

Bicarbonate Transport by Rabbit Cortical Collecting Tubules

EFFECT OF ACID AND ALKALI LOADS IN VIVO ON TRANSPORT IN VITRO

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ABSTRACT Rabbit cortical collecting tubules were perfused *in vitro* to investigate the control of bicarbonate transport. Bicarbonate was measured by microcalorimetry as total CO_2 . The perfusate and bath were identical solutions containing 25 mM bicarbonate at pH 7.4. The mean pH of the urine in the bladders of untreated rabbits at the time they were killed was 7.4. Their individual tubules, studied *in vitro*, either absorbed or secreted bicarbonate, and, combining the results, there was on the average no significant net transport. When the rabbits were treated with NH_4Cl the day before the experiment, their urine was acidic and their tubules studied *in vitro* absorbed bicarbonate (i.e., there was net lumen-to-bath transport). In contrast, when the rabbits were treated with NaHCO_3 , their urine was significantly more alkaline, and their tubules studied *in vitro* generally secreted bicarbonate (i.e., net bath-to-lumen transport). Thus, the direction of bicarbonate transport by cortical collecting tubules studied under standard conditions *in vitro* correlated with the urine pH and was determined by the preceding treatment of the animals *in vivo* with acidifying or alkalinizing salts. These results demonstrate a previously unrecognized mechanism which contributes to the control of urinary bicarbonate excretion.

INTRODUCTION

When mammals are given acidifying salts such as NH_4Cl , they excrete an acid urine that is virtually free of bicarbonate (1, 2). Conversely, when bicarbonate is given, the urine is alkaline and contains a

high concentration of bicarbonate (1, 3, 4). Fluid collected from the late distal convoluted tubules of alkalotic or acidotic animals by micropuncture does not reveal similar extremes of pH and bicarbonate concentration (1, 3). On the basis of these and similar studies, the large pH and HCO_3 gradients between urine and blood have been attributed to transport by the collecting ducts (2). Despite the possible importance of bicarbonate transport and acidification by collecting ducts, there have been no extensive direct studies of these processes. The purpose of the present experiments was to measure bicarbonate transport directly in rabbit cortical collecting tubules *in vitro* and to test whether prior treatment with alkalinizing or acidifying salts *in vivo* affected bicarbonate transport measured subsequently *in vitro*.

METHODS

The method for dissecting and perfusing isolated rabbit collecting tubules has been described previously (5, 6). The perfusions were at 37°C . The average tubule length was 2.9 mm. Previously, it was found that in the absence of an osmotic gradient virtually no fluid is absorbed from cortical collecting tubules (6). This was confirmed in the present studies. In five tubules perfused and bathed in the solution described below the mean rate of fluid absorption was -0.01 ± 0.01 nl/mm tubule length per min. Since there is no net fluid transport under the conditions of these experiments, the rate of total CO_2 transport, $^j\text{CO}_2$, was calculated as follows: $^j\text{CO}_2 = (C_o - C_L) V_L/L$, where C_o and C_L are the total CO_2 concentrations in the perfusate and collected fluid, respectively, V_L is the rate of collection of tubule fluid, and L is the length of the tubule. $(C_o - C_L)$ was found to be a function of perfusion rate, decreasing at high rates and vice versa. At the beginning of each experiment the perfusion rate was adjusted so that the absolute value of $(C_o - C_L)$ was approximately 5 mM which could be accurately measured, but was considerably less than the maximum absolute value of $(C_o - C_L)$ reached at the slowest perfusion rates (see footnote to Table 1). The average

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collection rate was 0.99 nl/mm per min. Total CO₂ was measured by the microcalorimetric method of Vurek et al. (7). For purposes of discussion the transport of bicarbonate will be considered to reflect that of total CO₂, neglecting the small error that is introduced.

Three or more collections of tubule fluid (beginning approximately 1 h after sacrifice of the animal) were made over a period of 10–15 min in each experiment. The concentration of total CO₂ and the rate of transport were determined for each collection. The mean for each tubule was calculated and used in the statistical analysis. The data are presented as the mean ± SEM (*n* = number of tubules). Statistical significance of differences was determined by the unpaired *t* test.

The pH of the urine present in the bladder of the rabbit at the time of death was measured using a Corning model 109 pH meter (Corning Medical, Corning Glass Works, Medfield, Mass.).

The same artificial solution was used as perfusate and bath. It contained (in mM) NaCl 114, NaHCO₃ 25, MgSO₄ 1.2, CaCl₂ 2.0, K₂HPO₄ 2.5, L-alanine 6.0, Na lactate 4.0, Na citrate 1.0, and glucose 5.5. Solutions were gassed with 95% O₂/5% CO₂ to maintain a pH of 7.4.

Some rabbits were treated on the day before the experiment either with 15 meq/kg NH₄Cl or 20 meq/kg NaHCO₃ by gavage under ether anesthesia.

RESULTS

The mean urinary pH of untreated rabbits (Table I) was 7.4. Tubules from 10 animals absorbed total CO₂ and those from 4 animals secreted it (Fig. 1), resulting in a mean rate of total CO₂ transport not significantly different from zero.

The mean urinary pH of rabbits treated with NH₄Cl was 5.0. All of the tubules from these animals absorbed total CO₂. The mean rate of absorption was 1.05 pmol/cm per s which is significantly different from the mean of the untreated animals.

The mean urinary pH of rabbits treated with NaHCO₃ was 8.1 which is significantly greater than that of the untreated animals. 15 tubules from these animals secreted total CO₂; 3 tubules absorbed it.

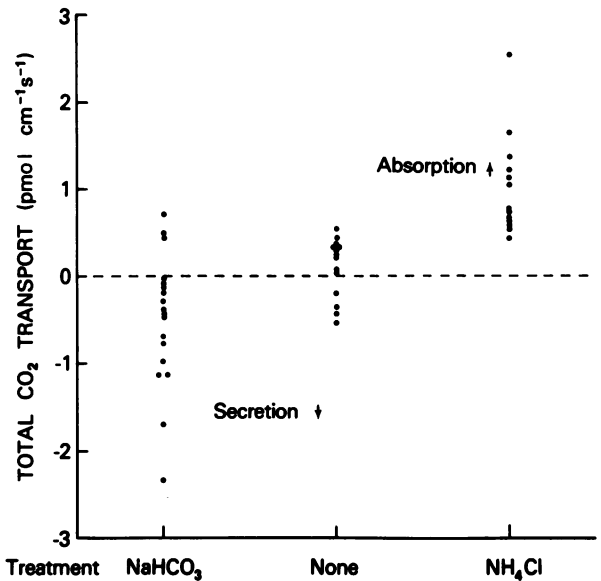


FIGURE 1 Effect of pretreatment of rabbits with NH₄Cl or NaHCO₃ on bicarbonate transport by their cortical collecting tubules in vitro. Treated animals received 15 meq/kg NH₄Cl or 20 meq/kg NaHCO₃ by gavage on the day preceding the experiment.

The mean rate of transport (secretion) was 0.51 pmol/cm per s which is significantly different from both the untreated rabbits and those given NH₄Cl.

DISCUSSION

It has been previously proposed that the collecting ducts participate in the control of urinary bicarbonate excretion and acidification. Altered transport in the collecting ducts has been attributed to changes in the acid-base state of the animal and in the composition of the fluid delivered from the more proximal segments, especially changes in sodium, anion, and

TABLE I
Effect of Pretreatment of Rabbits with NH₄Cl or NaHCO₃ on Their Urinary pH and on the Transport of Total CO₂ by Their Cortical Collecting Tubules Studied in Vitro under Standard Conditions

Pretreatment	^a CO ₂ *	P†	C _o - C _L ‡	Urinary pH [¶]	P‡
None	0.09 ± 0.09 (14)	—	5.7 ± 0.6	7.4 ± 0.15	—
NH ₄ Cl	1.05 ± 0.14 (13)	<0.001	6.1 ± 0.8	5.0 ± 0.07	<0.001
NaHCO ₃	-0.51 ± 0.18 (18)	<0.01	3.9 ± 1.0	8.1 ± 0.09	<0.001

* Rate of absorption of total CO₂ (lumen-to-bath), picomoles per centimeter per second. Negative values indicate secretion (i.e., net bath-to-lumen transport).

† Significance of difference compared to no pretreatment.

‡ Absolute difference in concentration of total CO₂ (mM) between perfused and collected fluid. At the beginning of each experiment the perfusion rate was adjusted so that the absolute value of |C_o - C_L| was approximately 5 mM (see Methods). In the course of adjustment when the perfusion rate happened to be low, higher absolute values of |C_o - C_L| were often observed, i.e., 9–15 mM in nine of the tubules that absorbed and 9–22 mM in six of the tubules that secreted total CO₂.

¶ Urine present in the bladder at the time the animals were killed.

buffer content (2). Based on the present studies we suggest that there is an additional factor, previously unrecognized, namely, an intrinsic change in the tubules that alters bicarbonate transport and acidification.

Pretreatment of the living rabbits with NH_4Cl caused their cortical collecting tubules to absorb bicarbonate when subsequently perfused in vitro. Pretreatment with NaHCO_3 caused bicarbonate secretion. Urine collected from the bladders of the animals at the time they were killed was correspondingly acidic or alkaline. Therefore, pretreatment with the acidifying or alkalinizing salts caused the pH of the urine from the living animals and bicarbonate transport by their cortical collecting tubules (studied later under standard conditions in vitro) to change in a correlated fashion.

What is the stimulus for the change in the collecting tubules? Since the pretreatment was designed to alter the acid-base status of the animals, we suggest this as an important factor. There are other possibilities, however, such as changes in effective intravascular volume, sodium and potassium balance, and hormone levels that may have also resulted from the pretreatment and we cannot determine which factor was most important, since these latter factors were not examined in the present study.

What was the nature of the change in the collecting tubules? Since the change persisted for up to 2 to 3 h after the tubules were removed from the animals, we suggest that some relatively stable component such as an enzyme was induced or repressed. It is of interest that NH_4Cl acidosis increases the activity of glucose 6-phosphate dehydrogenase in rat, mouse, and guinea pig kidney (8).

The rates of bicarbonate transport observed under the standard conditions in vitro probably correlate poorly with the actual rates in vivo. For example, in the acidotic animals the amount of bicarbonate delivered to the collecting tubules from the more proximal segments probably was reduced (1-3) which would cause a decreased rate of bicarbonate reabsorption by the collecting tubules associated with the lower bicarbonate concentration. In vitro, on the other hand, the rate of bicarbonate absorption was increased. We suggest that the increase observed in vitro reflects an increased capacity for bicarbonate absorption which may also have played a role in altering the pH of the urine in vivo. Intrinsic function of other tubule segments could also have been affected by the pretreatment, but this has not been tested yet.

The possibility of bicarbonate secretion by renal tubules apparently has not previously been seriously considered. It has been observed that treatment with NaHCO_3 causes the bicarbonate concentration in the urine to exceed that in the plasma (1, 3, 4) but

this could be explained by continued water reabsorption in the face of decreased bicarbonate reabsorption (3). In the present studies many of the cortical collecting tubules secreted bicarbonate, especially after pretreatment with NaHCO_3 . It is likely, therefore, that secretion of bicarbonate by collecting tubules may contribute to bicarbonate excretion after NaHCO_3 loading in vivo.

What are the mechanisms of bicarbonate absorption and secretion by the collecting tubules? Previous studies of the turtle urinary bladder may provide clues. The cells of the turtle bladder resemble those of the collecting tubule and the tissues have a similar embryologic origin. The turtle bladders acidify their contents by hydrogen ion secretion (9).¹ Turtle bladders also secrete bicarbonate, linked to chloride absorption via an electroneutral exchange that is interrupted by inhibitors both of metabolism and of carbonic anhydrase (9). It is not yet clear, however, whether the mechanisms of bicarbonate transport are the same in the turtle bladder and cortical collecting tubule.

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¹ There is not complete agreement on this point. Brodsky and Schilb (10) have propounded the contrary view, namely that direct bicarbonate absorption accounts for the acidification.