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J Clin Invest. 1993;91(6):2776-2784. https://doi.org/10.1172/JCI116519.

Research Article

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Renal Bicarbonate Reabsorption in the Rat

IV. Bicarbonate Transport Mechanisms in the Early and Late Distal Tubule

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Abstract

Bicarbonate transport was studied in vivo by separate microperfusion experiments of early and late distal tubules. Total CO_2 was measured by microcalorimetry and fluid absorption by ^{3}H-inulin. Significant bicarbonate absorption was observed in all experimental conditions. Bicarbonate transport was load-dependent upon increasing the luminal bicarbonate concentration from 15 to 50 mM in both early and late distal tubule segments and remained constant at higher concentrations at a maximum rate of 100–110 pmol/min per mm. At low lumen bicarbonate concentrations (15 mM), higher rates of bicarbonate absorption were observed in early (32.9±4.57 pmol/min per mm) as compared to late distal tubules (10.7±3.1 pmol/min per mm). Amiloride and ethyl-isopropylamiloride both inhibited early but not late distal tubule bicarbonate absorption whereas acetazolamide blocked bicarbonate transport in both tubule segments. Fluid absorption was significantly reduced in both tubule segments by amiloride but only in early distal tubules by ethyl-isopropylamiloride. Substitution of lumen chloride by gluconate increased bicarbonate absorption in late but not in early distal tubules. Bafilomycin A1, an inhibitor of H-ATPase, inhibited late and also early distal tubule bicarbonate absorption, the latter at higher concentration. After 8 d on a low K diet, bicarbonate absorption increased significantly in both early and late distal tubules. Schering compound 28080, a potent H-K ATPase inhibitor, completely blocked this increment of bicarbonate absorption in late but not in early distal tubule. The data suggest bicarbonate absorption via Na^+-H^+ exchange and H-ATPase in early, but only by amiloride-insensitive H^+ secretion (H-ATPase) in late distal tubules. The study also provides evidence for activation of K^+-H^+ exchange in late distal tubules of K depleted rats. Indirect evidence implies a component of chloride-dependent bicarbonate secretion in late distal tubules and suggests that net bicarbonate transport at this site results from bidirectional bicarbonate movement. (*J. Clin. Invest.* 1993. 91:2776–2784.) Key words: bicarbonate absorption • bicarbonate secretion • early distal tubule • late distal tubule • amiloride

Introduction

The superficial distal tubule, a heterogeneous nephron segment, contributes significantly to the retrieval of filtered bicarbonate from the tubule fluid (1–11). Several transport mechanisms have been implicated in this process including Na^+-H^+ exchange, electrogenic H^+ secretion and K-H exchange (12–15). In addition, bicarbonate secretion by electroneutral exchange of luminal Cl for HCO_3 also affects net bicarbonate transport (16). The distal tubule consists of three main segments, the distal convoluted tubule, the connecting tubule, and the initial collecting duct (17, 18). Little is known about the mechanisms of acidification in the distal convoluted tubule, an extension of the thick ascending limb in which Na^+-H^+ exchange has been observed in the rat kidney (19). On the other hand, the initial collecting tubule contains several populations of intercalated cells, known to be responsible for both H^+ and HCO_3^- secretion (15, 16, 20, 21).

In the present study we evaluate the mechanisms of bicarbonate transport by separate perfusion of early and late segments of the distal tubule. These studies provide evidence for different modes of H^+ and HCO_3^- transport in these tubule segments, for significant load dependence of bicarbonate absorption and for activation of K^+-H^+ exchange in K depletion. We also observed dependence of late distal bicarbonate absorption upon luminal chloride, suggesting chloride-dependent bicarbonate secretion. Thus, net absorption of bicarbonate along the distal tubule is likely to result from simultaneous transport of bicarbonate in opposite directions.

Methods

Preparation of animals. Experiments were carried out on male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN), weighing 200–300 g and kept on a commercial rat chow (Ralston Purina, St. Louis, MO) and tap water until the experiment. Low K rats were kept on low K diet for 8 d (Low K diet; Teklad, Madison, WI). After anesthesia by 100 mg/kg Inactin (Byk-Gulden, Konstanz, Germany), rats were prepared for microperfusion as previously described (7, 22). After performance of a tracheotomy, the left jugular vein was cannulated with two catheters, one for infusion of saline (0.9% NaCl) at a rate of 1.5 ml/h, the other for administering an additional 1 ml of saline during the surgical preparation. The left carotid artery was also cannulated and served for monitoring the blood pressure to ascertain that it was maintained during all experiments at levels > 90 mmHg.

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Received for publication 17 April 1991 and in revised form 5 January 1993.

J. Clin. Invest.

0021-9738/93/06/2776/09 $2.00
Volume 91, June 1993, 2776–2784

Table I. Composition of Perfusion Solutions

mM	1	1a	2	2a	3	3a	4	4a	5	5a
Na^+	70	70	70	70	70	70	146	72	146	75
Cl^-	59	2	49	14	24	14	90	14	75	2
HCO_3^-	15	15	25	25	50	50	60	60	75	75
K^+	2	2	2	2	2	2	2	2	2	2
Ca^{2+}	1	1	1	1	1	1	1	1	1	1
Urea	10	10	10	10	10	10	10	10	10	10
Gluconate	—	57	—	35	—	10	—	2	—	2

mM, Concentrations are given as in mM/liter; 1–5, solutions 1–5 contained high Cl^-; 1a–5a, solutions 1a–5a contained low Cl^-.

The carotid catheter was also used for collecting blood samples for chemical analyses.

The left kidney was exposed laterally by a flank incision, immobilized in a double cup, and covered with light mineral oil, which served to keep the temperature at 37°C throughout the experiments. The left ureter was catheterized with a short piece of PE-10 tubing to allow unimpeded flow of urine. A fiber-optic light source served to illuminate the surface of the kidney.

Microperfusion methods. Distal tubules were localized by pump perfusing late proximal segments with FDαC green colored perfusion solution. The procedure for selecting early and late distal tubule loops followed that described by Velazquez et al. (23). Briefly, an early or late distal tubule segment was impaled by the microperfusion pipette and perfused at a rate of 12 nl/min with a microperfusion pump (type III; W. Hampel, Frankfurt, Germany). The last surface segment of the proximal tubule was injected with Sudan black–colored castor oil to block fluid flow. A small hole was placed upstream of the oil block to allow proximal tubule fluid to escape. A collecting pipette filled with Sudan black–stained heavy mineral oil was then inserted into the second (for early distal perfusions) or last distal tubule segment (for late distal perfusions) provided the tubule under investigation had more than two loops on the surface. An oil block was carefully placed downstream and fluid collected while maintaining a stable oil block. Tubule fluid was collected for 6–8 min, and the pipette withdrawn into the oil covering the kidney surface to aspirate a small amount of oil into the pipette tip.

After the end of the experiment, tubules were filled with high viscosity Microfil (Canton Biomedical Products, Boulder, CO) and the kidney was excised and stored overnight in deionized water at 4°C. On the day after the experiment, the kidney was macerated in 25% of NaOH for 20 min. The latex casts of the kidney were dissected using a drawing device of a Wild stereoscopic microscope to measure the length of the perfused tubule segments. Three measurements were made: (*a*) the distance between the macula densa and the perfusion site; (*b*) the distance between the perfusion and collection site (perfused lengths of tubule); and (*c*) the distance between collection site and junction of the tubule with another nephron. The early distal tubule was defined as a segment within the first half and the late distal tubule was defined as a segment within the second half of the cortical distal tubule.

The method used to measure total CO_2 has been described previously and was carefully followed (4, 7). Collected samples were stored under mineral oil preequilibrated with a solution containing 100 mM Hepes and 25 mM $NaHCO_3$ at 9% CO_2. 10-nl aliquots were compared with 5, 10, 15, 25, 50, 60 and 80 mM sodium carbonate standards. The calibration curve was linear and its correlation coefficient always > 0.99.

The fluid volume of the samples was measured in a constant-bore glass capillary. The radioactivity of ^3H-inulin (New England Nuclear, Boston, MA) of the samples was measured in a liquid scintillation counter.

Net fluid absorption (J_V) was calculated according to the equation

$$J_V = V_O - VL,$$

where VL is the measured rate of fluid collection at length L, and

$$V_O = VL(INL/IN_O),$$

where INL/IN_O is the ratio of collected over perfused fluid radioactive inulin.

The net flux of bicarbonate (total CO_2) was obtained from

$$JHCO_3 = V_O(HCO_3)_O - VL(HCO_3)_L,$$

where $(HCO_3)_O$ is the concentration of bicarbonate in the original perfusion fluid, and $(HCO_3)_L$ is the concentration of HCO_3 in the collected fluid. J_V and $JHCO_3$ were expressed per millimeter of tubular length.

The solutions used in this study are given in Table I. Solutions used to measure net bicarbonate absorption at constant lumen perfusion rate but at different lumen bicarbonate concentrations contained 15, 25, 50, 60 or 75 mM $NaHCO_3$ and enough NaCl to achieve an osmolality of 150 mosmol. High Cl solutions containing 60 and 75 mM HCO_3 had their osmolality adjusted to 300 mosmol by NaCl. In addition, they contained 1 mM $CaCl_2$ and 2 mM KCl. Low chloride solutions (2–14 mM Cl) contained 15–75 mM $NaHCO_3$, 2 mM K-gluconate, and 2 mM $CaCl_2$. Its total osmolality was adjusted to 150 mosmol with sodium gluconate. Before use, they were bubbled at room temperature with 5% CO_2/95% O_2, except for solutions of 50–75 mM $NaHCO_3$, which were bubbled with 10 vs 90% O_2 to avoid large pH increases.

Amiloride and acetazolamide were obtained from Sigma Immunochemicals (St. Louis, MO). Ethyl-isopropylamiloride (EIPA)[1] was kindly provided by D. C. Batlle, Northwestern University (Chicago, IL). Bafilomycin A1 was a gift from Prof. K. Altendorf, Department of Microbiology, University of Osnabruck (Osnabruck, Germany) and Schering compound 28080 (SCH 28080) was obtained from Schering-Plough (Kenilworth, NJ).

Blood pH and P_{CO_2} were determined in a pH/blood gas analyzer (model 168; Corning Inc., Corning, NY). Plasma electrolytes were measured by flame photometry. Statistical analysis was performed by Student's *t* test, and by ANOVA with Scheffé contrasts when more than two groups were compared (24). A given group was compared by one ANOVA to all experimental conditions with the same HCO_3^- concentrations.

Results

Blood and urine acid-base status. Mean arterial blood pH in control animals was 7.37±0.02; arterial P_{CO_2} was 43.2±0.31 mmHg; plasma HCO_3 was 24.65±0.58 mEq/liter. Plasma Na was 144±0.9 mEq/liter; K was 4.3±0.2 mEq/liter. Low K rats

1. *Abbreviations used in this paper:* EIPA, ethyl-isopropylamiloride; $(HCO_3)_L$, concentration of HCO_3 in the collected fluid; $(HCO_3)_O$, concentration of bicarbonate in the original perfusion fluid; $JHCO_3$, net flux of bicarbonate (total CO_2); J_V, net fluid absorption.

Table II. Bicarbonate Reabsorption by Early and Late Distal Tubules at Different Luminal Bicarbonate Concentrations

$NaHCO_3$	S	T/R (*n*)	Vo	L	$(HCO_3)_o$	$(HCO_3)_L$	J_v	$JHCO_3$
mM			*nl/min*	*mm*	*mM*	*mM*	*nl/min per mm*	*Eq/min per mm*
Early DCT								
15	1	12/5	12.4±0.05	0.89±0.06	14.8±0.21	14.9±0.62	1.95±0.18	32.9±4.57
25	2	10/4	12.3±0.06	0.79±0.06	25.9±0.14	25.3±0.94	1.92±0.36	67.4±6.63
50	3	12/5	12.3±0.05	0.84±0.05	49.4±0.53	46.4±0.41	1.27±0.19	115.7±8.6
60	4	7/3	13.4±1.84	1.03±0.12	60.4±4.03	56.1±3.97	1.83±0.23	163.2±14.2
75	5	8/3	10.3±0.11	1.00±0.12	76.7±1.16	71.6±1.41	1.12±0.12	135.4±15.4
Late DCT								
15	1	13/6	12.2±0.01	0.83±0.07	15.4±0.16	16.5±0.44	1.72±0.17	10.7±3.10
25	2	8/4	12.4±0.11	0.75±0.09	25.7±0.13	25.1±0.40	1.44±0.13	45.8±3.39
50	3	11/4	12.4±0.05	0.91±0.09	49.1±0.38	45.5±1.63	0.91±0.18	96.5±11.8
60	4	6/3	13.8±2.11	1.05±0.10	63.4±4.13	59.3±4.87	1.91±0.32	143.3±11.6
75	5	8/4	10.2±0.12	0.99±0.08	75.2±1.85	69.1±1.48	0.85±0.11	124.0±14.1

S, solutions; N, number of perfused tubules; R, number of experimental rats; L, tubular length; V_O, perfusion rate. The osmolality of perfusates with 15–50 mM HCO_3 was 150 mosmol, and that of 60 and 75 mM was 300 mosmol.

had pH of 7.35±0.01, P_{CO_2} of 52.2±2.24 mmHg, plasma HCO_3 of 28.6±0.56 mEq/liter, and plasma K of 2.84±0.07 mEq/liter, Na of 142.5±0.2 mEq/liter.

Microperfusion experiments. A total of 326 distal tubules were perfused in 146 rats. The length of the perfused segments of early distal tubules ranged from 8 to 45% of total distal tubule length, and that of late distal tubules from 51.7 to 82.7%.

Load dependence of bicarbonate absorption in early and late distal tubules. Table II and Fig. 1, *A* and *B* summarize data of perfusion experiments in which the effect of increasing the bicarbonate concentration in the lumen upon bicarbonate transport was evaluated. Fig. 1 *A* provides a summary of data obtained in experiments in which the lumen Cl^- varied from 24 mM (at 50 mM HCO_3^-) to 90 mM (at 60 mM HCO_3^-). The statistically analysis by two-way ANOVA indicates concentration dependent bicarbonate absorption. Transport increases until the luminal bicarbonate concentration reaches 60 mM. We also note a statistically significant bicarbonate absorption that is less in late compared to early distal tubules, especially at low bicarbonate (high chloride) concentrations. Fig. 1 *B* depicts the relationship between luminal HCO_3^- concentration and net HCO_3^- reabsorption at low Cl^- concentration, (14 mM). It can be seen that the differences between early and late bicarbonate reabsorption rates disappear at low luminal Cl^-. This is consistent with reduced HCO_3^- secretion at low luminal Cl^- concentrations. Significant net bicarbonate absorption was present in all experimental conditions. Bicarbonate absorption increased with lumen bicarbonate concentration until a level of 60 mM was reached; bicarbonate absorption remained constant at higher concentrations. We have no explanation for the decline of $JHCO_3$ at the highest luminal bicarbonate concentrations. Fluid absorption was observed at all lumen bicarbonate concentrations and tended to decrease at higher bicarbonate concentrations in late distal tubules. Increased rates of bicarbonate absorption and transport saturation had also been observed in a previous rat microperfusion study in which the entire distal tubule was perfused (7). To test whether replacement of the gluconate could have lowered the luminal calcium concentration and thus effect bicarbonate transport, a perfusion experiment with Ca-free solution was also performed. We observed no effect on bicarbonate absorption of perfusion with Ca-free perfusion fluid: early distal tubule, J_V was 1.67±0.22

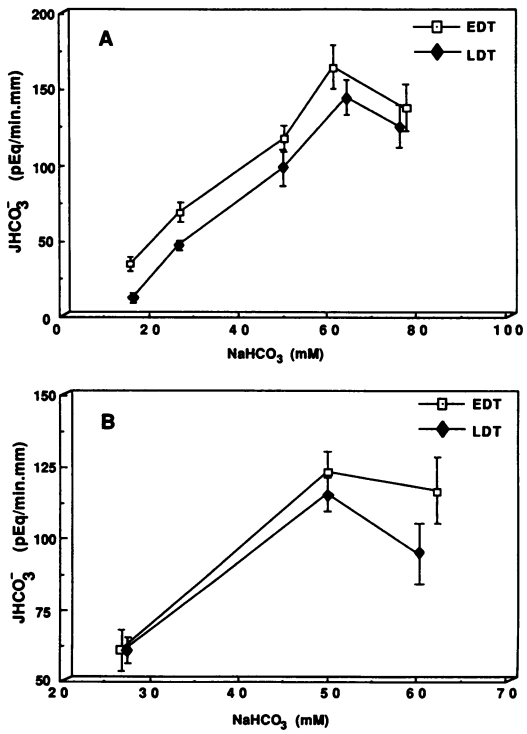

Figure 1. Early and late distal tubule bicarbonate reabsorption as function of luminal bicarbonate concentration. Data are means±SE. $JHCO_3$, bicarbonate reabsorption rate; *EDT*, early distal tubule; *LDT*, late distal tubule. (*A*) Elevated lumen chloride (24–90 mM, see Table V). Load-dependent bicarbonate absorption of EDT significantly different from LDT by two-way ANOVA test. (*B*) Low lumen chloride (14 mM, see Table V). Bicarbonate absorption curve of EDT not different from LDT.

Table III. Summary Data of the Effects of Amiloride and EIPA on Bicarbonate and Fluid Reabsorption in Early and Late Distal Convoluted Tubules

	T/R *n*	Vo	Tubular Length	$[HCO_3]_o$	$(HCO_3)_L$	J_v	$JHCO_3$
		nl/min	*mm*	*mM*	*mM*	*nl/min per m*	*pEq/min per mm*
Early DCT							
Control	12/5	12.4±0.05	0.89±0.06	14.9±0.62	14.9±0.62	1.95±0.18	32.9±4.57
AMIL	11/6	12.5±0.09	1.16±0.10	14.6±0.82	16.1±0.80	1.37±0.18*	6.5±3.70*
EIPA	8/4	12.4±0.10	1.00±0.14	14.9±0.20	14.9±0.61	0.95±0.28*	11.6±4.72*
Late DCT							
Control	13/6	12.2±0.05	0.83±0.07	15.4±0.16	16.5±0.44	1.72±0.17	10.7±3.10
AMIL	11/4	12.4±0.03	0.95±0.11	16.9±0.13	17.2±1.19	1.01±0.26*	14.2±3.14
EIPA	8/4	12.5±0.12	1.03±0.07	15.2±0.38	16.3±0.93	1.36±0.27	12.3±4.42

N, number of perfused tubules; R, number of experimental rats; L, tubular length; V_O, perfusion rate; AMIL, amiloride 10^{-3} M; EIPA ethyl-isopropylamiloride 10^{-4} M. Solution 1 was used in all perfusions; osmolality was 150 mosmol. * Significantly different from control volume $P < 0.05$ (comparison with respective control by ANOVA).

nl/min per mm in control and 1.76±0.45 nl/min per mm in 0 mM Ca^{++}, the corresponding bicarbonate absorption rates were 66.3±5.38 pEq/min per mm in control and 53.7±16.0 pEq/min per mm in 0 mM Ca^{++}. Late distal tubule J_V was 1.51±0.14 nl/min per mm in control and 0.98±0.13 nl/min per mm in 0 mM Ca^{++}, the corresponding bicarbonate absorption was 51.2±2.56 pEq/min per mm and 47.4±0.58 pEq/min per mm, respectively. There were no statistically significant changes between control and 0 mM Ca^{++}.

Effects of amiloride and EIPA on bicarbonate and fluid absorption in early and late distal tubules. To study the mechanism of bicarbonate absorption in early and late distal tubules, we tested the effects of substances known to inhibit Na-H exchange (25, 26). Table III and Fig. 2 summarize the effects of amiloride on bicarbonate and fluid transport in early and late distal tubules. Amiloride markedly inhibited bicarbonate transport in early but not in late distal tubules. J_V was decreased in both early and late distal tubule segments.

Table III and Fig. 3 provide information on the effects of EIPA on bicarbonate and fluid absorption in early and late distal tubules. EIPA was chosen because of its greater specificity for inhibition of Na-H exchange (25). Similar to the results of our amiloride experiments, bicarbonate absorption is strongly blocked in early but not in late distal tubules. Fluid absorption is markedly impaired in early but not in late distal tubules. This contrasts with the effects of amiloride, which affects late distal fluid reabsorption significantly.

Effects of bafilomycin A1, low K diet, and SCH 28080 on bicarbonate absorption in early and late distal tubules. Table IV shows the effects of bafilomycin, low K diet, SCH 28080 and acetazolamide on fluid and bicarbonate reabsorption in distal tubule segments. Bafilomycin A1 and SCH 28080 were dissolved in a stock solution of DMSO, and 1 μl of this solution was added per milliliter of perfusate. Control perfusions containing the same amount of DMSO were not different from control perfusions without DMSO; therefore, these data were pooled and used for comparison with the experimental groups by ANOVA. The data summarized in Table IV demonstrate that bafilomycin A1 (10^{-7} M) reduces bicarbonate reabsorption, a reduction that was significant only in late distal tubules; however, a significant reduction of both J_V and $JHCO_3^-$ were observed in early distal tubules when the dose of bafilomycin was increased to 5×10^{-7} M. J_V was decreased from 1.76±0.17 to 0.61±0.21 nl/min per mm ($n = 8$, $P < 0.001$), $JHCO_3^-$ was decreased from 65.5±10.28 to 34.32±7.79 pEq/min per mm ($n = 8$, $P < 0.05$). These results suggested the presence of a vacuolar-type H-ATPase in early and late distal tubules.

In low K rats, bicarbonate reabsorption was significantly enhanced in both early and late distal segments. The

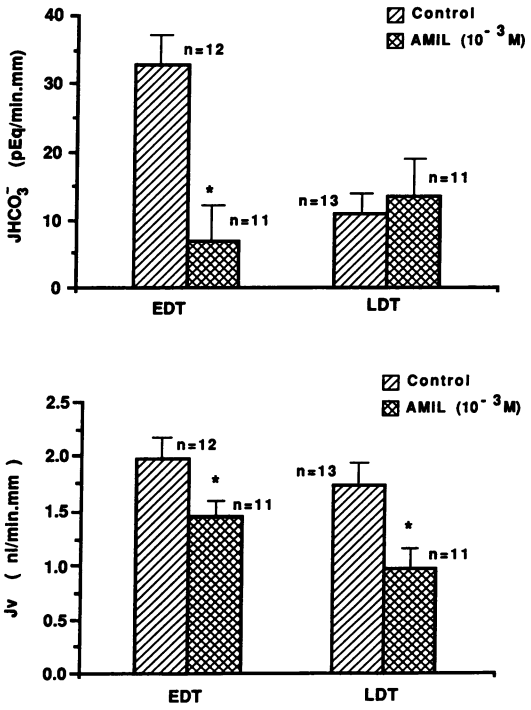

Figure 2. Effects of amiloride on bicarbonate and fluid reabsorption in early and late distal tubules. Data are means±SE. Perfusate contained 15 mM $NaHCO_3$ and 59 mM Cl. Concentration of amiloride added to the luminal perfusate was 10^{-3} M. *AMIL*, amiloride; *EDT*, early distal tubule; LDT, late distal tubule; *n*, number of perfused tubules. *Significant difference from control ($P < 0.05$).

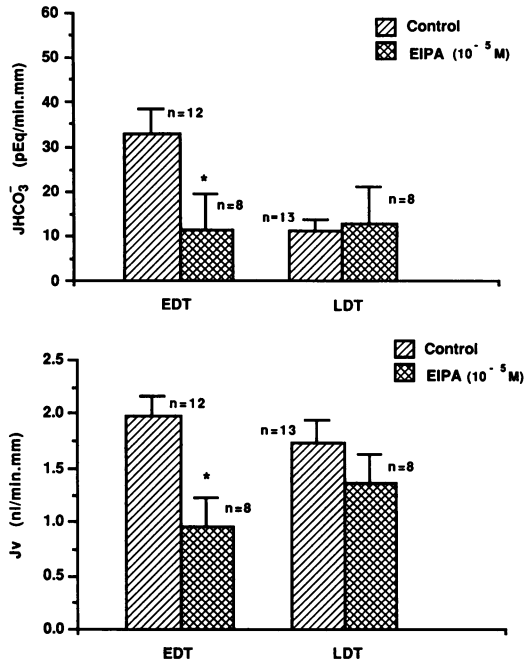

Figure 3. Effects of EIPA on bicarbonate and fluid reabsorption in early and late distal tubules. Data are means±SE. Perfusate contained 15 mM $NaHCO_3$ and 59 mM Cl. Concentration of EIPA added to the luminal perfusate was 10^{-5} M. *EDT*, early distal tubule; LDT, late distal tubule; *n*, number of perfused tubules. *Significant difference from control ($P < 0.05$).

H,K-ATPase inhibitor SCH 28080 reduced bicarbonate reabsorption in late distal tubules of K-depleted animals, but had no effect in control rats. Acetazolamide (10^{-4} M in tubule lumen) reduced bicarbonate reabsorption both in early and late distal segments. Fig. 4 summarizes the changes in bicarbonate reabsorption in the groups included in Table IV.

Table V summarizes the effect of different perfusate chloride concentrations on distal bicarbonate reabsorption at varying luminal bicarbonate levels. Comparing the effect of high (24–90 mM) and low (2–14 mM) Cl^- concentration, it is noted that a significant difference in bicarbonate reabsorption is obtained only in the late distal segment at the lower luminal bicarbonate concentration (15 mM), reabsorption increasing when Cl^- is lowered. These data are compatible with a secretory component of HCO_3^- transport at high Cl^-.

Discussion

Subdivisions of the cortical distal tubule. The heterogeneity of the distal tubule, as defined in micropuncture experiments, has posed some problems for defining the contribution of individual subdivisions of this nephron segment to the process of bicarbonate transport. The cortical distal tubule, consisting of the distal convoluted tubule, the connecting tubule, and the initial collecting tubule, is made up of several cell types (17, 18). Most previous microperfusion studies of bicarbonate transport have included the entire distal tubule segment and functional properties could not be assigned to individual morphologically defined segments (2, 6–8). Although several studies have succeeded in defining separately some transport properties of the early and late distal tubule (23, 27, 28), the properties of bicarbonate transport of the individual portions of this nephron segment remain poorly defined. In the present study, we were able to perfuse individual early and late distal tubule segments, taking advantage of the studies of Velazquez et al. who refined the methods for defining transport in early and late distal tubules (23). Thus, we could obtain information on the mechanism of bicarbonate transport in the subsegments of the distal tubule, an approach permitting assignment of transport

Table IV. Effect of Bafilomycin, K Depletion, Schering Compound, and Acetazolamide on Bicarbonate Reabsorption in Early and Late Distal Tubules

Groups	T/R (*n*)	Vo	L	$(HCO_3)_O$	$(HCO_3)_L$	J_v	$JHCO_3$
		nl/min	*mm*	*mM*	*mM*	*nl/min per mm*	*pEq/min per mm*
Early DCT							
Control	13/5	12.4±0.10	0.81±0.07	25.6±0.16	24.2±0.75	1.67±0.22	66.3±5.38
BAF (10^{-7} M)	9/4	12.5±0.17	0.93±0.08	25.2±0.19	23.9±0.81	1.03±0.17	42.6±6.38
Low K control	10/4	11.7±0.17	1.02±0.15	25.1±0.29	20.0±0.19	2.00±0.19	104.9±11.6*
Low K + SCH (10^{-5} M)	7/3	12.6±0.04	0.64±0.05	24.2±0.01	20.4±0.36	1.68±0.15	110.6±7.89*
SCH normal K	6/3	12.3±0.07	0.92±0.41	25.3±0.20	24.0±0.73	1.76±0.13	58.0±7.63
ACZ (10^{-4} M)	10/4	12.6±0.09	1.09±0.10	25.6±0.19	28.7±1.48	1.71±0.27	22.3±7.84*
Late DCT							
Control	12/6	12.5±0.12	0.93±0.12	25.5±0.15	24.8±0.54	1.51±0.14	51.2±2.56
BAF (10^{-7} M)	8/4	12.5±0.17	0.98±0.12	25.2±0.20	25.0±0.30	1.00±0.12	27.0±4.16*
Low K control	6/3	11.9±0.24	1.11±0.09	25.0±0.34	19.9±1.15	1.51±0.14	89.8±7.74*
Low K + SCH (10^{-5} M)	9/3	12.4±0.04	1.24±0.17	24.9±0.19	23.9±1.15	1.44±0.3	44.2±5.10‡
SCH normal K	7/3	12.4±0.09	1.27±0.10	24.9±0.34	24.6±1.81	1.40±0.29	42.8±9.30
ACZ (10^{-4} M)	10/4	12.6±0.09	1.23±0.14	25.6±0.19	27.3±1.05	1.3±0.31	12.9±7.56*

N, number of perfused tubules; R, number of experimental rats; L, tubular length; V_O, perfusion rate. The osmolality of perfusates was 150 mosmol. BAF, bafilomycin A1; SCH, schering compound; ACZ, acetazolamide. * Significantly different from control $P < 0.05$ (comparison with respective control by ANOVA). ‡ Significantly different from low K control $P < 0.05$. Solution 2 was used in all perfusions.

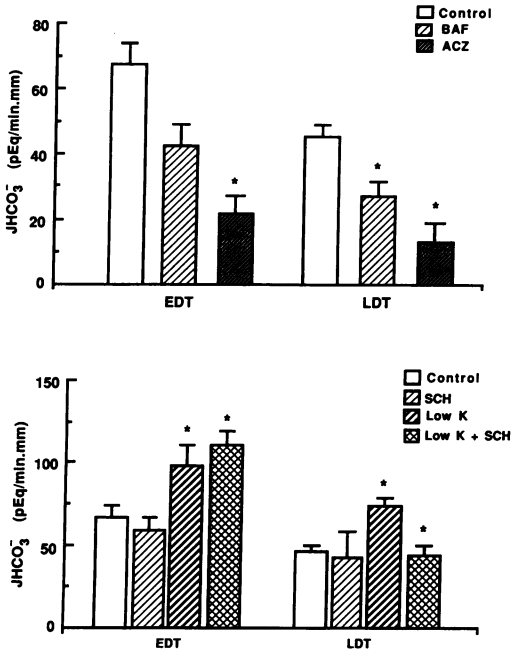

Figure 4. Effects of bafilomycin, low K diet, SCH 28080, and acetazolamide on early and late distal $JHCO_3$. Data are means±SE from perfusate containing 25 mM $NaHCO_3$ and 49 mM Cl. Concentrations of bafilomycin (BAF), acetazolamide (ACZ), and SCH 28080 (SCH) added to the luminal perfusate were 10^{-7}, 10^{-4}, and 10^{-5} M, respectively. Low K, after 8 d low K diet, plasma K was 2.84±0.07 mEq/liter. EDT, early distal tubule; LDT, late distal tubule. *Significant difference from control ($P < 0.05$).

mechanisms of acidification to a smaller number of cell types. consist of distal convoluted tubule cells, connecting tubule cells and principal and at least two types of intercalated cells (17, 18). Anatomical studies indicate that in the rat, the early distal tubule consists largely of distal convoluted cells, whereas the region making up the late distal tubule is lined mainly by principal and intercalated cells. Since the transition between individual cell types is gradual along the rat distal tubule, it is possible that portions of the perfused tubule segments include connecting tubule cells (29).

Fluid absorption in early and late distal tubule. A striking observation in our experiments was the highly significant rates of fluid absorption in early distal tubules. This segment is thought to have a low, vasopressin-independent water permeability (30), yet our data demonstrate significant solute-dependent fluid absorption. These findings indicate that at least a portion of the perfused early distal tubule must have a sizable water permeability. It appears that the transition between the water-impermeable ascending limb of Henle's loop and the water-permeable segments must be close to the site of origin of the distal convoluted tubule.

EIPA significantly suppressed fluid absorption in early distal tubules. These results are consistent with impairment of bicarbonate absorption by this specific inhibitor of sodium-hydrogen ion exchange. The observation that EIPA, a potent and specific blocker of sodium-hydrogen exchange, does not significantly affect late distal tubule fluid absorption is consistent with (*a*) the absence of an important component of sodium-hydrogen exchange; and (*b*) no significant action of EIPA on sodium channels (25).

Table V. Comparison of Early and Late DCT Bicarbonate Absorption during Perfusion with Solutions Containing High and Low Cl^- Conditions

Groups		S	T/R (*n*)	Vo	L	$[HCO_3]_o$	$[HCO_3]_L$	J_v	$JHCO_3$
			nl/min	*mm*	*mM*	*mM*	*nl/min per mm*	*pEq/min per mm*	
Early DCT									
15 mM HCO_3	59 mM Cl	1	12/5	12.4±0.05	0.89±0.06	14.8±0.21	14.9±0.62	1.95±0.16	32.9±4.57
	2 mM Cl	1a	10/4	12.3±0.01	1.22±0.14	15.1±0.30	14.6±1.21	1.11±0.28	29.2±5.75
25 mM HCO_3	49 mM Cl	2	10/4	12.3±0.06	0.79±0.06	25.9±0.14	25.3±0.94	1.92±0.36	67.4±6.63
	14 mM Cl	2a	8/3	12.2±0.14	1.11±0.14	26.1±0.17	24.6±0.64	1.58±0.22	60.2±7.13
50 mM HCO_3	24 mM Cl	3	12/5	12.3±0.05	0.84±0.05	49.4±0.53	46.4±0.41	1.27±0.19	115.7±8.6
	14 mM Cl	3a	7/3	12.4±0.10	1.15±0.12	49.3±0.39	44.2±1.61	1.42±0.20	122.3±7.5
60 mM HCO_3	90 mM Cl	4	7/3	13.4±1.84	1.03±0.12	60.4±4.03	56.1±3.97	1.83±0.23	163.2±14.2
	14 mM Cl	4a	9/4	12.2±0.06	1.24±0.33	61.7±0.91	58.2±1.97	1.32±0.24	116.1±11.5
75 mM HCO_3	75 mM Cl	5	8/3	10.3±0.11	1.00±0.12	76.7±1.16	71.6±1.41	1.12±0.12	135.4±15.4
	2 mM Cl	5a	9/4	12.6±0.09	1.25±0.21	76.4±1.55	74.9±2.71	1.65±0.17	111.0±11.8
Late DCT									
15 mM HCO_3	59 mM Cl	1	13/6	12.2±0.01	0.83±0.07	15.4±0.16	16.5±0.44	1.72±0.17	10.7±3.1
	2 mM Cl	1a	11.4	12.3±0.01	1.21±0.14	15.2±0.29	13.9±1.06	1.11±0.22	28.6±4.2*
25 mM HCO_3	49 mM Cl	2	8/4	12.4±0.11	0.75±0.09	25.7±0.13	25.1±0.40	1.44±0.13	45.8±3.4
	14 mM Cl	2a	8/3	12.2±0.13	1.01±0.13	26.4±0.18	24.6±0.85	1.79±0.13	60.1±5.9
50 mM HCO_3	24 mM Cl	3	11/4	12.4±0.05	0.91±0.09	49.1±0.38	45.5±1.63	0.91±0.18	96.5±11.8
	14 mM Cl	3a	5/3	12.5±0.10	0.94±0.17	49.3±0.39	44.3±1.33	1.09±0.16	114.7±10.5
60 mM HCO_3	90 mM Cl	4	6/3	13.8±2.11	1.05±0.10	63.4±4.13	59.3±4.87	1.91±0.32	143.3±11.6
	14 mM Cl	4a	8/4	12.3±0.05	1.20±0.14	59.5±1.67	55.4±0.75	0.99±0.34	94.1±10.1
75 mM HCO_3	75 mM Cl	5	8/4	10.2±0.12	0.99±0.08	75.2±1.85	69.1±1.48	0.85±0.11	124.0±14.1
	2 mM Cl	5a	10/4	12.6±0.12	0.82±0.05	76.6±1.88	74.4±1.68	1.09±0.18	102.9±11.6

S, solutions; low Cl^- solutions, Cl^- substituted by gluconate, see Table I for explanation. * Significantly different from high Cl^- perfusion ($P < 0.05$). Some of the data in this Table (high Cl perfusion experiments) are also included in Table II.

Bicarbonate transport in early and late distal tubules. Most free-flow micropuncture studies (see reference 3) have demonstrated significant bicarbonate absorption along the cortical distal tubule (1–11). Distal tubule bicarbonate reabsorption has been reported to increase with the delivery of bicarbonate to the distal tubule (4), with distal tubule flow rate (7), and during potassium depletion (5). Bicarbonate absorption is reduced by carbonic anhydrase inhibitors and induction of respiratory alkalosis (1). The situation is more complicated when entire distal tubules are perfused in vivo. Whereas net bicarbonate absorption has been reported in several microperfusion studies (6, 7, 22), bicarbonate secretion has also been observed (8, 11). Several factors have been suggested to affect the direction of bicarbonate transport in perfusion studies. These include the acid base status (2, 7, 9), the rate of distal tubule perfusion (10), the dietary and mineralocorticoid status of the animals (6, 11), the circulating levels of vasopressin (3), and the chloride concentration in the perfusion fluid (31). Bicarbonate absorption is enhanced by acidosis and reduced by metabolic alkalosis (7). Bicarbonate absorption is also stimulated by low tubule perfusion rates (10), by fasting (11), high aldosterone (Kunau, R., personal communication) and vasopressin levels (3), and by low luminal chloride (31).

An unresolved problem is the difference between the almost uniformly observed bicarbonate absorption in free-flow studies and the findings of bicarbonate secretion in microperfusion studies. This difference might be caused by technical aspects related to the microperfusion procedure; e.g., the nature of the perfusion fluids and the possibility that substances normally present in native tubule fluid but absent in artificial perfusion solutions stimulate bicarbonate absorption. A precedent for this possibility is the observation of a highly significant stimulation of distal tubule potassium secretion by perfusing distal tubules with proximal tubule fluid (32). Whether a similar mechanism accounts for the marked difference between the behavior of bicarbonate transport in free-flow and perfusion experiments remains to be explored.

Components of early and late distal tubule bicarbonate transport. Our perfusion studies allow the separate evaluation of the transport parameters of net absorption of bicarbonate in early and late distal tubules. Comparing early and late distal tubule bicarbonate absorption rates, (see Tables II and V and Fig. 1) we note similar concentration dependence of transport. Absorption increases strongly until concentrations of ~ 60 mM are reached and then attains a constant level. Transport rates of 120–160 pmol/min per mm are maintained at the highest bicarbonate concentrations in both segments. At similar bicarbonate loads, the presently reported transport rates are somewhat higher (140/160 vs 76 pmol/min per mm) than those in a previous study from our laboratory in which the entire distal tubule was perfused (7). However, whereas the bicarbonate load in the present study was increased by perfusing tubules with bicarbonate concentrations as high as 75 mM, load was increased in the earlier study by accelerating flow rate and keeping bicarbonate concentrations at 25 mM. It had been noted in proximal tubules that an increase of concentration is more effective in stimulating bicarbonate absorption than an increase of load achieved by enhancing luminal flow rate (7, 33, 34).

Bicarbonate absorption at low luminal bicarbonate concentrations is less in late than in early distal tubule segments. However, the observation that bicarbonate absorption is similar in early and late tubule segments when tubules are perfused with chloride-free solutions is consistent with similar rates of absorption but bicarbonate secretion in the late distal tubule. This would explain our observation of lower net absorption rates in the late distal tubule.

The components of distal bicarbonate fluxes during perfusion with solutions containing different bicarbonate concentrations can be approximated from the net rates of absorption given in Table II and from passive transepithelial bicarbonate fluxes calculated from bicarbonate permeability, transepithelial bicarbonate concentration differences, and transepithelial potential difference.[2] It is realized that the validity of the quantitative analysis of HCO_3^- fluxes depends on several assumptions, including (*a*) that the HCO_3^- permeability is the same in early and late distal segments; and (*b*) that the calculated HCO_3^- fluxes represent only active and passive HCO_3^- absorption and HCO_3^- diffusion. Accordingly secretory HCO_3^- fluxes are not taken into account, an assumption that limits the accuracy of these flux estimates, particularly in the late distal tubule. Thus in the *early* distal tubule, during perfusions with 15 mM bicarbonate, net bicarbonate absorption was 32.9 pmol/min per mm, passive backflux into the lumen was 7.1 pmol/min per mm, and active reabsorption was the sum of these values; i.e., 40.0 pmol/min per mm. In the same segment, during perfusion with 50 mM bicarbonate, net absorption was 115.7 pmol/min per mm, passive flux out of the tubule was 24.7 pmol/min per mm, and active absorption was the difference between net and passive flux (both in the same direction); i.e., 91.0 pmol/min per mm. In late distal tubules during perfusions with 15 mM bicarbonate, net absorption was 10.7 pmol/min per mm, passive backflux was 4.8 pmol/min per mm, and active absorption was 15.5 pmol/min per mm. During perfusion with 50 mM bicarbonate, net absorption was 96.5 pmol/min per mm, passive outflow was 34.2 pmol/min per mm, and active absorption was 62.3 pmol/min per mm. As expected, at high luminal bicarbonate perfusions, the passive outflux of bicarbonate along a favorable electrochemical potential gradient is of considerable magnitude and contributes significantly to net bicarbonate absorption. However, active bicarbonate absorption is also markedly increased because of the elevated luminal bicarbonate load.

Mechanism of bicarbonate transport in early and late distal tubules. The transport mechanisms of bicarbonate absorption in the two tubule segments adjoining the cortical distal tubule have been defined. In the cells of the thick ascending limb of Henle's loop they include sodium-hydrogen exchange (19) and possibly also a modest but significant component of hydrogen ATPase (35, 35a). In the cortical collecting tubule, electrogenic hydrogen ion secretion in alpha-intercalated cells (21) accounts for bicarbonate absorption and chloride-bicarbonate exchange in beta-intercalated cells for bicarbonate secretion (20, 36). Our results indicate that several different processes

2. Passive fluxes of bicarbonate were calculated from the equation

$$JHCO_3 = PHCO_3 \cdot [\Delta HCO_3] \cdot (EF/RT)/(\exp(EF/RT) - 1),$$

where $JHCO_3$ is the passive bicarbonate flux, $PHCO_3$ the transepithelial bicarbonate permeability (7), delta ΔHCO_3 is the log transepithelial bicarbonate concentration difference, E is the transepithelial potential difference of early (−10 mV) (Malnic, G., unpublished data) and late (7) distal tubule, and F, R, and T have their conventional meanings.

account for bicarbonate transport in the early and late distal tubule.

Based on our observations we propose that amiloride- and EIPA-sensitive sodium-hydrogen exchange mediates the bulk of bicarbonate absorption in the early distal tubule. The results with higher bafilomycin concentration suggest that ATP-dependent H^+ secretion may also be involved. On the other hand, the experiments with bafilomycin indicate that bicarbonate absorption in the late distal tubule is the result of amiloride-insensitive electrogenic hydrogen ion secretion (37, 38). The conclusion that electrogenic hydrogen ion secretion mediated bicarbonate reabsorption in late distal tubules is also supported by the observation that *N*-ethylmaleimide, a potent inhibitor of hydrogen ATPase (14, 39, 40), elevates lumen pH in this tubule segment (41).

It has been recently shown that gastric type K,H-ATPase is present in cells of the cortical and medullary collecting duct (42, 43), and functional evidence also suggests its presence in cortical distal tubules of rats fed a low K diet (44). In K-depleted animals we have observed in the present study stimulation of bicarbonate reabsorption in early and late distal tubules, which is compatible with free-flow micropuncture data obtained by Capasso et al. (4, 5) along the entire distal tubule. This stimulation is inhibited in late distal tubule by SCH 28080, a drug shown to inhibit with great specificity the gastric type K,H-ATPase (45). It has been reported that SCH 28080 also blocks H^+ secretion and K^+ reabsorption in K-depleted rats in the outer and inner medullary collecting duct (42, 46) and also in cortical collecting duct in HCO_3^--secreting conditions (46), and the present results are compatible with the existence of K,H-ATPase also in late distal tubules. On the other hand, no evidence for functionally measurable K^+/H^+ activity was found in our rats on a control diet.

Although we did not observe net secretion of bicarbonate in any of our perfusion experiments, we believe that nevertheless, a component of bicarbonate secretion may exist in the distal tubules. The observation that perfusion of late, but not early, distal tubules with low chloride solutions (2 mM Cl^-) at 15 mM HCO_3^- increases bicarbonate absorption is consistent with the view that a component of bicarbonate secretion opposed absorption and was responsible for lower net absorption of bicarbonate. Thus, net rates of bicarbonate transport are the result of simultaneously occurring processes of active bicarbonate absorption and secretion. The latter process is thought to occur by chloride-bicarbonate exchange in a subpopulation of intercalated cells (16, 43). It is unlikely that changes in transepithelial electrical potentials could have been responsible for the increase in bicarbonate absorption at low luminal chloride concentrations because gluconate replacement of chloride does not alter the electrical potential across the distal tubule (47). Our findings that bicarbonate secretion may modulate the apparent rate of net bicarbonate absorption support the view of Levine et al., who have assigned an important role to bidirectional bicarbonate transport in the distal tubule (31).

Acknowledgments

We would like to thank Dr. D. C. Batlle for providing the EIPA and Prof. K. Altendorf for providing the bafilomycin A1.

This work was supported by National Institutes of Health grants AM 17433 and by DK 27691. G. Malnic was supported by Fundacao de Amparo a Pesquisa do Estado de Sao Paulo and Conselho Nacional de Pesquisas, Brasil.

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