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Research Article

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Renal Bicarbonate Reabsorption in the Rat

III. Distal Tubule Perfusion Study of Load Dependence and Bicarbonate Permeability

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Abstract

Using continuous microperfusion techniques, we studied the load dependence of bicarbonate reabsorption along cortical distal tubules of the rat kidney and their bicarbonate permeability. Net bicarbonate transport was evaluated from changes in tracer inulin concentrations and total CO_2 measurements by microcalorimetry. Bicarbonate permeability was estimated from the flux of total CO_2 along known electrochemical gradients into bicarbonate-and chloride-free perfusion solution containing 10^{-4} M acetazolamide. Transepithelial potential differences were measured with conventional glass microelectrodes. Significant net bicarbonate reabsorption occurred at luminal bicarbonate levels from 5 to 25 mM, and at perfusion rates from 5 to 30 nl/min. Bicarbonate reabsorption increased in a load-dependent manner, both during increments in luminal bicarbonate concentration or perfusion rate, reaching saturation at a load of 250 pmol/min with a maximal reabsorption rate of approximately 75 pmol/min · mm. Rate of bicarbonate reabsorption was flow dependent at luminal concentrations of 10 but not at 25 mM. During chronic metabolic alkalosis, maximal rates of reabsorption were significantly reduced to 33 pmol/min · mm. The bicarbonate permeability was $2.32 \pm 0.13 \times 10^{-5}$ cm/s in control rats, and $2.65 \pm 0.26 \times 10^{-5}$ cm/s in volume-expanded rats. Our data indicate that at physiological bicarbonate concentrations in the distal tubule passive bicarbonate fluxes account for only 16–21% of net fluxes. At high luminal bicarbonate concentrations, passive bicarbonate reabsorption contributes moderately to net reabsorption of this anion.

Introduction

Bicarbonate reabsorption along the cortical distal tubule of the rat kidney has been studied by both in vivo micropuncture and microperfusion techniques (1–5). Owing to technical difficulties in isolating these segments, studies in isolated perfused mammalian distal tubules have been scarce (5), and no data are available on bicarbonate reabsorption and acidification. In early distal nephron segments of *Amphiuma,* acidification proceeds via a sodium-dependent mechanism (6, 7), similar to that found in the rat thick ascending limb of Henle (8). Investigations on cortical and medullary collecting ducts of rabbit kidney perfused in vitro have provided evidence for an ATP-dependent, electrogenic H-transport mechanism (9). Bicarbonate transport in the perfused cortical collecting duct of rats and rabbits depends on the metabolic state: bicarbonate is reabsorbed in acidotic and secreted in alkalotic animals; in control conditions significant transport of bicarbonate was not observed (10–14).

Free-flow micropuncture studies in rat kidneys, in conditions in which the bicarbonate concentration was measured by both pH microelectrodes and microcalorimetry, show that in the control acid–base status the concentration of this ion remains fairly constant along the distal tubule, whereas that of inulin increases approximately threefold. Thus, significant net bicarbonate reabsorption takes place along this tubule segment (1, 2). Distal reabsorption of bicarbonate is depressed by carbonic anhydrase inhibition (1) and enhanced during potassium depletion (2).

Microperfusion studies of distal bicarbonate transport in vivo have yielded conflicting evidence. Stationary microperfusion studies have shown that the distal tubule epithelium is able to generate significant pH gradients (15). Some recent pump-perfusion studies with calorimetric determination of total CO_2 have, however, failed to detect net bicarbonate reabsorption in control rats, whereas others have reported significant bicarbonate reabsorption in rats on a high protein diet or after overnight fasting (16, 17). Bicarbonate retrieval from the perfusion fluid was also reported in animals subjected to chronic metabolic acidosis (3, 4). Finally, bicarbonate secretion at high luminal flow rates has also been observed (18).

An important determinant of bicarbonate transport by a nephron segment is the passive permeability to this ion. Permeability values of the proximal tubule vary between laboratories, in a range of 10^{-5} to 10^{-4} cm/s (19–21). No comparable values for the distal tubule epithelium are available.

In this study we evaluated the mechanisms of bicarbonate reabsorption in the distal tubule and defined active and passive components of this transport process by determining the bicarbonate permeability and the relationships among flow rate, tubular bicarbonate load, and bicarbonate transport. We also have tested the effects of bicarbonate loading on distal bicarbonate transport.

Methods

Preparation of animals

Male Sprague-Dawley rats weighing 200–300 g (Harlan Sprague-Dawley, Indianapolis, IN) were allowed free access to standard Purina rat chow (Prolab, Waverly, NY; protein content 22%) and tap water until the time of the experiment. Rats were anesthetized by intraperitoneal injection of 100 mg/kg Inactin (Byk-Gulden, Konstanz, Federal Republic of Germany) and prepared for micropuncture as described previously (1, 2). The kidney was exposed by a lumbar approach and the

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left jugular vein and carotid artery cannulated for infusions and collection of blood samples. The rats were kept on a thermostatically heated surgical table, and the kidney immobilized by saline/agar and covered with preheated (37°C) light mineral oil. To minimize extracellular volume depletion, control rats received, during the experiment, an infusion of saline containing 3% mannitol at a rate of 0.05 ml/min. A second group of rats was volume-expanded by acute infusion of 0.2 ml/min saline (starting 1 h before the experiment). Other groups of animals (chronic bicarbonate loading) received a drinking solution of 50 mM $NaHCO_3^-$ for 7–10 d, or 50 mM $KHCO_3^-$ for 10–14 d. The latter group also received during the experiment an infusion of 0.3 M $NaHCO_3^-$ + 10 mM KCl at a rate of 0.05 ml/min. Potassium was administered to prevent the K depletion commonly observed during metabolic alkalosis.

Microperfusion methods

Distal tubules were localized by pump-perfusing late proximal segments with a FDC green-colored perfusion solution. The perfusion pipette was then transferred to an early distal loop and a single micropipette used to inject colored castor oil into the proximal tubule in order to block fluid flow. Fluid was collected for microcalorimetry and for [^{3}H]inulin analyses (21) from a late segment of the same distal tubule placing an oil block distally from the collection site. The principles of the perfusion technique and of measuring bicarbonate (total CO_2 transport) were similar to those described in detail in previous studies (2, 21).

The rate of fluid and bicarbonate transport were estimated according to the expression:

$$V_c = V_0\left(1 - \frac{In_c}{In_0}\right), \tag{1}$$

where V_0 is the perfusion rate (nanoliters per minute), In_0 is the concentration of inulin in the perfusion fluid, and In_c is the concentration of inulin in the collected perfusate. The perfusion pump was calibrated by timed collections of perfusion fluid that was directly delivered into counting vials for measuring of [^{3}H]inulin concentrations. The perfusion rates were confirmed in vivo by timed collections and inulin analysis. Whenever possible, paired perfusions were carried out on the same distal tubule. Net flux of bicarbonate (total CO_2) was derived from the expression:

$$J_{HCO_3^-} = (V_0C_0 - V_cC_c)/L, \tag{2}$$

where V_0 is the perfusion rate, V_c is the collection rate, C_0 is the initial concentration of CO_2, C_c is its concentration in the collected perfusate, and L is the tubule length (in millimeters).

Analytical methods

Collected tubule fluid samples were kept inside the micropipette until determination of total CO_2 in a picapnotherm (World Precision Instruments Inc., New Haven, CT) immediately after the experiment. At that time, the sample was split and part of it was separated for inulin measurements. The oil inside the micropipette and the light mineral oil under which the samples remained during microcalorimetry were preequilibrated with a solution containing 100 mM Hepes, 25 mM $NaHCO_3^-$, and equilibrated with 9% CO_2.

It is recognized that the measurement of total CO_2 at the low levels corresponding to permeability experiments is at the limit of the sensitivity of the picapnotherm method. We have measured a series of samples of water equilibrated with CO_2 and obtained the following results (mean±SD): equilibration with 4% CO_2 in oil, 0.89±0.042 mM (n = 5); equilibration with 6% CO_2 in oil: 1.25±0.27 mM (n = 5). Accordingly, total CO_2 can be measured in the millimolar range with an accuracy (SD/mean) of 5–20%. The samples used in these measurements were of the order of those used in the microperfusion experiments, i.e., 15 nl.

Other possible sources of error in total CO_2 determinations in the present setting of experimental conditions should also be considered. The diffusion of ammonia from blood into the tubule lumen could generate bicarbonate by interacting with carbonic acid (13). In order to prevent major effects due to ammonia addition, we have added 1 mM NH_4 to our perfusion solutions. Nevertheless, it appears prudent to consider the possibility that a small fraction of the bicarbonate in the collected samples may derive from processes not directly related to transepithelial bicarbonate entry.

At the end of the experiment, the perfused tubules were injected with Microfil (Canton Biomedical Products, Boulder, CO), incubated for 1 h with 25% NaOH, and dissected. Camera lucida drawings were used to measure the length of the perfused segments.

Permeability measurements

The permeability of the cortical distal perfused segments ($P_{HCO_3^-}$) was measured during perfusion with initially bicarbonate-free solutions from the following relationship:

$$P_{HCO_3^-} = \frac{J_{HCO_3^-}}{[HCO_3^-]} \times \frac{\exp(EF/RT) - 1}{EF/RT}. \tag{3}$$

Here $J_{HCO_3^-}$ is the passive bicarbonate backflux from blood to lumen, $[HCO_3^-]$ is the log mean bicarbonate concentration difference along the perfused segment, and E is the transepithelial potential difference (PD) measured during the experiment by means of Ling-Gerard microelectrodes filled with 1 M KCl. F, R, and T have their conventional meanings. Transepithelial electrical PD measurements were recorded in late distal tubule segments during the collection of the perfusate in experiments where bicarbonate permeabilities were measured. To minimize shunting through the puncture hole, they were performed at a site of approximately three to four tubule diameters upstream of the collection pipette. Resistance of the electrode was between 50 and 100 Ω, tip potentials < 5 mV. Corrections for tip asymmetries were done by recording the tip potentials in the perfusion solutions. In view of the fact that several sources of error tend to increase the bicarbonate concentration in the lumen (see above), the calculated bicarbonate permeabilities represent apparent permeabilities and what we consider maximal values.

Solutions

Two types of experiments were carried out, one to evaluate the relationship between luminal bicarbonate load and bicarbonate transport under conditions of different bicarbonate loading, and a second to measure the apparent bicarbonate permeability across the distal tubule.

In experiments designed to measure bicarbonate reabsorption, the following solutions were used:

Solution 1. This perfusion solution was chosen to investigate bicarbonate transport under conditions of minimal transepithelial fluid flow. It had the following composition: 25 mM NaCl, 25 mM $NaHCO_3^-$, 2 mM KH_2PO_4, 1 mM $CaCl_2$, 200 mM raffinose.

Solution 2. In experiments designed to measure net bicarbonate reabsorption at different flow rates and different luminal concentrations in the presence of fluid reabsorption we used the following solution: 5, 10, or 25 mM $NaHCO_3^-$, NaCl to complete 150 mM Na^+, 1 mM $CaCl_2$, and 4 mM KCl.

For the measurement of bicarbonate permeability, the following solution was used:

Solution 3. 50 mM Na gluconate, 2 mM KH_2PO_4, 1 mM $CaCl_2$, 1 mM $(NH_4)_2SO_4$, 200 mM raffinose, 0.1 mM acetazolamide. Gluconate substituted for Cl to exclude the possibility of bicarbonate secretion, in view of the observation that bicarbonate can be exchanged for chloride, leading to net secretion of this anion (10). Acetazolamide was added to the perfusion solution to minimize bicarbonate reabsorption and secretion (10, 11). Acetazolamide was also infused at a rate of 20 mg/kg after a prime of 20 mg/kg. This solution was preequilibrated with 9% CO_2.

Blood pH and P_{CO_2} were determined in a pH-meter/blood gas analyzer (Instrumentation Laboratory, Inc., Lexington, MA). Total CO_2 of plasma was measured by a CO_2 analyzer (Corning Medical,

Table I. Blood and Urine Data for the Experimental Groups Used

	Control	Chronic $NaHCO_3$	Chronic $KHCO_3$
Blood pH	7.37±0.01 (21)	7.40±0.01 (3)	7.46±0.01 (4)*‡
Blood P_{CO_2} (*mm Hg*)	44.0±0.45 (21)	42.8±0.02 (3)	48.2±0.34 (4)*‡
Plasma [HCO_3] (*mM*)	25.4±0.51 (21)	26.2±0.25 (3)	32.8±0.87 (4)*‡
Plasma [K^+] (*mM*)	4.00±0.05 (4)	3.74±0.09 (6)	4.55±0.03 (4)*‡
Urine pH	6.03±0.03 (8)	6.38±0.06 (6)*	6.33±0.03 (4)*

Chronic $NaHCO_3$: rats received 50 mM $NaHCO_3$ as drinking solution for 7–10 d. Chronic $KHCO_3$: rats received 50 mM $KHCO_3$ as drinking solution for 10–14 d, and 0.3 M $NaHCO_3$ plus 10 mM KCl at a rate of 0.05 ml/min during the experiment.
* $P < 0.05$ against control; ‡ $P < 0.05$ against $NaHCO_3$.

Medfield, MA). Statistical analysis was performed by Student's *t* test, and by analysis of variance and Scheffe contrasts when more than two groups were compared (22).

Results

Acid–base status of experimental animals. Blood and urine data obtained in animals on a control diet and in rats receiving HCO_3^- for different periods of time are summarized in Table I. The control group represents pooled data of all rats on a normal pellet diet. It is apparent that the two experimental conditions in which bicarbonate was administered led to a modest alkaline shift of pH and elevation of plasma bicarbonate concentration. We also note that plasma potassium levels were slightly increased in the group of animals receiving potassium.

Distal bicarbonate reabsorption. Table II shows results of experiments designed to analyze bicarbonate reabsorption along distal tubules during perfusion with raffinose-containing bicarbonate-Ringer's solution (solution 1), a condition in which fluid reabsorption was absent (23). Bicarbonate concentrations in the collected perfusate are uniformly reduced ($P < 0.01$), especially at low perfusion rates. These experiments demonstrate the ability of distal tubules of this group of control rats to lower luminal bicarbonate concentrations at perfusion rates ranging from 5 to 20 nl/min. It also demonstrates that bicarbonate reabsorption does not depend on net fluid transport.

The data summarized in Table III demonstrate that significant bicarbonate reabsorption is also present when distal tubules are perfused with isotonic Ringer's solution (solution 2) (*a*) at different bicarbonate concentrations and (*b*) at various perfusion rates. Bicarbonate concentrations in the collected perfusate are uniformly reduced, except at the highest perfusion rate (30 nl/min), at which a small, statistically not significant fall of bicarbonate reabsorption was observed, indicating that more bicarbonate than fluid is reabsorbed. Bicarbonate reabsorption increased markedly, both in experiments in which either bicarbonate concentrations were increased from 5 to 25 mM at a perfusion rate of 10 nl/min, or perfusion rates elevated from 5 to 20 nl/min at a concentration of 10 mM bicarbonate in the perfusion fluid. Only at the highest luminal bicarbonate concentrations (22.5–25.1 mM) was the situation different. As shown in Tables II and III, raising the perfusion rate from 5 to 30 nl/min at these high bicarbonate concentrations does *not* stimulate bicarbonate reabsorption significantly. Taken together the data indicate that with the exception of luminal concentration of 25 mM, bicarbonate reabsorption increases both with augmentation of flow and bicarbonate concentration.

Fig. 1 summarizes the relationship between the luminal load of bicarbonate and bicarbonate reabsorption. It is apparent that distal bicarbonate reabsorption is load-dependent up to ~ 250 pmol/min (e.g., 10 nl/min at 25 mM bicarbonate), reaching a reabsorption rate of 70–80 pmol/min · mm.

We have also investigated the behavior of the distal bicarbonate transport system during systemic bicarbonate loading (Table IV). It is apparent that a modest but significant increase in blood pH follows the bicarbonate loading maneuvers. This subgroup of control animals was mildly acidotic, whereas bicarbonate administration for 7–10 d, or $KHCO_3^-$ drinking for 10–14 d resulted in a progressive albeit modest increase in blood pH and bicarbonate. The main observation is a significant fall of distal bicarbonate reabsorption with elevation of plasma bicarbonate and pH. Net bicarbonate secretion along distal tubules could not be detected, even in animals exposed for as long as 2 wk to chronic and acute bicarbonate loading. The rationale for administering potassium was to prevent a state of potassium depletion that might accompany chronic bicarbonate loading. Although this has been achieved in our studies as reflected by the maintenance of higher plasma potassium levels, we cannot exclude a direct inhibitory effect of the potassium supplementation on bicarbonate transport.

Results of permeability measurements. Inspection of the data summarized in Table V indicates that during perfusion with initially bicarbonate-free perfusion solutions bicarbonate concentrations in collected distal fluid perfusates are uniformly low, indicative of only limited bicarbonate entry along the steep transepithelial bicarbonate gradient. Also summarized are passive bicarbonate fluxes from blood to lumen. These are only a modest fraction of net bicarbonate reabsorption (see Tables III and IV), of the order of 13 pmol/min · mm, compared with values of 50–80 pmol/min · mm for net reab-

Table II. Bicarbonate Reabsorption by Cortical Distal Tubules at Different Perfusion Rates in the Absence of Volume Flow (200 mM Raffinose in Perfusion Solution)

Perfusion rate	Length	Plasma HCO_3	Total CO_2 of perfusion solution	Total CO_2 of collected perfusion	J_{HCO_3}	Perfusion (*n*)	Rats (*n*)
nl/min	*mm*	*mM*	*mM*	*mM*	*pmol/min · mm*		
5	1.36±0.07	23.5±1.30	22.9±0.02	6.10±1.07	66.0±3.08	5	3
10	1.38±0.06	23.4±0.89	22.7±0.13	12.4±0.79	75.9±5.63	8	4
20	1.50±0.27	23.8±1.08	22.5±0.23	18.0±1.23	64.5±14.8	4	2

Perfusion with solution 1 (see Methods). Blood pH of rats in this table = 7.36±0.01 (9); P_{CO_2} = 42.5±0.24 (7) mm Hg.

Table III. Bicarbonate Reabsorption by Cortical Distal Tubules at Different Luminal Bicarbonate Concentrations and Flow Rates in Control Rats

Perfusion	Length	Plasma HCO_3	Total CO_2 of perfusion solution	Total CO_2 of collected fluid	J_{HCO_3}	J_v	Tubules perfused	Rats
nl/min	*mm*	*mM*	*mM*	*mM*	*pmol/min · mm*	*nl/min · mm*	*n*	*n*
Perfusion with 5 mM HCO_3								
9.9±0.04	1.22±0.06	23.6±0.12	5.2±0.04	3.85±0.15	17.1±0.50	1.66±0.06	10	3
Perfusion with 10 mM HCO_3								
5.0±0.03	1.16±0.06	23.8±0.43	10.1±0.02	7.87±0.12	17.0±0.74	0.89±0.02	9	3‡
10.1±0.02	1.23±0.05	22.9±0.32	10.2±0.07	8.96±0.11	26.1±0.50	1.76±0.05	9	3
20.3±0.20	1.16±0.06	23.8±0.43	10.1±0.02	7.92±0.10	55.2±3.05	2.02±0.11	9	3
Perfusion with 25 mM HCO_3								
10.1±0.18	1.45±0.07	24.4±1.30	25.1±0.09	21.6±0.47	70.2±4.11	2.06±0.05	9	3
20.4±0.74	1.38±0.13	24.4±1.30	23.9±0.44	21.3±0.56	76.7±7.56	1.72±0.05	5	4*
30.0±0.15	1.38±0.13	24.4±1.30	23.9±0.44	23.5±0.47	61.8±7.00	2.28±0.21	5	4*

Perfusion with solution 2 (see Methods). Mean blood pH of rats in this table = 7.36±0.015 (13); P_{CO_2} = 43.3±0.45 mm Hg (13). *‡ paired perfusions (different perfusion rates in the same tubule).

sorptive transport at a luminal concentration of 25 mM $NaHCO_3^-$. The mean value of the bicarbonate permeability is 2.32×10^{-5} cm/s, a value in the lower range of that observed in the proximal tubule (19–21). As discussed above, the value measured in these experiments is an upper limit of the distal bicarbonate permeability.

In similar studies on proximal tubules a modest increase in bicarbonate permeability was observed during extracellular volume expansion (24). Table VI summarizes relevant data for the distal tubule. The mean value of bicarbonate permeability of 2.65×10^{-5} cm/s is not significantly different from the control value. Accordingly, expansion of extracellular volume does not enhance passive fluxes of bicarbonate across distal tubule epithelium.

Discussion

Heterogeneity of the cortical distal tubule. An important question concerning the presently measured net bicarbonate transport rates and permeability values is the segment of the distal tubule under study. The cortical distal tubule is a heterogeneous segment, starting with the distal convoluted tubule, continuing with the connecting tubule and ending with the initial collecting tubule, a structure resembling the cortical collecting duct epithelium (25). In our studies perfusions were performed between an "early" and a "late" loop, the perfused length being of the order of 1.2–1.5 mm. This perfused segment includes at least parts of all of the described segments. Hence, the reported data reflect the overall behavior of the distal segments between an early and a late loop.

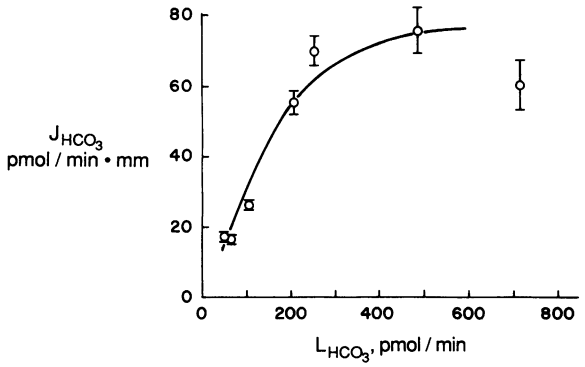

Figure 1. Bicarbonate reabsorption as function of bicarbonate load. Data from Table III summarizing experiments in which luminal flow rate or bicarbonate concentration were varied and in which net fluid reabsorption was observed.

Bicarbonate transport in distal tubules. The present data obtained in continuous perfusion studies confirm earlier free-flow micropuncture results from our laboratories that the distal tubule reabsorbs significant amounts of bicarbonate. As demonstrated in other perfusion studies in which a compara-

Table IV. Distal Bicarbonate Reabsorption in Control and Bicarbonate-loaded Rats

	Group		
	Control	B1	B2
Blood pH	7.34±0.02 (3)	7.40±0.01 (3)*	7.46±0.01 (4)*
P_{CO_2} (*mm Hg*)	46.9±0.90 (3)	42.8±0.02 (3)*	48.2±0.34 (4)‡
HCO_3 (*mM*)	24.4±1.30 (3)	26.2±0.25 (3)	32.8±0.87 (4)*‡
Perfusion rate (*nl/min*)	10.1±0.18 (9)	10.0±0.03 (9)	10.1±0.03 (11)
Length (*mm*)	1.45±0.07 (9)	1.32±0.09 (9)	1.44±0.04 (11)
J_v (*nl/min · mm*)	2.06±0.05 (9)	1.65±0.05 (9)*	1.20±0.02 (11)*‡
Total perfused CO_2 (*mM*)	25.1±0.09 (9)	25.1±0.04 (9)	25.2±0.09 (11)
Total collected CO_2 (*mM*)	21.6±0.47 (9)	23.0±0.18 (9)*	24.7±0.08 (11)*‡
J_{HCO_3} (*pmol/min · mm*)	70.2±4.11 (9)	53.9±0.87 (9)*	33.0±0.86 (11)*‡

Control group is part of data given in Table III. Groups: B1, chronic drinking of 50 mM $NaHCO_3$ for 7–10 d; B2, chronic drinking of 50 mM $KHCO_3$ for 10–14 d plus acute infusion of 0.3 M $NaHCO_3$ + 10 mM KCl at 0.05 ml/min. Perfusion with solution 2.
* $P < 0.05$ against control; ‡ $P < 0.05$ against B1.

Table V. Capillary–Lumen Bicarbonate Backflux ($-J_{HCO_3^-}$) and Bicarbonate Permeability ($P_{HCO_3^-}$) of Distal Tubules of Control Rats

Tubule	Perfusion rate	Length	Total perfused CO_2	Total collected CO_2	PD	$J_{HCO_3^-}$	$P_{HCO_3^-}$
	nl/min	*mm*	*mM*		*mV*	*pmol/mm · min*	*cm/s · 10^{-5}*
1	10.1	1.24	2.1	3.6	−26	12.6	2.08
2	10.0	1.34	2.1	4.0	−31	13.9	2.68
3	10.2	1.18	2.1	3.9	−23	15.3	2.45
4	9.9	1.42	1.9	3.8	−30	13.6	2.65
5	10.1	1.16	1.9	4.1	−22	18.9	3.18
6	9.8	1.13	1.8	3.2	−25	12.5	2.01
7	10.1	1.52	1.8	3.6	−32	11.6	2.28
8	10.1	1.28	1.8	3.3	−27	11.6	2.01
9	10.0	1.42	1.9	3.4	−28	10.9	1.87
10	10.0	1.61	1.9	3.5	−33	10.1	1.97
M	10.05	1.33	1.9	3.6	−27.7	13.1	2.32
SE	0.03	0.05	0.23	0.10	1.20	0.80	0.13

Mean blood pH: 7.39±0.01 (4); plasma HCO_3^-: 26.9±0.40 (4) mM; P_{CO_2}: 44.8±0.30 mm Hg (4). Perfusion with solution 3. Data obtained in four rats, two to three perfusions per rat.

ble load of bicarbonate entered this tubule segment, the absolute rate of bicarbonate reabsorption lies between that of the proximal tubule and the cortical collecting duct. The net reabsorption of bicarbonate ranged between 70 and 80 pmol/min · mm at a load of 250 pmol/min whereas the proximal tubule, at similar loads, reabsorbed 150 pmol/min · mm (26). This contrasts with the much lower reabsorption rates of 8.4 pmol/min · mm, at a load of 46 pmol/min, in the rat cortical collecting tubule in acidotic conditions (12). The presently found rate of 26.1 pmol/min · mm, at a load of 100 pmol/min (see Table III, 10 nl/min at a concentration of 10 mM HCO_3^-), compares with 49.4 pmol/min · mm, at a load of 95 pmol/min, obtained in free-flow studies in control rats (2). Thus, the value found in our perfusion study is lower than that under free-flow conditions but higher than other microperfusion studies where bicarbonate transport ranged from −11 to +16 pmol/min · mm (3, 4, 16, 17). This difference may be due to the absence of unknown solutes in the artificial perfusion solution. We have recently observed stimulation of distal tubule fluid and electrolyte transport during perfusion with native tubule fluid (Malnic, G., Berliner, and G. Giebisch, unpublished observations).

There has been controversy whether the rat distal tubule normally reabsorbs significant amounts of bicarbonate. Whereas evidence for significant bicarbonate reabsorption was obtained in free-flow (1, 2, 27), in stopped flow microperfusions (15), in the present continuous perfusion study and in similar studies of Kunau and Walker (16), it was not consistently detected during control acid-base conditions by Lucci et al. (3) and Levine (4). Also, in recent studies DuBose et al. (28) have not observed an acid disequilibrium pH in control acid-base conditions.

Table VI. Capillary–Lumen Bicarbonate Backflux ($-J_{HCO_3^-}$) and Bicarbonate Permeability ($P_{HCO_3^-}$) of Distal Tubules of Control Rats Undergoing Volume Expansion (Infusion of 0.2 ml/min NaCl 0.9%)

Tubule	Perfusion rate	Length	Total perfused CO_2	Total collected CO_2	PD	$J_{HCO_3^-}$	$P_{HCO_3^-}$
	nl/min	*mm*	*mM*		*mV*	*pmol/mm · min*	*cm/s · 10^{-5}*
1	10.2	1.12	1.9	3.1	−19	10.5	1.74
2	10.2	1.44	1.9	3.6	−24	15.3	2.14
3	10.0	1.08	1.9	3.2	−22	12.5	2.09
4	9.9	1.56	1.9	3.8	−32	12.0	2.42
5	10.2	1.18	1.9	3.7	−26	15.7	2.73
6	10.2	1.24	1.8	4.0	−34	18.1	4.29
7	10.2	1.51	1.8	3.9	−36	14.0	3.48
8	10.2	1.33	1.8	3.8	−26	15.0	2.56
9	10.1	1.16	1.8	3.5	−24	14.9	2.38
M	10.13	1.29	1.9	3.6	−27.0	14.2	2.65
SE	0.03	0.06	0.10	0.10	1.9	0.70	0.26

Cf. legend of Table V. Mean blood pH: 7.38±0.01 (4); plasma HCO_3^-: 25.6±0.90 (4) mM; P_{CO_2}: 43.9±0.50 mm Hg (4). Perfusion with solution 3. Data from four rats, two to three perfusions per rat.

Levine et al. (17) have recently drawn attention to the fact that fasting and correlated urine pH changes exert a significant influence on distal bicarbonate transport. Based on experiments in distal microperfusion studies, these investigators reported that fasting animals overnight induced distal bicarbonate reabsorption, whereas access to food (22% protein) led to the appearance of net bicarbonate secretion. Secretion of bicarbonate could also be induced in these studies in fasted animals given an acute bicarbonate load. In our experiments, animals also had access to food (22% protein) until the time of the experiment, yet we were unable to demonstrate net bicarbonate secretion under these conditions and even in animals receiving an exogenous bicarbonate load for 7–14 d. It is possible that subtle differences in the state of hydration (29)—high levels of vasopressin stimulate distal bicarbonate reabsorption —in protein intake (16), in the acid–base status, may account for the discrepancies that have been reported by various investigators. However, we note that the presence of significant bicarbonate reabsorption in the present perfusion studies and those of Kunau and Walker (16) are entirely compatible with all reported free-flow micropuncture results that have demonstrated brisk bicarbonate reabsorption in experimental conditions very similar to those employed in the present perfusion studies (1, 2).

Flow and load dependence of bicarbonate transport. We observed that bicarbonate reabsorption was markedly flow-dependent in the low but not the high range of luminal bicarbonate concentrations. A similar behavior had been observed by Alpern et al. (26) in perfusion studies of proximal bicarbonate transport. As a possible explanation, these investigators advanced the hypothesis that radial diffusion limitation of bicarbonate ions occurs at low flow rates at an unknown site along the transepithelial pathway for bicarbonate movement. It was further postulated that either high concentrations of bicarbonate in the lumen or high flow rates reduce the diffusion limitation of bicarbonate transport, but that these events are not additive. If this situation were to apply to the distal tubule, one might similarly expect that bicarbonate reabsorption would not be further stimulated by enhanced flow rates once the luminal bicarbonate concentration had been significantly increased.

A significant reduction of bicarbonate reabsorption was observed in rats that had been exposed to a chronic bicarbonate load. Since care was taken to prevent reduction in plasma potassium levels and K depletion (group B2 in Table IV), a condition that stimulates distal H^+ secretion (27), it is unlikely that bicarbonate secretion had been masked by enhanced H^+ secretion due to K^+ deficiency. Of course, our experiments do not exclude the possibility that the observed fall in net bicarbonate transport in chronic bicarbonate loading might have been caused by stimulation of bicarbonate secretion at a time when bicarbonate reabsorption was still large enough to result in the observed overall net reabsorption.

Bicarbonate permeability in distal tubules. The bicarbonate permeability measured in our experiments was derived from assessing the net flux of bicarbonate along a known electrochemical potential gradient of this ion. We have attempted to minimize several possible sources of error. First, if H ion secretion by the distal tubule were to titrate any of the bicarbonate entering the tubules this would result in an underestimate of the permeability. This possibility has been eliminated by the application of luminal and systemic acetazolamide, in concentrations known to inhibit cellular H ion secretion (1, 30). Secondly, if bicarbonate were to enter the tubule lumen by a chloride-bicarbonate secretory mechanism such as has been demonstrated in the cortical collecting tubule, a tubule segment also lined with principal and intercalated cells (10), such nondiffusive anion movement would lead to an overestimate of bicarbonate permeability (10). By removing chloride from the luminal perfusion fluid, this source of error has also been minimized in the present series. Finally, secretion of ammonia may generate luminal bicarbonate if it were to interact with carbonic acid. The appearance of bicarbonate in the lumen not derived from transepithelial diffusion would similarly result in an overestimate of bicarbonate permeability. Addition of ammonia to our perfusion solutions had the purpose of avoiding this error. Accordingly, we believe that our measurements of distal bicarbonate permeability, despite some remaining uncertainties, represent realistic estimates of the physiologically relevant bicarbonate permeability. We realize that errors in the permeability measurements will affect the magnitude of these fluxes.

It is most reasonable to assume that the transport path of bicarbonate across the distal tubule epithelium is through the paracellular shunt pathway. This view is based on observations of the apical permeability properties of principal and intercalated cells, both of which do not indicate measurable anion permeabilities (31, 32). Although not extensively studied, it is also not likely that cell membranes of the distal convoluted tubule have significant bicarbonate permeabilities.

Measurements of the overall distal tubule NaCl permeability, either by monitoring NaCl appearance into raffinose-containing perfusion fluids (23) or from estimates of tracer experiments (33) yield data of the order of $1.4–5 \times 10^{-5}$ cm/s, of similar magnitude of the value of 2.32×10^{-5} cm/s observed in the present series of experiments. Converting this value to a conductance term of the distal epithelium (34) yields a specific resistance of 285 Ω/cm^2. This value compares to the range of 40–180 Ω/cm^2 reported by DeBermudez and Windhager (35), the lower data corresponding to late distal segments. Our bicarbonate conductance thus represents 14–60% of total distal epithelial conductance.

Active and passive components of distal bicarbonate transport. The results obtained in this study provide the means of analyzing the components of bicarbonate transport in the distal tubule. Fig. 2 summarizes relevant transport parameters. The following values were used: the control bicarbonate permeability, early (8.0 mM) and late (7.7 mM) distal tubule bicarbonate concentrations (from Eq. 2), a transepithelial PD of −20 mV for early and −40 mV for late distal tubules, and bicarbonate reabsorption rates from control free-flow conditions (2). Passive backflux of bicarbonate was calculated by Eq. 3. At physiological distal bicarbonate concentrations in a normal acid–base balance (Fig. 2) the passive backflux component of bicarbonate is 14–18% of the rate of active bicarbonate reabsorption.

A maximal net bicarbonate reabsorption of the order of 80 pmol/min · mm was observed in our experiments. Since this value was found in the absence of a transepithelial bicarbonate concentration gradient (see Table III) the maximal active H-ion secretory rate must be of similar magnitude. This maximal transport rate compares to a proximal maximum of 200 pmol/min · mm (26). In the free-flow micropuncture studies of Capasso et al. (2), acute bicarbonate loading resulted in a net

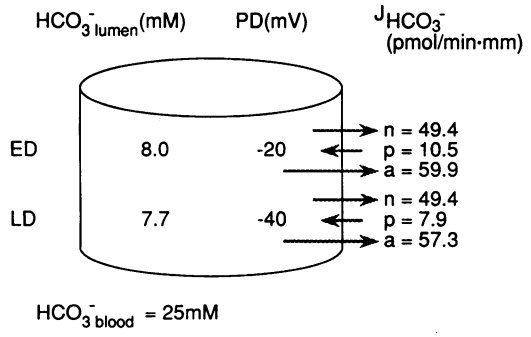

Figure 2. Schematic representation of components of bicarbonate reabsorption in control distal tubule. Net flux and lumen concentrations from Capasso et al. (2). Passive bicarbonate transport is 21% of net reabsorption in early distal and 16% in late distal tubule. Abbreviations: ED, early distal; LD, late distal; a, active component, p, passive component; n, net flux (reabsorption).

reabsorptive rate of 138 pmol/min · mm. Under these conditions, distal bicarbonate levels as high as 70 mM, at a plasma level of 40 mM, were observed (1, 2). This transepithelial concentration gradient, at a PD of −40 mV, could promote an additional component of passive reabsorption of 60 pmol/min · mm of bicarbonate across the distal epithelium. Total net reabsorption would be 140 pmol/min · mm, a value approaching that found in free-flow experiments. We conclude that in bicarbonate-loaded rats passive bicarbonate reabsorption contributes significantly to net transport of this anion along the cortical distal tubule.

Conclusion. Our studies demonstrate significant reabsorption of bicarbonate along the distal tubule in control acid-base conditions. Elevation of perfusion rate and of luminal bicarbonate concentrations stimulate bicarbonate reabsorption rates in a load-dependent manner. The magnitude of distal bicarbonate permeability is in the lower range of that in the proximal epithelium. Passive bicarbonate fluxes play only a modest role in modulating distal bicarbonate transport with the exception of conditions in which the distal bicarbonate concentrations significantly exceed those in plasma. The active transport component of bicarbonate saturates at 80 pmol/min · mm; this value is significantly reduced by metabolic alkalosis.

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