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Secretion of HCO_3^-/OH^- in Cortical Distal Tubule of the Rat

R. Fernandez*, G. Malnic

Department of Physiology and Biophysics, Instituto de Ciências Biomédicas, USP, University of São Paulo, 05508-900, São Paulo, Brazil

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Abstract. Secretion of bicarbonate has been described for distal nephron epithelium and attributed to apical Cl^-/HCO_3^- exchange in beta-intercalated cells. We investigated the presence of this mechanism in cortical distal tubules by perfusing these segments with acid (pH 6) 10 mM phosphate Ringer. The kinetics of luminal alkalinization was studied in stationary microperfusion experiments by double-barreled pH (ion-exchange resin)/1 M KCl reference microelectrodes. Luminal alkalinization may be due to influx (into the lumen) of HCO_3^- or OH^-, or efflux of H^+. The magnitude of the Cl^-/HCO_3^- exchange component was measured by perfusing the lumen with solutions with or without chloride, which was substituted by gluconate. This component was not different from zero in control and alkalotic (chronic plus acute) Wistar rats. Homozygous Brattleboro rats (BRB), genetically devoid of antidiuretic hormone, were used since this hormone has been shown to stimulate H^+ secretion, which could mask bicarbonate secretion. In these rats, no evidence for Cl^-/HCO_3^- exchange was found in control BRB and in early distal segments of alkalotic animals, but in late distal tubule a significant component of 0.14 ± 0.033 nmol/cm^2 · sec was observed, which, however, is small when compared to the reabsorptive flow found in control Wistar rats, of 0.95 ± 0.10 nmol/cm^2 · sec. In addition, 5×10^{-4} M SITS had no effect on distal bicarbonate reabsorption in controls as well as on secretion in alkalotic Wistar and Brattleboro rats, which is compatible with the absence of effect of this drug on the apical Cl^-/HCO_3^- exchange in other tissues. It is concluded that most distal alkalinization is not Cl^- dependent, and that Cl^-/HCO_3^- exchange may be found in cortical distal tubule, but its magnitude is, even in alkalosis, markedly smaller than the reabsorptive flux, which predominates in the rats studied in this paper, keeping luminal pH lower than that of blood.

Key words: Bicarbonate secretion — Cl^-/HCO_3^- exchange — SITS — Stationary microperfusion

Introduction

The observation that the luminal bicarbonate concentration in cortical distal tubule remains almost constant while inulin concentration increases at least threefold [5, 23] is evidence for the presence of significant net bicarbonate reabsorption at this site. Estimates of net bicarbonate reabsorption, based on "in vivo" micropuncture techniques, have yielded a reabsorption rate of 5 to 8% of filtered bicarbonate along this segment.

The only study in which modest net secretion of bicarbonate was observed in free-flow experiments was in animals undergoing hypotonic volume expansion, a situation which reduced plasma levels of ADH [1]. The situation is more complicated when bicarbonate transport is studied in perfused distal tubules. Net bicarbonate reabsorption or secretion in pump-perfused tubules depends critically on experimental conditions such as protein content of the diet, the acid-base status and luminal flow-rate [12, 34]. In tubules that under normal conditions secreted bicarbonate, fasting induced bicarbonate reabsorption [18]. On the other hand, both net reabsorption [34, 35] and net secretion of bicarbonate [17] have been reported in metabolic and in chloride depletion alkalosis.

A satisfactory explanation for the apparent discrepancy between the demonstration of significant net reabsorption of bicarbonate under free-flow conditions and net bicarbonate secretion in some tubule perfusion studies is presently not available. Capasso et al. [4] attributed this difference to the absence of some transport

* *Present address:* Depto. de Fisiologia, Facultad de Medicina, Universidad de la Republica, Montevideo, Uruguay

Correspondence to: G. Malnic

stimulating substance in artificial perfusion studies, present either in the filtrate or added to the tubule fluid in vivo. Evidence for the presence of a transport stimulating substance has been obtained in perfusion studies of distal tubules with native proximal tubule fluid [22].

Replacing luminal chloride with gluconate inhibits bicarbonate secretion and enhances bicarbonate reabsorption, while high chloride perfusates elicit net bicarbonate secretion in usually reabsorbing distal tubules [19]. These results are evidence for Cl^-/HCO_3^- exchange modulating bicarbonate secretion in this segment. On the other hand, studies with stationary microperfusion and direct recording of lumen pH do not demonstrate bicarbonate secretion in control rats [10].

The purpose of the present investigation was to study the presence of net bicarbonate secretion in distal tubule of rats subjected to situations in which this secretion is stimulated: chronic metabolic alkalosis and reduced levels of ADH. The flux of bicarbonate was deduced from the continuous record or lumen pH in experiments of stationary microperfusion.

Materials and Methods

Two populations of male rats were used in the experiments: Wistar and homozygous Brattleboro. Wistar rats were obtained from Instituto de Ciencias Biomédicas, and Brattleboro rats from Escola Paulista de Medicina. They were divided into two groups: control and chronic metabolic alkalosis. Control rats received tap water and a standard pellet diet containing 10% protein up to the time of the experiment. These rats received isotonic saline plus 3% mannitol at a rate of 0.05 ml/min during the experiments. Control Brattleboro rats received 75 mM NaCl plus 2% mannitol (243 mOsm) at 0.2 ml/min. In the second group, chronic metabolic alkalosis was induced by adding 60 mM $NaHCO_3$ plus 20 mM $KHCO_3$ for three days to the drinking water, diluted to one-half for Brattleboro rats, and infusion during the experiment of 70 mM $NaHCO_3$, 6 mM $KHCO_3$, 70 mM NaCl plus 3% mannitol for Wistar, and 30 mM $NaHCO_3$, 4 mM $KHCO_3$, 70 mM NaCl plus 2% mannitol for Brattleboro rats. The final osmolality of the infusion for Wistar rats was 290 mOsm/kg H_2O, and that for Brattleboro rats was hypotonic, i.e., 250 mOsm/kg H_2O.

After anesthesia by 100 mg/kg Inactin (Byk-Gulden, Konstanz, Germany) the rats were prepared for micropuncture as described previously [25]. In brief, the left jugular vein and carotid artery were cannulated for infusions and blood withdrawal, respectively. A tracheostomy was performed. The kidney was isolated by a lumbar approach, and immobilized "in situ" by Ringer-agar in a Lucite cup. It was superfused with mammalian Ringer solutions at 37°C containing 20 mM phosphate at pH adjusted to 6.0, 7.0 and 7.5 for electrode standardization. The bladder was catheterized for urine collection. The urine was collected under oil.

Stopped-Flow Microperfusion

The microperfusion procedure, which has been previously described [10], involved impalement of a proximal loop with a double-barreled micropipette made out of theta glass tubing (R&D Optical Systems, Spencerville, MD), one barrel filled with Sudan black-colored castor oil used to block fluid columns to keep them stationary in the tubule lumen, and the other with the control perfusion solution colored with 0.05% FDC-green, which was used to detect early and late distal tubule loops. Further downstream in the proximal tubule a single micropipette containing the perfusion solution with the test substance was inserted. A double-barreled microelectrode was then inserted into an early or late distal loop. Perfusions were performed by applying pressure to the double-barreled micropipettes via hand-held oil-filled syringes. This method allowed for perfusions at a rate sufficient to achieve luminal pH near that of the original solution, minimizing the function of the segments between site of perfusion and measurement. Following the manual perfusion, a column of oil was injected into the late proximal or early distal tubule lumen, blocking the flow of fluid.

After control perfusions, the experimental solution without Cl^- was used, followed again by the control solution. pH was measured as the voltage difference between the two barrels of an asymmetric double-barreled microelectrode made out of Hilgenberg (Malsfeld, Germany) double-barreled glass capillaries. The larger barrel contained an H-ion-sensitive ion-exchange resin (Fluka, Buchs, SW), and the smaller one, 1 M KCl colored by FDC green (reference barrel). With this technique, the slope of the electrode in the pH range 6.5 to 7.5 was 58.4 ± 1.85 mV per pH unit in phosphate-buffered solutions [21]. Transepithelial electrical potential difference (*Vt*) was the difference between the reference barrel and ground. Voltages were read by a WPI (New Haven, CT) model FD 223 differential electrometer, the output of which was recorded on a Beckman model R511A Dynograph, and digitized in 1 sec intervals by means of an AD conversion board (Data Translation model DT 2801, Marlborough, MS) mounted on a 386 DX (DELL 333D) IBM-PC compatible microcomputer.

The tubules were perfused with phosphate or bicarbonate Ringer solutions at pH 6 or 8, respectively. After blocking fluid in the lumen, the pH of the perfusate approached the stationary value (pHs) due to fluxes of H^+, OH^- or HCO_3^- across the tubule epithelium.

From the time course of the pH change the concentration of NaH_2PO_4 or $NaHCO_3^-$ was calculated by the Henderson-Hasselbalch equation using a pKa of 6.8 or 6.1. These concentrations are obtained assuming either constant total phosphate content in the lumen, or a pCO_2 equivalent to that in systemic blood [24*a*]. To calculate alkalinization rates, the log of $(NaH_2PO_4)_t - (NaH_2PO_4)_s$, or $(HCO_3^-)_t - (HCO_3^-)_s$ was plotted against time in seconds. The subscript *t* refers to time *t*, and *s* to the stationary situation. In contrast to pH changes, such a plot generated a straight line, indicating that the buffer concentrations approach their steady-state value in an exponential manner. The half-time ($t½$) of the approach of acid phosphate or bicarbonate concentrations to their steady-state value was calculated from the slope of these lines.

The rationale for the detection of bicarbonate secretion was to measure the rate of alkalinization of this fluid during stationary perfusion when Cl^- was the dominant anion, and after its substitution by gluconate. Bicarbonate secretion dependent on Cl^-/HCO_3^- exchange is the difference between the alkalinization rates obtained during these two perfusions. Bicarbonate flux ($JHCO_3^-$) was calculated by [6]:

$$J_{OH^-} = \ln 2/t½ \cdot (NaH_2PO_4o - NaH_2PO_4s) \cdot r/2$$

where $t½$ is the alkalinization half-time, *r* is the tubule radius, NaH_2PO_4o and NaH_2PO_4s are initial and stationary acid phosphate concentrations. The decrease in these concentrations is equivalent to elevation in OH^- or HCO_3^- concentrations. Bicarbonate reabsorption is calculated in a similar manner, based on initial (HCO_3^-o) and stationary (HCO_3^-s) concentrations.

Solutions

The control luminal perfusion solution contained 80 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgSO_4$, raffinose to isotonicity and was

Table 1. Acid-base data in Wistar and Brattleboro (BRB) rats

	Wistar Contr.	Wistar Alk.	BRB Contr.	BRB Alk.
B pH	7.41 ± 0.021	7.52 ± 0.02	7.40 ± 0.014	7.56 ± 0.02
	(13)	(20)**	(12)	(15)***
B PCO_2	32.4 ± 3.47	45.1 ± 2.95	32.4 ± 1.26	40.1 ± 2.26
(mmHg)	(12)	(18)*	(11)	(13)***
P HCO_3^-	21.0 ± 1.93	36.8 ± 2.68	19.2 ± 0.98	34.1 ± 2.23
(mM)	(11)	(18)	(11)	(13)***
U pH	6.66 ± 0.073	8.15 ± 0.06	6.61 ± 0.103	7.27 ± 0.14
	(30)	(13)***	(24)	(21)***
U PCO_2	23.8 ± 1.95	41.46 ± 2.16	18.0 ± 2.26	48.8 ± 5.36
(mmHg)	(30)	(12)***	(22)	(15)***
U HCO_3^-	2.97 ± 0.44	177.53 ± 21.3	3.04 ± 0.77	20.0 ± 3.66
(mM)	(30)	(12)***	(22)	(15)
U HCO_3^--V	0.23 ± 0.038	10.41 ± 2.01	0.37 ± 0.077	1.78 ± 0.77
(μEq/min)	(26)	(12)***	(18)	(15)
AT (μEq/min)	0.68 ± 0.084		1.06 ± 0.114	
	(18)		(24)*	
NH_4^+	30.9 ± 3.03	18.04 ± 2.71	59.0 ± 2.71	
(μEq/min)	(17)	(12)***	(12)**	

Values are mean ± SE; (number of observations); B, blood; P, plasma; U, urine; AT, urine titratable acid; NH_4^+, urine ammonium. Differences with control Wistar rats, * $P < 0.05$, ** $P < 0.01$; Differences with the respective control group, *** $P < 0.01$.

buffered with 10 mM phosphate at pH 6, below the physiological level. The zero chloride solution contained: 80 mM Na^+ gluconate, 5 mM K^+ gluconate, 3 mM Ca^{2+} gluconate, 1.2 mM $MgSO_4$, raffinose to isotonicity, buffered with 10 mM phosphate at pH 6. Bicarbonate buffered fluid contained 80 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1.2 $MgSO_4$, 25 mM $NaHCO_3^-$ plus raffinose to isotonicity.

Analytical Methods

Blood and urine pH and pCO_2 were measured by means of an Instrumentation Laboratory model 11/329 blood gas system. K^+ and Na^+ in blood and urine were measured by flame photometry (Micronal B262, Brazil). Osmolality was measured by freezing point depression (Fiske Om Osmometer). Inulin in plasma and urine was measured by the anthrone method. Urinary titratable acid was determined by titration of 50 μl urine samples to the turning point of phenolphthalein. Ammonium in urine was determined spectrophotometrically by nesslerization.

Statistical Analysis

Statistical comparisons were made by the paired *t*-test or by analysis of variance followed by the Scheffé contrast test when more than two groups were compared.

Results

Whole Animal Data

Brattleboro rats have an elevated urine flow rate (0.166 ± 0.017 ml/min, $n = 23$, against 0.086 ± 0.009 ml/min, $n = 26$, in controls), and a low urine osmolality (223.3 ± 11.8 mOsm, $n = 23$) compared to Wistar rats (582.2 ± 17.1 mOsm, $n = 26$). Mean GFR was in the range of 8 to 9.5 ml/min · kg, not significantly different between the groups. At the same time, urine excretion of sodium (UNaV) was reduced in Brattleboro rats (2.98 ± 0.38 μEq/min against 6.16 ± 1.73 in controls). Both alkalotic groups showed decreased urine flow rate (0.059 ± 0.011 ml/min, $n = 13$ in Wistar, 0.099 ± 0.025 ml/min, $n = 24$, in Brattleboro), and increased urine excretion rates of sodium (10.78 ± 1.69 μEq/min in Wistar, and 4.85 ± 1.18 μEq/min in Brattleboro) compared to the respective control groups.

Blood acid-base parameters obtained in animals on a control diet and in rats receiving $NaHCO_3$ for 72 hr are summarized in Table 1. Alkalotic animals showed a significant increment in PCO_2, pH and HCO_3^- when compared with control animals. Wistar control animals and Brattleboro rats showed no difference in their acid-base parameters.

In alkalotic animals, urinary acidification was reduced markedly (Table 1). Urine pH increased from 6.66 ± 0.073 to 8.15 ± 0.06 in Wistar rats, and from 6.61 ± 0.103 to 7.27 ± 0.14 in Brattleboro rats. Titratable acid excretion was significantly higher in control Brattleboro rats compared with control Wistar rats (1.06 ± 0.114 *vs.* 0.68 ± 0.084 μEq/min). The urinary ammonium excretion was also augmented in control Brattleboro rats compared with control Wistar (59.0 ± 2.71 vs 30.9 ± 3.03 μEq/min). As a result of these effects, the urinary net acid excretion is significantly increased in control Brattleboro rats compared with control Wistar. These data are compatible with a stimulated urinary acidification in Brattleboro rats, possibly due to the increased urine flow rate and higher distal buffer load.

Table 2. Stationary microperfusion of cortical distal tubules of Wistar rats

	n	pHs	*t½* (sec)	J_{OH} (nmol · cm^{-2} · sec^{-1})	PD (mV)
ED					
Control	21/6	7.20 ± 0.08	13.84 ± 2.01	0.230 ± 0.037	−8.05 ± 1.18
Cl^- 0	17/4	7.21 ± 0.11	12.64 ± 1.29	0.238 ± 0.035	−7.8 ± 1.73
LD					
Control	13/4	6.75 ± 0.05	15.13 ± 3.09	0.179 ± 0.015	−27.0 ± 6.09
Cl^- 0	12/4	6.70 ± 0.03	16.20 ± 2.35	0.165 ± 0.03	−15.8 ± 2.22
ED					
Alkalosis	35/6	7.26 ± 0.06	11.98 ± 1.24	0.473 ± 0.048	−8.20 ± 1.07
A + Cl^- 0	32/7	7.15 ± 0.07	12.93 ± 1.51	0.465 ± 0.064	−8.06 ± 1.23
LD					
Alkalosis	19/6	7.13 ± 0.06	13.84 ± 0.89	0.302 ± 0.029	−27.0 ± 3.47
A + Cl^- 0	23/6	7.15 ± 0.07	12.91 ± 1.01	0.361 ± 0.04	−26.5 ± 3.0

Values are means ± SE; *n*, number of perfusions/number of tubules; pHs, stationary pH; *t½*, alkalinization half-time; J_{OH}, rate of alkalinization; PD, transepithelial potential difference; ED, Early distal tubules; LD, Late distal tubules; Control, perfusion with control Ringer solution; Alkalosis (A), alkalotic rats; Cl^- 0, perfusion without chloride.

Microperfusion Data

Tables 2 and 3 show results of stationary microperfusion experiments designed to analyze bicarbonate secretion along distal tubules during perfusion with phosphate-buffered solutions, at normal and zero chloride levels. This design allows an analysis of the chloride dependence of bicarbonate transport in this segment.

Table 2 shows the results of microperfusions in control Wistar rats. There were no alterations with zero chloride perfusions in early and late distal tubules when compared to control perfusions. (*See also* Fig. 1). The results of similar experiments (Table 3) in control Brattleboro rats show that the stationary pH tends to be more alkaline when compared with Wistar rats (7.32 ± 0.05 *vs.* 7.20 ± 0.08 in early distal and 7.00 ± 0.11 *vs.* 6.75 ± 0.05 in late distal, $P < 0.01$). The perfusion with zero chloride solution did not alter the stationary pH as well as J_{OH^-}.

To stimulate bicarbonate secretion, a group of rats was subjected to chronic metabolic alkalosis. Table 2 and Fig. 2 show that in alkalotic Wistar rats the results are similar to the control group, no chloride dependence of bicarbonate transport being detected. Table 3 and Fig. 3 summarize the results of stationary microperfusion in cortical distal tubule of alkalotic Brattleboro rats. In the early distal tubule no alterations were observed when comparing control and zero chloride perfusions. On the other hand, the zero chloride perfusion caused a significant decrease in the stationary pH of late distal tubule (7.18 ± 0.08 *vs.* 6.75 ± 0.11). A reduction of almost 50% in the net flux of base was observed during perfusion with zero chloride solution (0.183 ± 0.025 *vs.* 0.091 ± 0.016 nmol · cm^{-2} · s^{-1}).

Experiments with SITS

The previous results are consistent with the existence of chloride-dependent bicarbonate transport in cortical distal tubule. With the aim of investigating if this transport mechanism is dependent on Cl^-/HCO_3^- exchange at the apical membrane, similar to the Band 3 transporter of red blood cells [3], the tubular lumen of control Wistar rats was perfused with bicarbonate Ringer containing 5 × 10^{-4} M of SITS. SITS is a known inhibitor of the anion exchanger present in the basolateral membrane of intercalated cells [14]. Stationary luminal microperfusion of cortical distal tubule with 5 × 10^{-4} M SITS yielded the following bicarbonate reabsorption rates (in nmol/cm^{-2}sec^{-1}): 0.75 ± 0.07, $n = 11$ in controls and 0.56 ± 0.07, $n = 12$ with SITS in early distal tubule, and 0.95 ± 0.103, $n = 11$ in controls and 0.82 ± 0.08, $n = 19$ with SITS in late distal tubule. Thus, there was a tendency to reduce bicarbonate reabsorption, but the observed difference was not statistically significant. If SITS were to inhibit bicarbonate secretion, an increase of net bicarbonate reabsorption would be expected under these conditions which was definitely not observed. Figure 4 gives alkalinization half-times and rates of alkalinization in early and late distal tubules of alkalotic Wistar rats during control and 5 × 10^{-4} M SITS perfusions. Also, in this group no significant differences between controls and SITS perfused tubules were obtained. Table 4 shows that in alkalotic Brattleboro rats, late distal perfusion with zero Cl^- Ringer containing 5 × 10^{-4} M SITS reduces luminal alkalinization in a similar way as in the absence of the drug (*see* Table 3), confirming that the Cl^- dependent alkalinization (HCO_3^- secretion) is not affected by this agent.

Discussion

The ability of distal epithelia to secrete bicarbonate was first demonstrated by McKinney and Burg [24] in cortical collecting duct of alkalotic rabbits, an observation

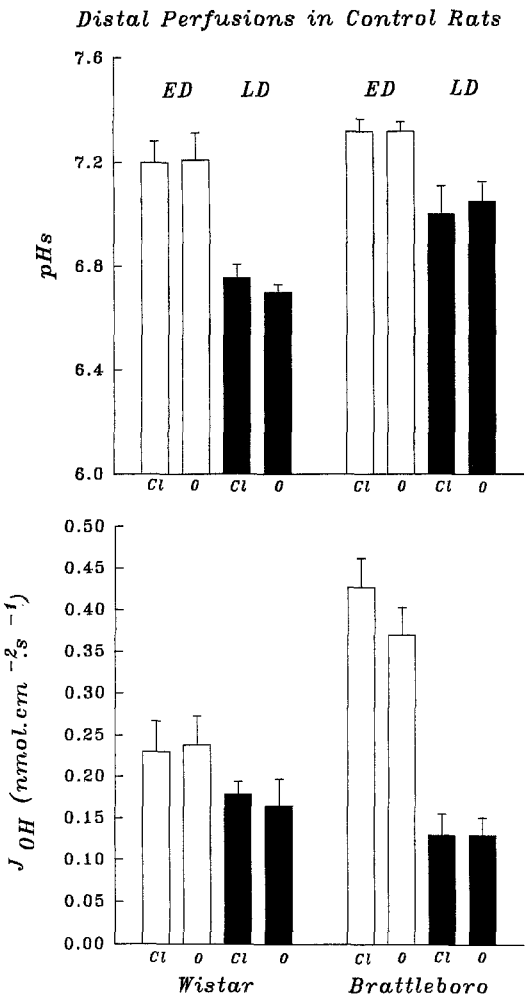

Fig. 1. Stationary pH and net fluxes of base (J_{OH}) in early (*ED*) and late (*LD*) distal tubule segments of control Wistar and Brattleboro rats. (*Cl*) Perfusion with normal chloride Ringer solution. (*Cl 0*) Perfusion with 0 Cl Ringer (Cl substituted by gluconate).

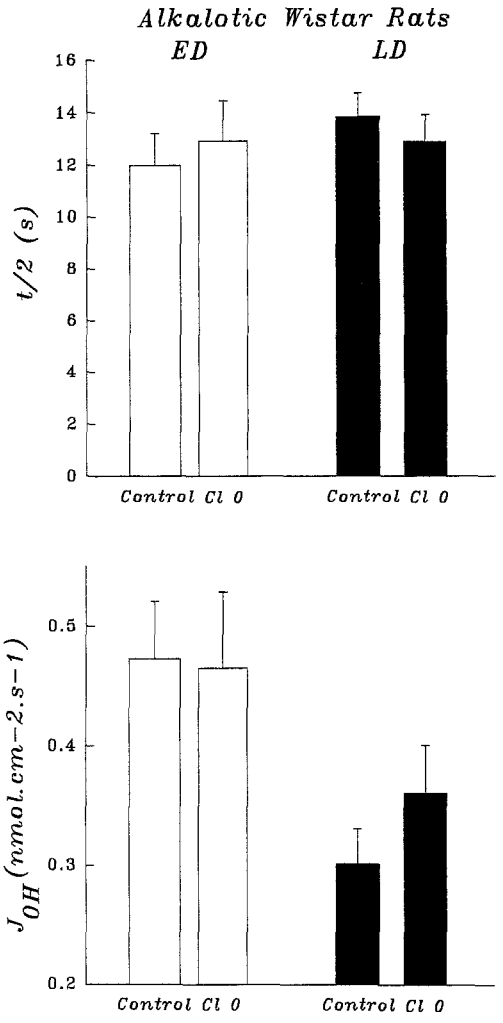

Fig. 2. Distal tubule perfusions in alkalotic Wistar rats. (*t/2*) Alkalinization half-time; (J_{OH}) base flux. Control, perfusion with normal chloride Ringer; (*Cl 0*) perfusion with zero Cl. (*ED*) early distal; (*LD*) late distal segments.

later extended to rat CCD [13]. This finding has raised interest in detecting a similar process in cortical distal tubule, since it is known that the late distal tubule of micropuncturists corresponds morphologically to the initial collecting duct [16]. This segment, as well as the connecting segment, contains intercalated cells (IC), rich in carbonic anhydrase and therefore responsible for acid-base transport [20]. Studies on the cells of CCD have evidenced at least two types of such cells, alpha-intercalated cells, responsible for H^+ secretion, and beta-intercalated cells, with an inverted cell polarity, capable of bicarbonate secretion [11, 27]. Immunocytochemical studies have shown the presence of vacuolar H^+-ATPase on the luminal membrane of alpha cells, and on the basolateral membrane of beta cells both in cortical collecting duct and in late distal tubule [2], indicating reversal of H^+-ion flow in these cell types. At the basolateral cell membrane of alpha-IC cells evidence for the presence of a Cl^-/HCO_3^- exchanger sensitive to a derivative of stilbene (SITS) has been obtained [29]. It is thought that such a transporter is also responsible for bicarbonate transfer into the lumen in beta-IC cells [27, 28].

There is evidence that in control rats H^+-ion secretion predominates over bicarbonate secretion in cortical distal tubules. Recent studies from our and other laboratories have found evidence for the presence of Na^+/H^+ exchange mostly in early distal tubule, and of vacuolar H^+-ATPase mostly in late distal tubule [10, 33]. The presence of a gastric-type H^+/K^+-ATPase has also been detected in late distal tubule of K depleted rats, as had been found particularly in medullary collecting duct [37, 38].

In the present studies, secretory base fluxes (J_{OH^-}) in control and alkalotic Wistar rats were not different during perfusion with and without chloride; therefore, no evidence for Cl^-/HCO_3^- exchange was obtained in these

Table 3. Stationary microperfusion of cortical distal tubules of Brattleboro rats

	n	pHs	*t½* (sec)	J_{OH} (nmol · cm^{-2} · sec^{-1})	PD (mV)
ED					
Control	23/7	7.32 ± 0.05	5.67 ± 0.29	0.427 ± 0.035	−4.5 ± 0.62
Cl^- 0	20/6	7.32 ± 0.03	6.95 ± 0.62	0.370 ± 0.033	−5.8 ± 0.81
LD					
Control	11/5	7.00 ± 0.11	11.97 ± 1.28	0.130 ± 0.025	−29.2 ± 1.78
Cl^- 0	19/7	7.05 ± 0.07	17.63 ± 2.24	0.130 ± 0.021	−25.5 ± 2.44
ED					
Akalosis	20/6	7.44 ± 0.04	7.62 ± 0.74	0.370 ± 0.026	−4.4 ± 0.94
A + Cl^- 0	18/6	7.43 ± 0.03	7.85 ± 1.26	0.374 ± 0.026	−4.7 ± 0.96
LD					
Alkalosis	25/6	7.18 ± 0.08	13.4 ± 1.50	0.183 ± 0.025	−34.1 ± 2.21
A + Cl^- 0	18/6	6.75 ± 0.11*	15.7 ± 3.24	0.091 ± 0.016*	−43.1 ± 1.57*

Values are mean ± SE; *n*, number of perfusions/number of tubules; pHs, stationary pH; *t½*, alkalinization half-time; J_{OH}, rates of alkalinization; PD, transepithelial potential difference; ED, Early distal tubules; LD, Late distal tubules; Control, perfusion with control Ringer solution; Alkalosis (A), alkalotic rats; Cl^- 0, perfusion without chloride; * $P < 0.01$ compared with control perfusion.

rats (*see* Table 3, and Figs. 1 and 2). Nevertheless, net base fluxes into the lumen were significant in these rats, being maximally of the order of approximately 50% of net acidification fluxes (as given in Fig. 5). These data suggest that significant passive fluxes of OH^-, H^+ or paracellular HCO_3^- are present in distal tubules.

When comparing our findings with those of other investigators a complicating factor relates to the different experimental conditions that have been studied. Evidence suggesting bicarbonate secretion has been reported in cortical distal tubule of fed rats under a normal diet [18]; net secretion was observed in rats undergoing two-day chloride depletion plus bicarbonate drinking, while at normal chloride no secretion was observed [17]. Rats subject to chloride and potassium depletion followed by bicarbonate addition to the diet reabsorbed bicarbonate when the distal tubule was perfused with 5 mM bicarbonate, and secreted bicarbonate during perfusion with 0 bicarbonate [36]. We have not been able to detect luminal pH more alkaline than blood in control and chronically alkalotic rats, including in animals receiving a potassium supplement [21]. In chronic plus acute metabolic alkalosis, increased bicarbonate reabsorption rather than secretion was observed both during free-flow and "in vivo" microperfusion experiments [4, 8]. To follow up on these studies, we investigated the presence of bicarbonate secretion perfusing cortical distal tubules with phosphate-buffered solutions at a pH below stationary levels (pH 6), a condition where, after blocking fluid flow with oil, the lumen pH alkalinizes toward its stationary level. We have shown before that in proximal tubule the flux of acid as well as alkaline phosphate out of the lumen is considerably slower than lumen acidification or alkalinization, and concluded that major pH changes due to differential transfer of phosphate salts (NaH_2PO_4 faster than Na_2HPO_4) are unlikely [7]. Since

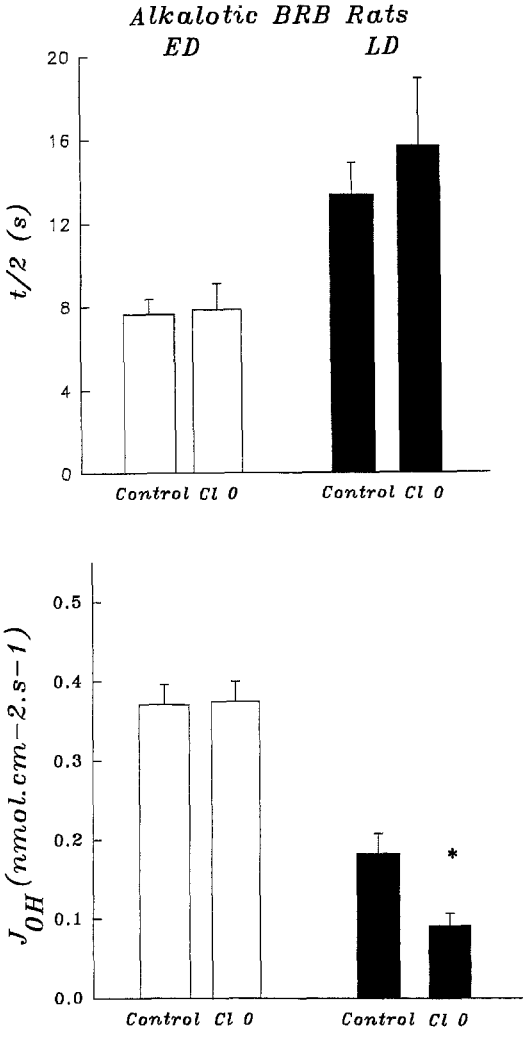

Fig. 3. Distal tubule perfusions in alkalotic Brattleboro (BRB) rats. Symbols as in Fig. 2. *$P < 0.05$ compared to control Cl.

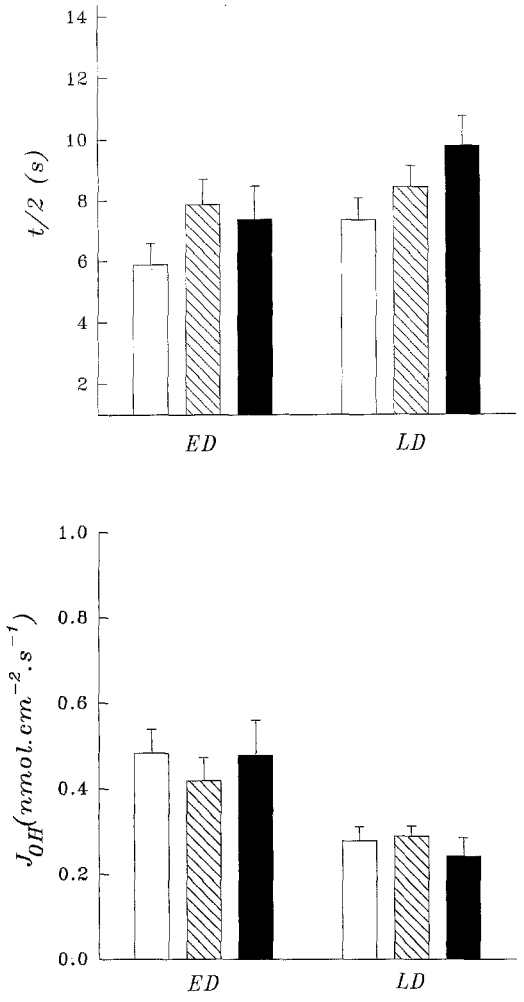

Fig. 4. Alkalinization half-times (*t/2*), *s*, and net base flux into the lumen (J_{OH}) in alkalotic Wistar rats during distal perfusion with 5×10^{-4} M SITS. (□) Control; (▨) SITS 5×10^{-4} M; (■) recovery.

Table 4. Effect of 5×10^{-4} M SITS on bicarbonate secretion in late distal tubules of alkalotic Brattleboro rats

	n	pHs	t/2 (sec)	J_{OH^-} (nmol · cm^{-2} · sec^{-1})	PD (mV)
Control	26/12	7.46 ± 0.037	10.6 ± 0.958	0.25 ± 0.023	24.1 ± 2.05
$Cl^- = 0$	26/12	7.49 ± 0.044	17.9 ±* 1.94	0.15 ±* 0.014	24.3 ± 2.51

Values are means ± SE; *see* legends Tables 2 and 3 for additional explanations; 5×10^{-4} M SITS was added to perfusion fluid of both groups. *$P < 0.01$ compared to controls (normal Cl^-).

distal epithelium is markedly tighter than proximal, this conclusion applies even more to this segment. Thus, the observed alkalinization might proceed mostly by influx into the lumen of HCO_3^-, NH_3 or OH^-, or by outflux of H^+. Bicarbonate flux into the lumen could occur via paracellular flux or by Cl^-/HCO_3^- exchange at the luminal cell membrane. Inhibition of bicarbonate secretion during luminal perfusion with low chloride solutions constitutes evidence for this anion exchange both in CCD and cortical distal tubule [19, 28].

The experiments with Brattleboro rats were designed to explore the genetically conditioned absence of antidiuretic hormone (ADH) in these animals [32]. This hormone had been shown to stimulate H^+ secretion (i.e., bicarbonate reabsorption) in cortical distal tubules [1] and in cortical collecting duct [26, 30]. It was expected that in these animals bicarbonate secretion might be unmasked due to reduction of H^+ secretion. The analysis of whole animal data, however, shows that urinary acidification is in the range of control Wistar rats, the increased titratable acid and ammonia excretion possibly being due to the markedly increased urine flow and the consequent increase of buffer excretion.

In control Brattleboro rats, perfusion with low chloride solutions did not alter base flux into the lumen. However, in late distal tubule of alkalotic Brattleboro rats low chloride perfusion reduced stationary luminal pH, increased alkalinization half-times and in consequence reduced net J_{OH^-} into the lumen. These data suggest the presence of a component of Cl^-/HCO_3^- exchange in alkalotic Brattleboro rats (*see* Table 3). Luminal perfusion with low chloride has been shown to increase late distal bicarbonate reabsorption in "in vivo" pump perfusion studies in Sprague-Dawley rats, which also constitutes evidence for a component of distal bicarbonate secretion [33]. On the other hand, it should be noted that even in alkalotic BRB rats, stationary luminal pH was still less alkaline than blood pH, indicating that bicarbonate reabsorption is still predominant in this condition.

Cl^-/HCO_3^- exchange is in general mediated by a transporter akin to the Band 3 anion exchanger of red blood cells [31]. This is true for basolateral anion exchange in most cells; the exchanger is specifically inhibited by stilbene compounds such as SITS and DIDS [9, 14]. On the other hand, the apical anion exchanger of turtle urinary bladder is largely insensitive to these drugs [15]. This observation is compatible with our data (Fig. 4), showing that SITS does not affect bicarbonate transport significantly when applied at the apical cell surface. If bicarbonate secretion were inhibited in these experiments, an increase in net bicarbonate reabsorption and a reduction in the rate of luminal alkalinization as well as an obliteration of the difference in bicarbonate transport rates in the presence and absence of Cl^- would be expected, which was not the case. Similar observations have been made in cortical collecting duct [11].

Figure 5 summarizes some of the results of this work. Net rates of bicarbonate reabsorption in control rats are compared to chloride-dependent bicarbonate secretion in control and alkalotic Brattleboro rats. Chloride-dependent bicarbonate secretion was obtained from

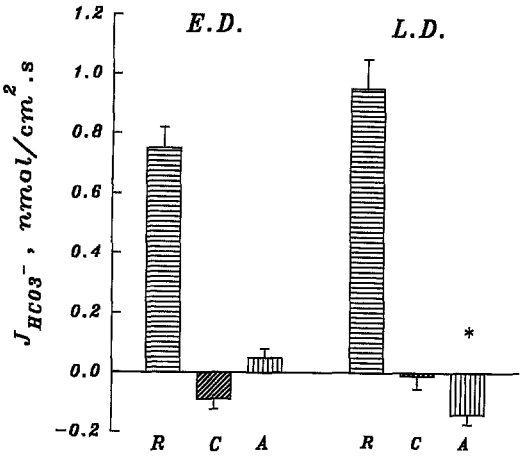

Fig. 5. Bicarbonate transport in cortical distal tubules. (*R*) bicarbonate reabsorption in control Wistar rats in *E.D.* (early) and *L.D.* (late) distal tubule. (*C*) Cl-dependent bicarbonate transport in control, and (*A*) in alkalotic Brattleboro rats. + $J_{HCO_3^-}$, bicarbonate reabsorption; − $J_{HCO_3^-}$, bicarbonate secretion. (*) $P < 0.05$ compared to zero secretion.

individual tubules subtracting base fluxes measured in the absence of chloride from fluxes measured in the presence of this anion (data from Tables 2 and 3). It is clearly noted that in early and late distal tubule of control rats and in early distal tubule of alkalotic rats no significant anion exchange component was detected. Such a component was only found in late distal loops of alkalotic Brattleboro rats. It is also interesting to compare the magnitude of this component (0.14 nmol · $cm^{-2}sec^{-1}$) with that of early (0.75) and late (0.95) distal bicarbonate reabsorption in control Wistar rats. A general conclusion that may be reached is that, under the experimental conditions studied in this work, bicarbonate secretion is only a minor component of the ion fluxes responsible for tubule acid-base transport in cortical distal tubule.

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