electroneutral Cl^-/base exchanger in brush border vesicles. Subsequent vesicle studies either confirmed (3–5) or failed to obtain (6–9) similar results. In contrast to the apical membrane, the presence of Na^+-independent Cl^-/base exchange in basolateral membrane vesicles (10–12) and whole tubule studies (13–17) is less controversial.

As in brush border vesicle studies, there is conflicting evidence for an apical Cl^-/base exchanger in whole tubule experiments. Schwartz (18) failed to detect apical Cl^-/base exchange by measuring the effect of luminal stilbenes on luminal pH in rabbit proximal tubules. In contrast, Yoshitomi and Hoshi (19) demonstrated apical Cl^-/base exchange in Triturus proximal tubules by measuring intracellular Cl^- activity. An inhibitory effect of luminal stilbenes on electroneutral proximal tubule NaCl reabsorption has been observed in several studies, suggesting a role for apical Cl^-/base exchange (20–22). Against a role for apical Cl^-/HCO_3^- (but not incompatible with Cl^-/OH^-) exchange was the finding that the carbonic anhydrase inhibitor methazolamide failed to alter J_{Cl} in the rat proximal convoluted tubule (23).

In 1983, Guggino et al. (24) first suggested that apical Cl^-/organic anion exchange may mediate transcellular Cl^- absorption in the proximal tubule. Karniski and Aronson (25) and subsequently Chen et al. (5) provided evidence for a Cl^-/formate exchanger in rabbit renal cortical brush border membrane vesicles. The authors hypothesized that coupled apical Na^+/H^+ and Cl^-/formate exchange with apical formic acid recycling mediated apical NaCl uptake. Alpern (26) and Baum (27), using intracellular pH (pH_i)[1] measurements, detected minimal apical Cl^-/base exchange activity in the absence of exogenous formate. However, Sasaki and Yoshiyama (14) failed to find an effect of luminal 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid on rabbit proximal straight tubule intracellular Cl^- activity even in the presence of 1 mM luminal formate. Schild et al. (22) first showed that formate stimulated transepithelial NaCl absorption in the rabbit proximal tubule. These authors demonstrated that 0.5 mM formate in the perfusate and bathing solutions stimulated volume absorption when the lumen was perfused with 5 mM HCO_3^-, but not in the presence of 25 mM HCO_3^-. An unfavorable lumen-to-cell pH gradient was postulated to be present with 25 mM HCO_3^-, thereby impairing

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A portion of this study was presented at the Annual Meeting of the American Society of Nephrology in 1990-1992 and at the International Union of Physiological Sciences in 1993.

Received for publication 27 December 1993 and in revised form 4 March 1994.

J. Clin. Invest.

0021-9738/94/07/0173/11 $2.00
Volume 94, July 1994, 173–183

1. *Abbreviations used in this paper:* β_i, intrinsic cell buffer capacity; β_T, total buffer capacity; EBF, equivalent base flux; FDH, formate dehydrogenase; H_2DIDS, 4,4′-diisothiocyanatodihydrostilbene-2,2′-disulfonic acid; pH_i, intracellular pH.

Table I. Solutions

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
NaCl	140	—	135	134	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Na gluconate	—	140	—	—	135	—	—	—	—	—	—	—	—	—	—	—	—	—
TMACl	—	—	—	—	—	140	—	115	—	140	—	115	—	140	—	115	—	120
TMAOH	—	—	—	—	—	—	140	—	115	—	140	—	115	—	140	—	115	—
Gluconic acid lactone	—	—	—	—	—	—	140	—	115	—	140	—	115	—	140	—	115	—
K_2HPO_4	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	2.5
$CaCl_2$	1	—	1	1	—	1	—	1	—	1	—	1	—	1	—	1	—	1
Ca gluconate	—	3.5	—	—	3.5	—	3.5	—	3.5	—	3.5	—	3.5	—	3.5	—	3.5	—
$MgHPO_4 \cdot 3H_2O$	—	—	—	—	—	—	—	—	—	1	1	1	1	1	1	1	1	—
$MgCl_2$	1	—	1	1	—	1	—	1	—	—	—	—	—	—	—	—	—	1
Mg gluconate	—	1	—	—	1	—	1	—	1	—	—	—	—	—	—	—	—	—
Glucose	5	5	—	—	—	5	5	5	5	5	5	5	5	5	5	5	5	5
Alanine	5	5	—	—	—	5	5	5	5	5	5	5	5	5	5	5	5	5
Hepes acid	5	5	—	—	—	5	5	—	—	5	5	—	—	5	5	—	—	5
$NaHCO_3$	—	—	5	5	5	—	—	—	—	—	—	—	—	—	—	—	—	—
TMA HCO_3	—	—	—	—	—	—	—	25	25	—	—	25	25	—	—	25	25	—
K formate	—	—	—	—	—	—	—	—	—	1	1	1	1	—	—	—	—	—
KCN	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	1	1	—
KCl	—	—	—	—	—	—	—	—	—	1	—	1	—	1	—	1	—	—
K gluconate	—	—	—	—	—	—	—	—	—	—	1	—	1	—	1	—	1	—
Na formate	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Formic acid	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	19.5

All concentrations are in mM. All HCO_3^--containing solutions were bubbled with 6.5% CO_2/93.5% O_2, pH 7.4 except solutions C, D, and E, which were bubbled with 1% CO_2/99% O_2, pH 7.4. In experiments where solutions containing 1, 10, and 100 μM Na formate were used, the appropriate concentration of Na formate was added to solution C and E keeping the NaCl and Na gluconate concentration at 135 mM, respectively. When solutions containing 1 mM Na formate were used, the NaCl concentration was decreased to 134 mM (solution D). The 20 mM formic acid stock solution (Sigma Chemical Co.) was 97.3% pure; therefore, the actual formate concentration used in the formic acid permeability experiments was 19.5 mM.

the passive diffusion of formic acid from the lumen to the cell. Whether the magnitude of formic acid flux is sufficient to mediate apical Cl^-/base exchange has been questioned (28). Two studies have examined the effect of formate on volume absorption in the rat proximal tubule. Preisig and Rector (23) failed to find an effect of 0.5 mM luminal formate on J_V or J_{Cl}. In contrast, Wang et al. (29) found that 0.5 mM luminal formate stimulated J_V by 45%.

Given the conflicting results in the literature, the present study was performed in the rabbit superficial S_2 proximal tubule to determine (*a*) the magnitude and mechanism of base transport via the apical and basolateral Na^+-independent Cl^-/base exchangers, (*b*) the dose-dependent effect of physiological concentrations of formate on Cl^-/base exchange and transepithelial NaCl transport, and (*c*) whether lumen-to-cell formic acid uptake is an important mechanism for cellular formate accumulation.

Methods

Isolation and perfusion of proximal straight tubules. The methodology for isolating and perfusing proximal tubules in this laboratory has been described previously (30). Experiments were performed on rabbit superficial S_2 proximal straight tubules. S_2 tubules were dissected from the proximal end of a medullary ray. The solutions used in the various study protocols are depicted in Table I. All bathing solutions containing HCO_3^- were continuously bubbled with either 6.5% CO_2/93.5% O_2 or 1% CO_2/99% O_2 as indicated. The bathing solutions were delivered to the perfusion chamber through glass tubing to minimize gas loss. Bathing solutions containing Hepes were continuously bubbled with 100% O_2. The bathing solution flowed continuously at ~ 4 ml/min. Luminal solutions were bubbled with the appropriate gas and then sealed in syringes and were delivered to the perfusion chamber using a syringe infusion pump (model 22; Harvard Apparatus, South Natick, MA). Luminal solutions were changed completely in < 1 s. The perfusion rate was > 50 nl/min unless otherwise stated. Tetramethylammonium bicarbonate was made by bubbling tetramethylammonium hydroxide with 100% CO_2. Gluconate replaced Cl^- in the Cl^--free solutions. Because gluconate lowers the ionized Ca^{2+} concentration, the total Ca^{2+} concentration was increased from 1 to 3.5 mM to keep the ionized Ca^{2+} concentration constant.

Measurement of equivalent base flux. pH_i was measured in isolated perfused tubules using 2′,7′-bis(carboxyethyl)-5,6-carboxyfluorescein acetoxymethyl ester (BCECF-AM) as described previously (30). The rate of data acquisition was one excitation ratio per second unless otherwise stated. Intrinsic cell buffer capacity (β_i) was measured using the NH_4Cl prepulse technique (31) where various concentrations of NH_4Cl replaced TMACl (solution F). β_i was measured with 1 mM 4,4′-diisothiocyanatohydrostilbene-2,2′-disulfonic acid (H_2DIDS) in the luminal and bathing solutions at various pH_i values. In S_2 tubules, β_i was 25.6±3.8 mM/pH between pH_i of 6.7 and 7.1 (n = 14). This value is similar to the value of β_i previously obtained in rabbit S_3 proximal tubules, 21.5±3.3 mM/pH (16). In HCO_3^--containing solutions, total buffer capacity (β_T) was equal to the β_i plus the HCO_3^- buffer capacity ($\beta_{HCO_3^-}$) calculated as $2.3 \times [HCO_3^-]_i$ (31). Equivalent base flux (EBF)

was calculated as $EBF = dpH_i/dt \times \beta \times V$. dpH_i/dt represents the initial rate of change of pH_i calculated in the first 10 s after an experimental maneuver. β in the above equation signifies β_i in experiments where Hepes was used, and β_T when HCO_3^--buffered solutions were used. V is the cell volume per tubule length that was calculated by measuring the inner and outer tubule diameter according to the following equation: $V = \pi[(D_o)^2 - (D_i)^2]/4$, where D_o is the tubule outer diameter and D_i is the tubule inner diameter (32).

Measurement of total formate concentration. A new fluorometric assay for measuring total formate was developed using the enzyme formate dehydrogenase (FDH) derived from *Methylosinus trichosporium* OB3b (33), which was kindly provided by Dr. D.R. Jollie. In the presence of FDH, formate is converted to CO_2 with the concomitant reduction by NAD^+ to NADH, which is measured fluorometrically. Formate was measured according to the following protocol. Serum samples were diluted 10-fold with 0.15 M potassium phosphate buffer. Standards were prepared from sodium formate. 0.3 ml of a degassed 0.15 M potassium phosphate buffer (pH = 7.5) containing 1 mM NAD^+ was mixed with 5 μl of a stock solution of FDH (100 U/ml). 90 μl of degassed standard or sample was added to the microtube containing the FDH–NAD^+–phosphate buffer mixture and the fluorescence at 340 nm excitation wavelength was immediately read in a Turner model 110 fluorometer (Abbott Diagnostics, Mountain View, CA) fitted with a microtube holder. After an additional 6 min, the fluorescence was remeasured. The increment in fluorescence was a measure of the NADH generated from NAD^+ and was stoichiometrically related to the concentration of formate present in the sample or standard.

Measurement of tubule formate production. To measure formate production, the tubules were perfused and bathed in solution F. Because the distal end of the perfused segment remained open to the bath medium, formate entered the bath solution via the fluid leaving the distal end of the perfused segment and via the basolateral aspect of the perfused tubule segment. Therefore, the rate of total formate production equaled the rate of accumulation of formate in the bath medium. Formate production by the perfused proximal tubule segment was assayed by sampling the bath solution at various time points over 60 min. In these experiments the tubule was bathed in 200 μl of solution, which was covered with light mineral oil to prevent evaporation and bubbled continuously with 100% O_2 to ensure mixing.

Measurement of apical formic acid permeability. The apical formic acid permeability was measured by perfusing 19.5 mM formate in the lumen at pH 7.0, bath 7.6, solution R. At a pH of 7.0, assuming a pK of 3.76 (28), the calculated formic acid concentration is 0.011 mM. The apical membrane permeability was calculated by measuring the rate of change of pH_i in the first second (10 excitation ratios per second) after exposure to luminal formic acid. The rate of change of pH_i was converted to an equivalent acid influx rate according to the following equation: $EAF_{FH} = dpH_i/dt \times \beta_i \times V$. The formic acid permeability (P_{FH}) was calculated according to $P_{FH} = EAF_{FH}/[FH]_L$, where $[FH]_L$ is equal to the luminal formic acid concentration at $t = 0$. An apical membrane folding factor of 36 was used to calculate the formic acid permeability per folded surface area (34).

Measurement of J_V (volume absorption). The net fluid absorption rate (J_V) was determined by adding extensively dialyzed [*methoxy*-^{3}H]inulin (15 μCi/ml) (Du Pont–New England Nuclear, Boston, MA) to the perfusate as an impermeant volume marker. Volume flux per tubule length (J_V) in nl/min/mm was calculated as $J_V = (V_P - V_C)/L$, where V_P is the perfusion rate (nl/min), V_C is the collection rate (nl/min), and L is the tubule length (mm). The perfusion rate (V_P) was calculated according to $V_P = (I_C/I_P)/T$, where I_C is the [^{3}H]inulin count (cpm) of the collected samples, I_C is the perfusate [^{3}H]inulin concentration (cpm/nl), and T is the collection time (min). The collection rate (V_C) was calculated according to $V_c = V/T$, where V is the volume of the collecting pipette (nl). For these studies, both the perfusate and bathing solutions were identical except when formate and/or H_2DIDS were added to the perfusate. J_V measurements were initiated after the tubules were perfused for 15 min at 37°C, and ≥10 min after a luminal solution change. Because of the time constraint imposed by tubule deterioration after 1 h of in vitro perfusion, these studies were limited to two experimental periods. Tubules were perfused at a rate of 17.4±0.3 nl/min ($n = 65$).

Materials. BCECF-AM and H_2DIDS were purchased from Molecular Probes, Inc. (Eugene, OR). Nigericin, amiloride, furosemide, DMSO, iodoacetic acid, and formic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Light mineral oil was purchased from Fisher Scientific Co. (Pittsburgh, PA). H_2DIDS was added to the solutions at the final concentration used experimentally. A 0.5 M furosemide stock in DMSO was made and diluted 1:500 for a final concentration of 1 mM. The tubules were exposed to furosemide and H_2DIDS for 20 min before an experimental maneuver. Amiloride was dissolved in DMSO (stock concentration 1 M) and was diluted 1:500 for a final concentration of 2 mM.

Statistics. Results are reported as mean±SEM. A P value of < 0.05 was considered significant. Student's unpaired t test was used when comparing two protocols. When more than one experimental group was compared with a control group, Dunnett's t test was used.

Results

Luminal Cl^- removal; Hepes; pH 7.4 (lumen/bath). These experiments were performed to determine whether apical Cl^-/base exchange could be detected in S_2 tubules. S_2 tubules were perfused and bathed in solutions containing Na^+ and Cl^- (solution A). In this protocol, Na^+-dependent and/or -independent Cl^-/base exchange is assayed after the removal of luminal Cl^-. After luminal Cl^- removal (solution B), pH_i increased by only 0.04 pH units with an EBF of 2.76 pmol/min per mm (Table II). To enhance the measured effect on EBF induced by luminal Cl^- removal, an attempt was made to inhibit Na^+-dependent transport processes as previously described (26, 27). S_2 tubules were perfused with 2 mM luminal amiloride and 1 mM basolateral H_2DIDS. Under these conditions, EBF significantly increased to 7.26 pmol/min per mm (Table II). In these studies, because all apical and basolateral Na^+-dependent H^+/base transport processes may not have been completely inhibited despite the presence of the two inhibitors, subsequent experiments were performed in Na^+-free solutions to both completely inhibit Na^+-dependent H^+/base transport and to ensure that only Na^+-independent Cl^-/base exchange was studied.

S_2 tubules were perfused and bathed in Na^+-free, Cl^--containing solutions (solution F). After luminal Cl^- removal (solution G), pH_i increased at a rate of 1.28 pH/min, with an EBF of 36.2 pmol/min per mm (Fig. 1 *a*, Table II). Luminal H_2DIDS (0.25 mM) decreased dpH_i/dt after luminal Cl^- removal to 0.77±0.13 pH/min and EBF to 21.8±3.6 pmol/min per mm, $n = 4$, $P < 0.02$. 1 mM luminal H_2DIDS decreased dpHi/dt to 0.41±0.09 pH/min and EBF to 11.9±2.5 pmol/min/per mm, $n = 3$, $P < 0.001$. 1 mM luminal furosemide decreased dpH_i/dt to 0.48±0.11 and EBF to 18.4±4.18, $n = 6$, $P < 0.01$.

The results indicate that apical Cl^-/base exchange is demonstrable in S_2 tubules perfused and bathed in Na^+-free, Hepes-buffered solutions, in the absence of SO_4^{2-} and exogenous organic anions and is greater in magnitude than previously thought. To determine the effect of formate on apical Cl^-/base exchange, S_2 tubules were perfused and bathed in Na^+-free, Cl^--containing solutions with 1 mM formate (solution J). After luminal Cl^- removal (solution K), EBF was not significantly different than control (Fig. 1 *b*, Table II). Therefore, exogenous formate failed to alter the rate of apical Cl^-/base exchange under the conditions of these experiments.

Table II. Effect of Luminal Cl^- Removal on Apical Cl^-/Base Exchange in Tubules Perfused and Bathed at pH 7.4

Solution	Steady state pH_i	ΔpH_i	dpH_i/dt	Cell volume	Equivalent base flux	n
			pH/min	*$\times 10^{-10}$ liters/mm*	*pmol/min/mm*	
$+Na^+$; $+Cl^-$ (lumen, bath)						
1. Hepes	6.83±0.03	0.04±0.01	0.09±0.02	11.7±0.39	2.76±0.48	4
2. Hepes; 2 mM amiloride (lumen); 1 mM H_2DIDS (bath)	7.18±0.08*	0.25±0.04‡	0.23±0.03‡	12.4±0.26§	7.26±1.11‡	5
$-Na^+$; $+Cl^-$ (lumen, bath)						
3. Hepes	6.94±0.06	0.45±0.04	1.28±0.10	11.0±1.7	36.2±2.7	11
4. Hepes; 1 mM formate (lumen, bath)	6.96±0.06‖	0.51±0.04‖	1.33±0.20‖	10.5±1.7‖	35.8±5.3 ‖	12
$-Na^+$; $+Cl^-$ (lumen, bath)						
5. HCO_3^- (25 mM)	7.00±0.05	0.21±0.02	0.43±0.04	12.4±0.9	27.9±3.2	17
6. HCO_3^- (25 mM); 1 mM formate (lumen, bath)	6.97±0.05¶	0.23±0.03¶	0.51±0.06¶	10.6±1.0¶	26.5±3.8 ¶	14

* $P < 0.001$ vs. Hepes (protocol 1). ‡ $P < 0.02$ vs. Hepes (protocol 1). § P = NS vs. Hepes (protocol 1). ‖ P = NS vs. Hepes (protocol 3). ¶ P = NS vs. HCO_3^- (protocol 5).

Luminal Cl^- removal; HCO_3^- (25 mM, pH 7.4, lumen/bath). To determine whether the apical Cl^-/base exchanger was HCO_3^- dependent, the tubules were perfused and bathed in 25 mM HCO_3^-, pH 7.4, in Cl^--containing solutions (solution H). After luminal Cl^- removal (solution I) the EBF was 27.9 pmol/min per mm (Fig. 1 *c*, Table II). This value is approximately the value measured in Hepes-buffered solutions, 36.2 pmol/min per mm (Table II). Although the EBF tended to be greater in Hepes than in HCO_3^--buffered solutions, this difference was not significant at the $P < 0.05$ level. Because of the possibility that increased basolateral base efflux as HCO_3^- minimized dpH_i/dt and EBF in HCO_3^--buffered solutions, similar experiments were performed in the presence of 0.25 mM basolateral H_2DIDS to inhibit basolateral base flux. Under these conditions, after luminal Cl^- removal, pH_i increased at a rate of 0.36±0.06 pH/min, and the EBF was 26.6±5.1 pmol/min per mm (n = 5), which was not significantly different from the results obtained in HCO_3^--containing solutions in the absence of basolateral H_2DIDS. These experiments rule out the possibility that basolateral HCO_3^- transport accounted for the lack of a stimulation of EBF in HCO_3^--buffered solutions. Further experiments were done to determine whether enhanced metabolic proton production could have prevented a stimulation of EBF in HCO_3^--containing solutions. S_2 tubules were perfused and bathed with 1 mM KCN and 0.5 mM iodoacetate in HCO_3^--containing solutions (solution P) to inhibit metabolic proton production. After luminal Cl^- removal (solution Q) EBF was 26.4±8.1 pmol/min per mim (n = 5), which was not significantly different than in control HCO_3^- solutions (Table II). The results indicate that exogenous HCO_3^- fails to stimulate apical Cl^-/base exchange in S_2 tubules. In tubules perfused and bathed in HCO_3^--containing solutions with 1 mM formate, dpH_i/dt and EBF were not significantly different than in control HCO_3^--buffered solutions (Fig. 1 *d*, Table II). These results complement the findings in Hepes-buffered solutions and demonstrate that exogenous formate fails to alter the rate of apical Cl^-/base exchange in the presence or absence of HCO_3^-.

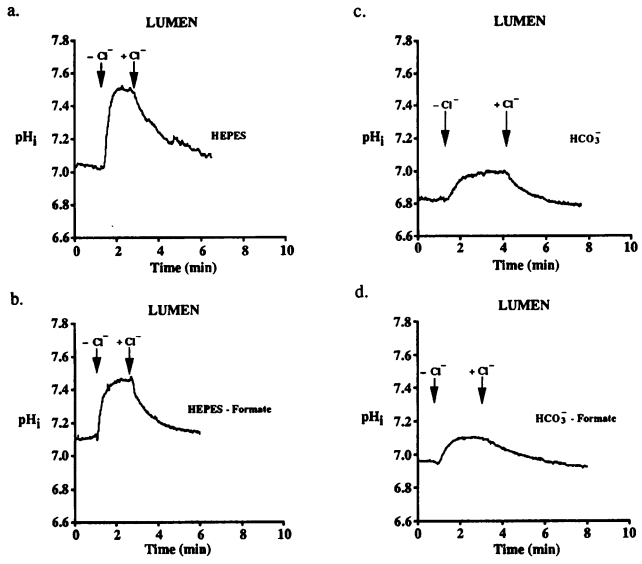

Figure 1. Effect of luminal Cl^- removal and subsequent addition on pH_i: tubules were initially perfused and bathed in Na^+-free, Cl^--containing solutions, pH 7.4 (lumen, bath). (*a*) Hepes-buffered solutions, (*b*) Hepes plus 1 mM formate (lumen, bath) (*c*) HCO_3^- (25 mM) buffered solutions, (*d*) HCO_3^- (25 mM) plus 1 mM formate (lumen, bath).

Luminal Cl^- removal; Hepes; lumen pH 7.0/bath pH 7.6. In free-flow micropuncture studies in the rat, the luminal pH at the end of the accessible proximal tubule is ~ 6.7 (35, 36). Although the in vivo luminal pH in the rabbit proximal straight tubule is unknown, in vitro the luminal pH is ~ 7.0 (37). Formic acid recycling from lumen to cell would be predicted to be enhanced as the luminal pH is decreased axially in the proximal tubule (22). In the previous experiments that failed to detect an effect of formate on apical Cl^-/base exchange the lumen was perfused at pH 7.4. Therefore, further experiments were

Table III. Effect of Lumen Cl^- Removal on Apical Cl^-/Base Exchange in Tubules Perfused at pH 7.0 and Bathed at pH 7.6

Solution	Steady state pH_i	ΔpH_i	dpH_i/dt	Cell volume	Equivalent base flux	n
			pH/min	*$\times 10^{-10}$ liters/mm*	*pmol/min/mm*	
1. Hepes	6.90±0.03	0.27±0.05	0.63±0.05	13.5±1.1	21.6±1.7	7
2. Hepes; 1 mM formate (lumen, bath)	6.87±0.05*	0.29±0.05*	0.63±0.08*	12.2±2.3*	19.6±2.4*	6
3. Hepes; 1 mM KCN/0.1 mM acetazolamide (lumen, bath)	6.71±0.04‡	0.38±0.04*	0.55±0.05*	14.2±0.7*	20.0±1.7*	14

All studies were performed in tubules perfused and bathed in Na^+-free, Cl^--containing solutions. * P = NS vs. Hepes (protocol 1). ‡ $P < 0.01$ vs. Hepes (protocol 1).

performed in Hepes-buffered solutions at a luminal pH of 7.0 (solution F). In preliminary studies with a luminal pH of 7.0 and a bath pH of 7.4, pH_i decreased to ~ 6.6. Therefore, in subsequent studies, the bath pH was increased to 7.6 to increase pH_i to approximately the value obtained when the perfusate and bath pH were 7.4. After luminal Cl^- removal, EBF was 21.6 pmol/min per mm (Table III). Both dpH_i/dt and EBF in these experiments were significantly less ($P < 0.01$) than in the previous studies where luminal Cl^- was removed with the tubule perfused and bathed at pH 7.4 (Table II). The difference in dpH_i/dt and EBF is likely due to the lower luminal concentration of base at pH 7.0. As summarized in Table III, at a luminal pH of 7.0, 1 mM formate (lumen, bath) again failed to alter EBF after luminal Cl^- removal.

Luminal Cl^- addition; Hepes; lumen pH 7.0/bath 7.6. In previous studies demonstrating an effect of exogenous formate on luminal Cl^--induced changes in pH_i, the tubules had been perfused initially in the absence of Cl^-, and Cl^- was then added to the lumen (26, 27). However, in the results described thus far, EBF was calculated after Cl^- removal from the lumen in tubules initially perfused and bathed in Cl-containing solutions. To determine whether the direction of apical Cl^-/base transport altered the results, S_2 tubules were perfused and bathed in Na^+-free, Cl^--free solutions (solution G). Cl^- was then added to the lumen (solution F). Fig. 2 *a* shows the effect of luminal Cl^- addition in S_2 tubules perfused and bathed in the absence of Cl^-. Luminal Cl^- addition caused pH_i to decrease with an EBF of −38.0 pmol/mm/min (Table IV). Both dpH_i/dt and EBF after lumen Cl^- addition were significantly greater than following luminal Cl^- removal at lumen pH 7.0 and bath pH 7.6, $P < 0.05$ (Table III). The tubules were then perfused and bathed in Cl^--free solutions containing 1 mM formate (solution K). After Cl^- addition to the lumen (solution J), EBF was −42.0 pmol/min per mm (Fig. 2 *b*, Table IV). These results are not significantly different from those obtained in the absence of formate (Fig. 2 *a*, Table IV).

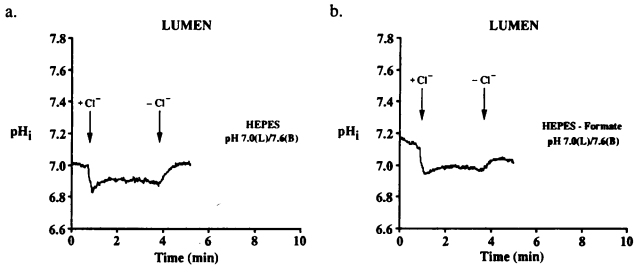

Figure 2. Effect of lumen Cl^- addition and subsequent removal on pH_i: tubules were perfused initially in Na^+-free, Cl^--free solutions, at pH 7.0 (lumen)/7.6 (bath) (*a*) Hepes-buffered solutions, (*b*) Hepes with 1 mM formate (lumen, bath).

Luminal Cl^- removal; Hepes; inhibition of metabolic HCO_3^- production. In Hepes-buffered nominally HCO_3^--free solutions, metabolic CO_2 production can generate intracellular HCO_3^- (38). To determine whether HCO_3^- produced metabolically contributed to base flux via the apical Cl^-/base exchanger in tubules perfused and bathed in Hepes-buffered solutions, metabolic CO_2 production was inhibited with KCN, and the rate of CO_2 hydration to HCO_3^- was decreased with acetazolamide. Tubules were perfused at pH 7.0 and bathed at 7.6, with 1 mM KCN and 0.1 mM acetazolamide in the lumen and bath (solution N). Apical Cl^- removal (solution O) induced an EBF of 20.0 pmol/min per mm, P = NS versus control (Table III). These results, in addition to absence of an effect of exogenous formate on apical Cl^-/base exchange, suggest that either OH^- ions or an endogenously produced base such as formate mediate base flux via the apical Cl^-/base exchanger.

Measurement of formate production in isolated perfused S_2 tubules. In experiments where the tubules were perfused without exogenous formate, endogenous cell formate production could have increased the formate concentration in the perfusate and/or bathing solutions, supplying sufficient formate to be transported on the apical Cl^-/base exchanger. Therefore, further studies were performed to measure formate production in isolated perfused S_2 tubules under the same conditions where apical Cl^-/base activity had been measured in the absence of exogenous formate. A newly developed fluorescent assay using formate dehydrogenase derived from methylosinus trichosporium was used to measure formate production (see Methods). An in vitro calibration curve using sodium formate standards is shown in Fig. 3. The assay was linear over the range of standards tested. Five S_2 tubules were perfused and bathed in solution F, pH 7.4, without mounting the opposite end of the tubule. The detection limit of the assay is 0.6 pmol/min per mm. In all five tubules formate production was undetectable. Therefore, under

Table IV. Effect of Lumen Cl^- Addition on Apical Cl^-/Base Exchange in Tubules Perfused at pH 7.0 and Bathed at pH 7.6

Solution	Steady state pH_i	ΔpH_i	dpH_i/dt	Cell volume	Equivalent base flux	n
			pH/min	*$\times 10^{-10}$ liters/mm*	*pmol/min/mm*	
1. Hepes	7.13±0.05	−0.19±0.02	−1.28±0.21	11.6±0.8	−38.0±6.5	8
2. Hepes; 1 mM formate (lumen, bath)	7.09±0.06*	−0.15±0.03*	−1.21±0.25*	13.5±1.3*	−42.0±8.7*	7

All studies were performed in tubules initially perfused and bathed in Na^+-free, Cl^--free solutions. * P = NS vs. Hepes.

the conditions of these experiments, endogenous formate production was < 0.6 pmol/min per mm.

Measurement of arterial formate concentration in rabbits. Although the formate production rate in these experiments was low, in vivo formate could enter the luminal fluid through glomerular filtration. Using the fluorescence assay, the formate concentration in rabbit arterial serum was 6.0±0.1 μM, n = 6. (Similar results have been obtained in rat arterial serum: 7.4±0.8 μM, n = 5.) These results indicate that blood formate levels are not in the 0.1–1.0 mM range in rabbits as thought previously (22, 39). Therefore, in separate studies, tubules were perfused at pH 7.0 and bathed at pH 7.6 (solution F) with 10 μM formate (lumen, bath) to approximate the formate concentration in rabbit arterial serum. After removal of lumen Cl^-, dpH_i/dt was 0.54±0.09 (n = 7) with an EBF of 18.0±3.2 pmol/min per mm, which was not significantly different from control: dpH_i/dt 0.63±0.05 and EBF 21.6±1.7 pmol/min per mm, n = 7. Therefore, the addition of exogenous formate at a concentration approximating rabbit arterial serum does not stimulate apical Cl^-/base exchange.

Measurement of apical membrane formic acid permeability and flux. Given the low formate concentration in arterial serum and the low tubule formate production rate, the apical membrane formic acid permeability in S_2 tubules was measured to determine whether apical formic acid recycling could contribute to base flux via the apical Cl^-/base exchanger. S_2 tubules were perfused at a luminal pH 7.0, bath pH 7.6 (solution F). 19.5 mM formate was added acutely to the lumen (solution R). The formic acid concentration in this solution at pH 7.0 was 0.011 mM, assuming a pK of 3.76 (28). As depicted in Fig. 4, pH_i decreased rapidly at a rate of −7.60±0.16 pH/min, n = 4. The calculated formic acid flux in these studies was 262.6±5.53 pmol/min per mm. The apical formic acid permeability was 2.35×10^4±0.05 nl/min per mm.

The proximal tubule luminal formic acid concentration in vivo is unknown. If the luminal formate concentration is the same as rabbit arterial serum, ~ 6 μM, at a luminal pH of 7.0 the formic acid concentration is 3.45×10^{-6} pmol/nl. Assuming an apical formic acid permeability of 2.35×10^4 nl/min/mm, the predicted lumen to cell formic acid flux is 8.11×10^{-2} pmol/min per mm. This value is too small for apical formic acid recycling to be incorporated into a model of formate stimulated NaCl transport in the superficial S_2 proximal tubule.

Effect of luminal formate on J_V. In a previous study, 0.25 or 0.5 mM formate added to the lumen and bath stimulated J_V in the rabbit proximal tubule by ~ 60% (22). Given that the blood formate concentration is in the micromolar range, further studies were performed to determine the dose dependence of luminal formate on proximal tubule J_V. S_2 tubules were perfused and bathed in high Cl^-/low HCO_3^- solutions (solution C) bubbled with 1% CO_2 (a solution simulating late proximal tubule fluid). Under these conditions (absent transepithelial electrochemical gradient for Na^+ and Cl^-), J_V is due to active NaCl transport (21, 22). J_V was measured in paired studies under control conditions and in the presence of 1, 10, 100, and 1,000 μM luminal sodium formate. The dose-dependent formate stimulation of J_V is depicted in Fig. 5 *a–d*). 10 μM formate, which approximates the arterial formate concentration, increased J_V by 81%. As shown in Fig. 6 *a*, 1 mM H_2DIDS (lumen) decreased J_V in the absence of exogenous formate from 0.62±0.07 (n = 3) to 0.25±0.08 nl/min per mm (n = 5), $P < 0.05$. The stimulation of J_V by 100 μM formate was completely inhibited by 1 mM H_2DIDS (lumen): 1.32±0.26 nl/min per mm, n = 6 versus 0.26±0.04, n = 5, $P < 0.01$ (Fig. 6 *b*). In a separate study, luminal Cl^--induced EBF was measured in tubules perfused and bathed in solution C (high Cl^-/low HCO_3^- solutions, bubbled with 1% CO_2, i.e., the same solutions used to measure J_V). Steady state pH_i was 6.85±0.04 (n = 5).

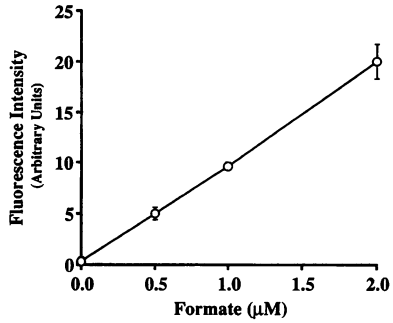

Figure 3. In vitro calibration curve for fluorescence assay of formate: fluorescence intensity was plotted as a function of sodium formate standards. The assay is linear over the range of standards used. Each point represents the mean±SEM of three measurements.

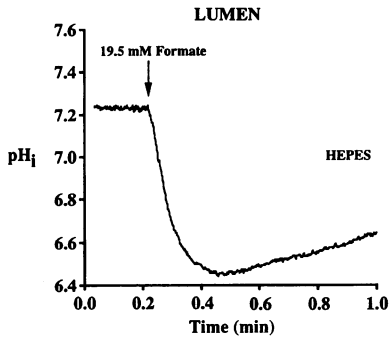

Figure 4. Apical formic acid permeability: tubules were perfused in Hepes-buffered solutions at pH 7.0 (lumen)/7.6 (bath). The luminal fluid was changed acutely to a solution containing 19.5 mM formate. pH_i decreased rapidly due to formic acid influx. The apical formic acid permeability in these studies was 2.35×10^4±0.05 nl/min per mm (n = 4).

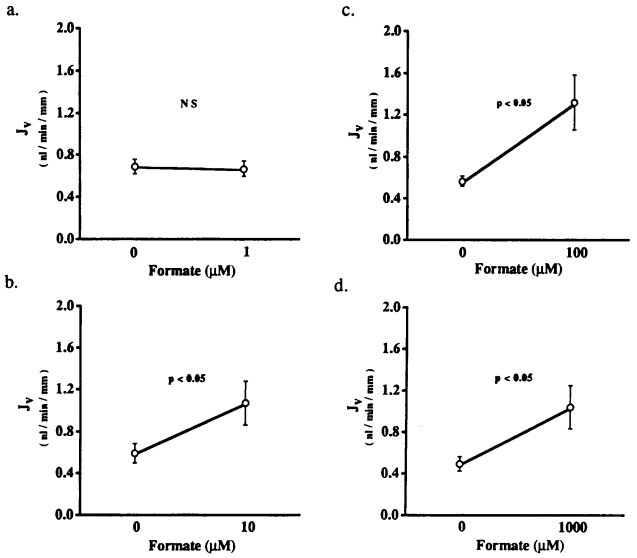

Figure 5. (*a–d*) Effect of luminal formate on J_V: J_V was measured in tubules perfused and bathed in the absence of transepithelial Na^+ and Cl^- gradients in identical high Cl^-/low HCO_3^- solutions, bubbled with 1% CO_2. The tubules were initially perfused in the absence of formate followed by the luminal addition of 1 (n = 6), 10 (n = 7), 100 (n = 6), and 1,000 μM (n = 7) sodium formate. Paired experiments were performed on each tubule because of the variation between tubules in J_V. 10, 100, and 1000 μM formate stimulated J_V by 81%, 136%, and 112%, respectively ($P < 0.05$).

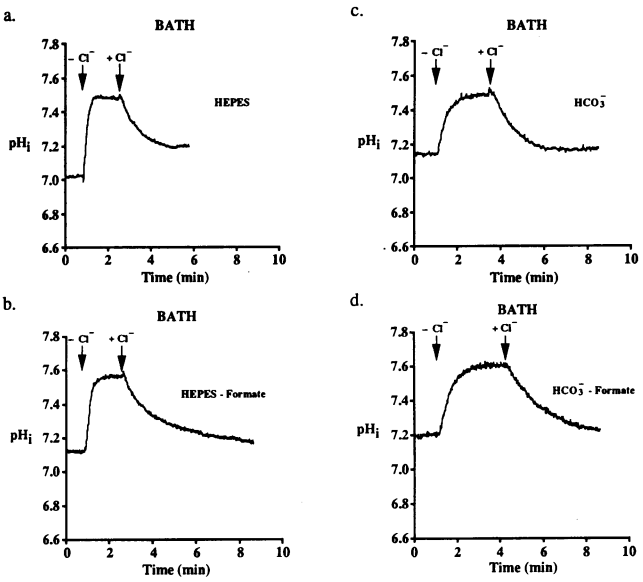

Figure 7. Effect of basolateral Cl^- removal and subsequent addition on pH_i: tubules were perfused and bathed in Na^+-free, Cl^--containing solutions, pH 7.4 (lumen, bath). (*a*) Hepes-buffered solutions, (*b*) Hepes plus 1 mM formate (lumen, bath), (*c*) HCO_3^- (25 mM) buffered solutions, (*d*) HCO_3^- (25 mM) plus 1 mM formate (lumen, bath).

After luminal Cl^- removal (solution E), pH_i increased at a rate of 0.13±0.01 pH/min with an EBF of 3.90±0.47 pmol/min per mm (n = 5). S_2 tubules were then perfused and bathed in solution C with 100 μM sodium formate added to the perfusate. Steady state pH_i was 6.84±0.03. Lumen Cl^- removal (solution E with 100 μM sodium formate) caused pH_i to increase at a rate of 0.15±0.05 pH/min with an EBF of 4.10±1.25 pmol/min/mm (n = 4). These results were not significantly different than control. Therefore, under the same conditions in which formate stimulated active transepithelial NaCl transport, an effect of formate on apical Cl^--induced EBF was not detectable.

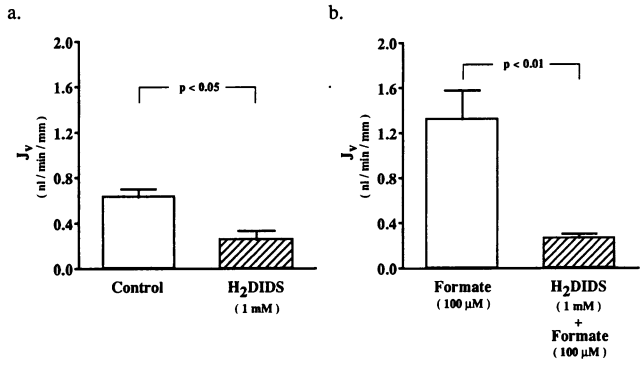

Figure 6. Inhibition of J_V by 1 mM H_2DIDS (lumen) in the absence and the presence of 100 μM luminal formate. (*a*) 1 mM H_2DIDS (lumen) decreased control J_V from 0.73±0.12 (n = 4) to 0.25±0.08 (n = 5) $P < 0.02$. (*b*) in the presence of 100 μM formate (lumen), 1 mM luminal H_2DIDS decreased J_V from 1.32±0.26 (n = 6) to 0.26±0.04 (n = 5) $P < 0.01$.

Basolateral Cl^- removal; Hepes; pH 7.4 lumen/bath. Further studies were done to determine the HCO_3^- and formate-dependence of basolateral Na^+-independent Cl^-/base exchange. S_2 tubules were perfused and bathed in Na^+-free, Cl^--containing solutions (solution F). After basolateral Cl^- removal (solution G), EBF was 37.2 pmol/min per mm (Fig. 7 *a*, Table V). In tubules perfused and bathed in 1 mM formate (lumen/bath), the EBF was not significantly different than control (Fig. 7 *b*, Table V). Therefore exogenous formate failed to stimulate basolateral Cl^-/base exchange.

Basolateral Cl^- removal; HCO_3^- (25 mM, pH 7.4, lumen/bath). Fig. 7 *c* shows the effect of luminal Cl^- removal in S_2 tubules perfused and bathed in Na^+-free, HCO_3^--buffered solutions (solution H). After basolateral Cl^--removal (solution I), EBF was 73.0 pmol/min per mm (Table V). The results indicate that unlike the apical Cl^-/base exchanger, base transport via the basolateral Cl^-/base exchanger is significantly greater in 25 mM HCO_3^--buffered solutions than in Hepes-buffered solutions (Table V), $P < 0.05$. In separate studies the effect of 1 mM formate (lumen/bath) on basolateral Cl^-/base exchange was studied (Fig. 7 *d*). As shown in Table V, EBF was not different from control HCO_3^--buffered solutions. Therefore the basolateral Cl^-/base exchanger (like the apical Cl^-/base exchanger) was not stimulated by formate in the presence or absence of exogenous HCO_3^-. However, EBF in the presence of 1 mM formate and 25 mM HCO_3^- was significantly greater than in tubules perfused and bathed in 1 mM formate and Hepes-buffered solutions (Table V), $P < 0.05$. Therefore, exogenous HCO_3^- (25 mM) stimulates the basolateral Cl^-/base exchanger, unlike the apical Cl^-/base exchanger.

Effect of basolateral furosemide and H_2DIDS on basolat-

Table V. Effect of Bath Cl^- Removal on Basolateral Cl^-/Base Exchange in Tubules Perfused and Bathed at pH 7.4

Solution	Steady state pH_i	ΔpH_i	dpH_i/dt	Cell volume	Equivalent base flux	n
			pH/min	*$\times 10^{-10}$ liters/mm*	*pmol/min/mm*	
$-Na^+$; $+Cl^-$ (lumen, bath)						
1. Hepes	7.06±0.02	0.57±0.08	1.32±0.12	11.0±1.7	37.2±3.2	6
2. Hepes;						
1 mM formate (lumen, bath)	7.09±0.06*	0.63±0.06*	1.29±0.20*	10.5±1.7*	34.7±5.4*	6
$-Na^+$; $+Cl^-$ (lumen, bath)						
3. HCO_3^- (25 mM)	7.10±0.04	0.43±0.04	0.87±0.14	13.4±1.7	73.0±11.5	8
4. HCO_3^- (25 mM);						
1 mM formate (lumen, bath)	7.17±0.05‡	0.38±0.04‡	0.86±0.09‡	10.6±1.2‡	65.2±8.6‡	9

* P = NS vs. Hepes (protocol 1). ‡ P = NS vs. HCO_3^- (protocol 3).

eral Cl^-/base exchange; Hepes; pH 7.4 lumen/bath. Given the difference in the effect of exogenous HCO_3^- on apical and basolateral Cl^-/base exchange, further studies were done to determine whether the exchangers differed in their inhibitor sensitivity. S_2 tubules were perfused and bathed in solution F. Basolateral furosemide (1 mM) significantly decreased dpH_i/dt after basolateral Cl^- removal (solution G) to 0.15±0.04 pH/min, and EBF to 4.17±1.19 pmol/min per mm, $n = 10$, $P < 0.001$. Basolateral H_2DIDS (0.1 mM) significantly decreased dpH_i/dt to 0.04±0.02 pH/min and EBF to 1.24±0.59 pmol/min per mm, $n = 7$, $P < 0.001$. Therefore, both the apical and basolateral Na^+-independent Cl^-/base exchangers are inhibited by furosemide and H_2DIDS. The basolateral transporter has a greater sensitivity to both inhibitors.

Discussion

The present study demonstrates that rabbit superficial S_2 proximal tubules possess apical and basolateral Na^+-independent Cl^-/base exchangers that function in the nominal absence of HCO_3^-. Base transport by the basolateral transporter, unlike the apical transporter, is enhanced in HCO_3^--buffered solutions and, like the apical transporter, is stilbene and furosemide sensitive. However, the basolateral transporter is more sensitive to these inhibitors. Formate in the micromolar range stimulates active transcellular NaCl transport without affecting apical Cl^-/base exchange. The results suggest that lumen-to-cell formic acid flux is insufficient to be incorporated into a cell model that accounts for the stimulation by formate of transcellular Cl^- transport. We propose that transcellular NaCl transport in the superficial S_2 proximal tubule is mediated by an apical Na^+/H^+ exchanger in parallel with a Cl^-/OH^- exchanger and that the secreted H^+ and OH^- ions form H_2O in the tubule lumen.

Previous studies have failed to measure appreciable apical Cl^-/base exchange in the intact tubule using cell pH measurements in the absence of exogenous formate (26, 27). Several factors and methodological differences distinguish the studies of Alpern (26) and Baum (27) from the present study and may account for the marked Cl^--induced changes in pH_i observed. Alpern (26) found that in rat proximal tubules perfused and bathed in the absence of Cl^-, pH_i decreased by 0.02 pH units after luminal Cl^- addition in the absence of formate and by 0.06 pH units in the presence of 1 mM formate. In rabbit superficial proximal convoluted tubules, Baum (27) measured a decrease in pH_i of 0.03 pH units after luminal Cl^- addition in the absence of formate and 0.11 pH units in the presence of formate (1 mM). In the proximal straight tubule, pH_i failed to change in the absence of formate; however, with 1 mM formate, pH_i decreased by 0.02 pH units after luminal Cl^- addition. Equivalent base flux was not reported in either study. In both studies the solutions contained Na^+, SO_4^{2-}, and HCO_3^-. Na^+-dependent H^+/base transport processes could have minimized the Cl^--induced pH_i changes despite the use of luminal amiloride (27) and basolateral SITS (26, 27) to inhibit H^+/base transport. It is also unlikely that luminal amiloride completely inhibited the apical Na^+/H^+ antiporter (40). In red blood cells the anion exchanger can function in a $H^+-SO_4^{2-}/Cl^-$ exchange mode (41). If this mode of anion exchange was functioning in the previous studies where the solutions contained SO_4^{2-}, the Cl^--induced pH_i changes would have been minimized because of the opposing effects on pH_i mediated by Cl^-/base and H^+-SO_4^{2-}/Cl^- exchange. In addition, HCO_3^--buffered solutions were used, which decreased the luminal Cl^--induced pH_i changes because of the increased cell buffer capacity. The present study documents the difficulty of observing apical Cl^-/base exchange in the rabbit S_2 proximal tubule in Na^+-containing solutions. In Hepes-containing solutions, luminal Cl^- removal caused pH_i to increase by 0.04 pH units with an EBF of ~3 pmol/min per mm. In the presence of amiloride (lumen) and H_2DIDS (bath) after apical Cl^- removal, both the change in pH_i of 0.25 pH units and the EBF of ~7 pmol/min per mm were significantly greater than control. In the absence of Na^+, both the change in pH_i and EBF were increased further to 0.45 pH units and ~36 pmol/min per mm, respectively. Interestingly, luminal Na^+ removal decreases intracellular Cl^- activity in the S_2 proximal tubule, which would potentially diminish Cl^- efflux (and Cl^-/base exchange) after luminal Cl^- removal (42). However, in the present study, EBF was greater in the Na^+-free solutions because it is likely that Na^+ removal more completely inhibits Na^+-dependent H^+/base transport than the use of inhibitors.

In HCO_3^--containing solutions, both the change in pH_i and dpH_i/dt after apical Cl^- removal were less than in Hepes-buffered solutions. However EBF was ~ 28 pmol/min per mm in

HCO_3^-, which was similar to the result obtained in Hepes. Therefore, exogenous HCO_3^- failed to stimulate apical Cl^-/base exchange in contrast to the stimulation of Cl^-/base exchange by HCO_3^- reported in other cell types (15, 16, 43–47). The lack of stimulation of apical Cl^-/base exchange could have been due to enhanced basolateral base efflux in HCO_3^--containing solutions. However, in the presence of 0.25 mM basolateral H_2DIDS, EBF in HCO_3^--containing solutions was not different from the control value. Another possible mechanism for the lack of HCO_3^- stimulation of apical Cl^-/base exchange is enhanced cellular acid loading due to an increase in lactic acid production in HCO_3^-. However, since in the proximal tubule metabolic ATP production is coupled to ATP used primarily by the basolateral Na^+-K^+-ATPase (48), it is likely that in Na^+-free solutions metabolic proton production was low. In addition, a previous study in rabbit proximal tubules has documented a greater rate of glycolytic lactate production in Hepes than in HCO_3^--containing solutions (49). In the present study when metabolic proton production was inhibited with iodoacetic acid and KCN in tubules perfused and bathed in 25 mM HCO_3^-, EBF was not increased above the control value.

In Hepes-containing solutions, metabolic CO_2 production can increase the cell HCO_3^- concentration (38). Therefore, further studies were done to inhibit metabolic HCO_3^- production using KCN and acetazolamide. The finding of significant Cl^-/base exchange in Hepes-buffered solutions when metabolic HCO_3^- production was inhibited and a lack of stimulation of apical Cl^-/base exchange by exogenous HCO_3^- suggests that OH^- ions or an endogenously produced base, such as formate, mediated base flux via the apical anion exchanger. The finding that formate production is undetectable under the present study conditions suggests that OH^- ions are the species transported in exchange for Cl^-. These results are compatible with the study of Preisig and Rector (23), who demonstrated that the carbonic anhydrase inhibitor methylzolamide had no effect on Cl^- absorption in the rat proximal tubule and Chen et al. (5) who failed to detect a stimulation by HCO_3^- of brush border vesicle Cl^-/base exchange. In addition, Yoshitomi and Hoshi (19) postulated that the Triturus proximal tubule possesses an apical Cl^-/OH^- rather than a Cl^-/HCO_3^- exchanger. A recent study of the physiological properties of the band 3 protein in red blood cells also demonstrated the importance of Cl^-/OH^- rather than Cl^-/HCO_3^- exchange (50).

Coupled apical Na^+/H^+ and Cl^-/base exchange in the proximal tubule will result in the absorption of NaCl and the generation of an acid in the tubule lumen. The type of acid will depend on the base secreted by the anion exchanger (i.e., OH^- [H_2O]; formate [formic acid]; HCO_3^- [carbonic acid]). The results of the present study are compatible with the hypothesis that H_2O is formed in the lumen of the superficial S_2 proximal tubule as a result of H^+ and OH^- secretion. OH^- secretion into the tubule lumen will diminish net proximal tubule HCO_3^- absorption. In the rat, transepithelial HCO_3^- absorption is greatest in the first millimeter after the glomerulus (51). Whether the Cl^-/base exchange rate is lower in the superficial S_1 proximal tubule compared with the S_2 segment has not been determined. It will be important to document the axial distribution of the apical Cl^-/base exchange, Na^+/H^+ exchange and H^+-ATPase activity to understand more completely how the proximal tubule accomplishes both net transcellular $NaHCO_3$ and NaCl absorption.

The apical Cl^--induced EBF was greater in tubules initially perfused and bathed in the absence of Cl^- after luminal Cl^- addition, when compared with experiments where the luminal Cl^- was removed in tubules initially perfused and bathed in Cl^--containing solutions (Tables III and IV). However, the magnitude of change in pH_i was less. Presumably the gradient for apical Cl^- entry was greater in these studies because it is likely that the intracellular Cl^- activity was decreased in the Cl^--free solutions below the control value. In addition, a favorable OH^- gradient from cell to lumen was present because the lumen was perfused at pH 7.0. A likely explanation for the smaller change in pH_i is that a new steady state was achieved more rapidly because the basolateral Cl^-/base transporter mediated base influx and Cl^- efflux sooner after luminal Cl^- addition than in the experiments where luminal Cl^- was initially removed. It is also possible that the intracellular and/or extracellular Cl^- or OH^- concentration affect the kinetic properties of the apical and basolateral Cl^-/base exchangers.

It is of interest that the apical and basolateral Cl^-/base exchangers differ in their response to exogenous HCO_3^-. 25 mM HCO_3^- stimulated basolateral Cl^--induced EBF by approximately twofold. In the S_3 proximal straight tubule, HCO_3^- also stimulates basolateral Cl^-/base exchange (15, 16). This finding has been reported in other cell types where Cl^-/base exchange has been measured in the presence and absence of HCO_3^- (43–47). Whether HCO_3^- is transported per se or is stimulating Cl^-/OH^- exchange has not been determined. A family of anion exchange genes exist (AE1, AE2, and AE3); however, their base transport properties have not been clearly defined. It is unknown whether the apical and basolateral transporters in the S_2 proximal tubule represent different members of the anion exchange gene family and/or whether postranslational modification accounts for the difference in HCO_3^- dependence.

Despite the absence of an effect of formate apical or basolateral Cl^-/base exchange, micromolar formate was found to stimulate J_V (which in the absence of a transepithelial Na^+ and Cl^- concentration gradient reflects transcellular NaCl absorption). The importance of a lumen-to-cell formic acid gradient to drive net formic acid recycling has been emphasized previously (22). In two previous studies, where the effect of luminal Cl^--induced changes in pH_i was examined, formate increased the magnitude of acidication after luminal Cl^- addition despite the absence of a marked lumen-to-cell pH gradient (pH 7.4 lumen/7.45 cell in the rat proximal convoluted tubule [26]; pH 7.4 lumen/7.35 cell in the rabbit proximal straight tubule [27]). In the present study several protocols were performed to modify the cell to lumen formic acid gradient and the direction of Cl^-/base exchange: (*a*) lumen Cl^- removal, pH 7.4 lumen (Table II); (*b*) lumen Cl^- removal, pH 7.0 lumen (Table III); and (*c*) lumen Cl^- addition, pH 7.0 lumen (Table IV). In all protocols, in the presence of formate, Cl^--induced EBF was not different from control. However, 10 μM luminal formate, which approximates the concentration measured in rabbit and rat arterial blood, increased J_V by 0.48 nl/min per mm. This represents an increase in J_{Cl} of ~ 72 pmol/min/mm. Schild et al. (22) have reported previously a stimulation of J_V by 0.2 mM and 0.5 mM formate of ~ 60% in rabbit proximal tubules. Wang et al. (29) reported that 0.5 mM luminal formate increased J_V in the rat proximal tubule by ~ 1.1 nl/min per mm, which is equivalent to an increase in J_{Cl} of ~ 160 pmol/min per mm. However, Preisig

and Rector (23) failed to find an effect of 1 mM luminal formate on J_{Cl} or J_V in the rat proximal tubule. The reason for the discrepancy between these studies may be that, in the latter study, the peritubular capillaries were not perfused such that sufficient formate may have been present basolaterally to maximally stimulate transcellular NaCl transport. In the steady state, for net lumen-to-cell formic acid flux to be the primary process contributing to cell formate accumulation and subsequent apical Cl^-/formate exchange, net apical formic acid absorption must equal the rate of Cl^- and base transport via the apical Cl^-/base exchanger. This model also implicitly assumes that renal cell metabolism of formate is insignificant. Given an apical formic acid permeability of 2.35×10^4 nl/mm and assuming (*a*) a cell formate concentration of zero, (*b*) a lumen formate concentration of 100 μM ($\sim 15 \times$ the measured arterial concentration); and (*c*) a lumen pH of 6.8, the apical formic acid absorption rate would only be 2.1 pmol/min per mm, which is not sufficient to account for the formate-induced increase in J_V observed in the present studies. In addition, a recent mathematical analysis of a proximal tubule villous membrane concluded that unstirred layer effects cannot account for the formic acid recycling rate required to stimulate J_V (52). The finding in the present study of a stimulation of J_V by formate in the micromolar range indicates that lumen to cell formic acid flux can no longer be considered an important mechanism to supply formate for an apical Cl^-/formate exchanger in the superficial S_2 proximal tubule.

Using a new fluorescence assay for measuring formate we observed that the arterial serum formate concentration, ~ 6 μM in rabbits, is much lower than recognized previously. The formate dehydrogenase enzyme used in the present study is advantageous in that the reaction catalyzed by the enzyme is essentially unidirectional favoring the formation of CO_2 from formate. The first fluorescence assay for formate used a coupled enzymatic system (53, 54). An improved coupled enzymatic fluorescence method for measuring blood formate was developed by Shahangian and Ash (55) by eliminating the use of exogenous diaphorase. However, the authors addressed several methodological difficulties resulting from a 10-fold variation in the calibration slope and errors due to variation in the blank fluorescence. These difficulties have been overcome by the single enzyme assay developed in the present study because an end point reaction was used and the blank fluorescence was subtracted from each sample. In the only previously reported study of arterial blood formate levels in rabbits, a less accurate gas liquid partition chromatography method was used and a wide range of results was obtained (0.05–0.46 mM) (39). In rats, McMartin et al. (56) using the coupled enzymatic assay, reported a blood formate level of zero in two normal rats and 0.04 and 0.11 mM at 24 h after methanol poisoning. The present results indicate that the concentration of formate used in previous proximal tubule NaCl transport studies likely exceeds the concentration of formate to which the proximal tubule in exposed in vivo in both the luminal and peritubular solutions. It would be of interest to determine the in vivo proximal tubule luminal formate concentration by micropuncture.

In the absence of a transepithelial Na^+ and Cl^- gradient (and exogenous formate), 1 mM luminal H_2DIDS inhibited J_V by 60%. This is almost identical to the degree of inhibition (67%) of apical Cl^-/base exchange induced by 1 mM H_2DIDS. In addition, the stimulation of J_V by 100 μM luminal formate was completely inhibited by 1 mM luminal H_2DIDS. Interestingly, both in the absence and presence of luminal formate, J_V after the addition of H_2DIDS (lumen) was ~ 0.25 nl/min per mm. These results are compatible with the presence of an apical Cl^-/OH^- exchanger that functions under steady state conditions in the absence of exogenous formate. The finding that luminal stilbenes inhibit J_V without exogenous formate has been reported in the rabbit proximal tubule both in the absence (21) and in the presence of a transepithelial Cl^- concentration gradient (22). In the rat proximal tubule, luminal DIDS inhibited J_V only in the presence of formate (29). Whether this is due to the lower dose of DIDS (0.1 mM) used in the latter study and/or species differences remains to be determined.

The mechanism(s) for the stimulation of J_V by exogenous formate remains unexplained. The results of the present study rule out lumen-to-cell formic acid uptake as an important mechanism for cellular formate accumulation. Whether apical membrane transport processes such as formate/HCO_3^- (OH^-) exchange or Na^+/formate cotransport mediate lumen-to-cell formate uptake in the S_2 proximal tubule despite the failure to detect these transporters in brush border vesicles (25) is unknown. Finally, as has been described in other tissues (57), it is possible that intracellular formate may alter cellular metabolism and indirectly stimulate S_2 proximal tubule NaCl transport. Further studies are needed to examine these possibilities.

Acknowledgments

Funding support was provided by National Institutes of Health grant DK41212-03 (I. Kurtz), Iris and B. Gerald Cantor Foundation (I. Kurtz), NIH grant DK44122-03 (G. Nagami), NIH grant DK40973-03 (N. Yanagawa), American Heart Association Greater Los Angeles Affiliate Award (L. Li), Veterans Affairs Career Development Research Associate Award (C. Emmons), and an American Heart Association Greater Los Angeles Affiliate Grant-in-Aid Award (C. Emmons). Dr. Kurtz is an Established Investigator of the American Heart Association.

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