Bicarbonate Secretion by Rabbit Cortical Collecting Tubules in Vitro

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We previously reported that rabbit renal cortical collecting tubules can secrete bicarbonate in vitro (i.e., there can be net transport from bath to lumen, causing the concentration in the lumen to increase). Net bicarbonate secretion was observed most often when rabbits had been pretreated with $NaHCO_3$ and were excreting alkaline urine before being killed for experiments. The purpose of the present studies was to elucidate the mechanism involved by testing the effects of ion substitutions and drugs on collecting tubules that were secreting bicarbonate. Acetazolamide inhibited net bicarbonate secretion, suggesting that the process is dependent upon carbonic anhydrase. Net bicarbonate secretion also decreased when sodium in the perfusate and bath was replaced by choline, but not when chloride was replaced by nitrate or methylsulfate. Ouabain had no significant effect. Amiloride caused net bicarbonate secretion to increase. The rate of net secretion did not correlate with transepithelial voltage. The results are compared to those in turtle urinary bladders that also secrete bicarbonate. There are no direct contradictions between the results in the two tissues, i.e., in turtle bladders acetazolamide also inhibited bicarbonate secretion and ouabain had no effect. Nevertheless, it seems unlikely that net secretion of bicarbonate by collecting tubules involves specific exchange for chloride, as has been proposed for turtle bladders, because replacement of chloride by other anions did not […]

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ABSTRACT We previously reported that rabbit renal cortical collecting tubules can secrete bicarbonate in vitro (i.e., there can be net transport from bath to lumen, causing the concentration in the lumen to increase). Net bicarbonate secretion was observed most often when rabbits had been pretreated with $NaHCO_3$ and were excreting alkaline urine before being killed for experiments. The purpose of the present studies was to elucidate the mechanism involved by testing the effects of ion substitutions and drugs on collecting tubules that were secreting bicarbonate. Acetazolamide inhibited net bicarbonate secretion, suggesting that the process is dependent upon carbonic anhydrase. Net bicarbonate secretion also decreased when sodium in the perfusate and bath was replaced by choline, but not when chloride was replaced by nitrate or methylsulfate. Ouabain had no significant effect. Amiloride caused net bicarbonate secretion to increase. The rate of net secretion did not correlate with transepithelial voltage. The results are compared to those in turtle urinary bladders that also secrete bicarbonate. There are no direct contradictions between the results in the two tissues, i.e., in turtle bladders acetazolamide also inhibited bicarbonate secretion and ouabain had no effect. Nevertheless, it seems unlikely that net secretion of bicarbonate by collecting tubules involves specific exchange for chloride, as has been proposed for turtle bladders, because replacement of chloride by other anions did not inhibit bicarbonate secretion by collecting tubules. It was previously shown that the collecting tubules in vitro also may absorb bicarbonate, especially when the rabbits have been treated with NH_4Cl and are excreting acid urine before being killed. The effects of drugs on net bicarbonate secretion found in the present studies are compared to their previously reported effects on net bicarbonate absorption and the possibility is discussed that bicarbonate absorption and secretion are independent processes, as was previously proposed for turtle bladders.

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INTRODUCTION

Cortical collecting tubules dissected from rabbit kidneys and perfused in vitro may either absorb or secrete bicarbonate (1). The direction of transport correlates with treatment given to the animals and with the pH of the urine in their bladder at the time they are killed. Rabbits given ammonium chloride on the day before the experiment had acid urine and their collecting tubules in vitro regularly absorbed bicarbonate (i.e., net lumen-to-bath flux). In contrast, when sodium bicarbonate had been given, the urine was alkaline and the tubules in vitro generally secreted bicarbonate (i.e., net bath-to-lumen flux). It was proposed that the bidirectional bicarbonate transport demonstrated in vitro might also occur in vivo and contribute to the control of urinary acidification and alkalinization.

Net secretion of bicarbonate by renal tubules was not previously recognized, but the process had been identified in turtle (2) and toad (3) urinary bladders. The purpose of the present studies was to investigate the mechanism of net bicarbonate secretion in collecting tubules by determining the effect of conditions previously found to modify net bicarbonate absorption in collecting tubules and bicarbonate secretion in turtle bladders.

METHODS

The method of perfusion of individual nephron segments of rabbit kidneys used in these studies has been described previously (4–6), and is briefly summarized below. Young (1–2 kg) female New Zealand white rabbits were killed by decapitation. Kidney slices were prepared and placed in the control solution described below. A cortical collecting tubule was dissected and perfused at 37°C with concentric glass

TABLE I
Total CO_2 Transport by Cortical Collecting Tubules

Experiment	Transport rate*		Change	*P* value	Perfusion rate‡		No. of tubules
	Control	Experimental			Control	Experimental	
	pmol/cm/s				*nl/mm tubule length/min*		*n*
No sodium	−0.96±0.25	0.71±0.26	1.67±0.46	<0.02	2.4±0.4	2.1±0.2	7
Ouabain, 10 μM, in bath	−2.05±0.61	−1.55±0.73	0.50±0.45	>0.3	2.2±0.3	2.0±0.3	5
Amiloride, 10 μM, in perfusate	−0.46±0.11	−0.83±0.19	−0.38±0.11	<0.02	0.8±0.1	0.8±0.2	6
No chloride	−0.56±0.19	−0.55±0.19	0.01±0.20	>0.4	0.9±0.1	0.8±0.1	9
Acetazolamide, 0.1 mM, in bath and perfusate	−1.31±0.38	−0.25±0.24	1.06±0.34	<0.05	1.9±0.2	1.9±0.3	5

* Negative values indicate net bath-to-lumen transport, i.e., secretion. Values represent mean±1 SEM.
‡ Values represent mean±1 SEM.

pipettes (5). The average tubule length was 2.8 mm. Transepithelial potential difference was measured as previously described (7).

The solution used for the control perfusate and bath contained the following (in mM): NaCl, 114; $NaHCO_3$, 25; K_2HPO_4, 2.5; $MgSO_4$, 1.2; $CaCl_2$, 2.0; Na lactate, 4.0; Na citrate, 1.0; *l*-alanine, 6.0; and glucose, 5.5. When chloride or sodium was omitted, a solution was prepared that was otherwise similar but which contained nitrate or methylsulfate in place of chloride or choline in place of sodium.[1] Solutions were gassed with 95% O_2/5% CO_2 and were at pH 7.4.

Total CO_2 concentration of the perfusate and collected fluid was measured by the microcalorimetric method of Vurek et al. (8) as reported in previous studies (9). The concentration of bicarbonate was assumed to equal that of total CO_2, neglecting the small error introduced by this approximation. Previously, it was shown that collecting tubules do not absorb fluid in the absence of an osmotic gradient (1, 6, 10–12). Therefore, the net rate of total CO_2 transport (J_{CO_2}) was calculated from the change in total CO_2 concentrations between the perfusate and collected fluid, as follows:

$$J_{CO_2} = (C_O - C_L)V_L/L,$$

where C_O and C_L are the total CO_2 concentrations of the perfused and collected fluids, respectively, V_L is the rate of collection of tubule fluid and L is the tubular length. Negative values of J_{CO_2} indicate secretion (i.e., net transport of total CO_2 from bath to lumen). Positive values indicate absorption, (i.e., net lumen-to-bath transport). Theoretically, J_{CO_2} might be reduced if the transport of bicarbonate resulted in such large concentration differences across the epithelium that there was a significant back flux or the transport mechanism became concentration limited. Therefore, a perfusion rate was chosen during the control period that was slow enough to result in accurately measurable changes in total CO_2 concentration, but was fast enough to prevent large changes in concentration, and the same rate was used in the experimental periods (Table I). The mean change in concentration was approximately 5 mM.

Collections began after 20–30 min of perfusion with the control solutions. After three or more control collections, the experimental solutions were substituted and the collections repeated beginning 10 min later. In some cases collections were repeated with the control solutions at the end of the experiment. Total CO_2 concentration and J_{CO_2} were calculated for each collection and the mean values were used in the statistical analysis. When control observations were made both at the beginning and end of an experiment, the mean of the two sets of controls were used in the statistical analysis. The summary data is presented as the mean of the results in individual tubules ±SEM (n = number of tubules). Statistical significance of differences was determined by the Student's paired t test.

Tubules were accepted for study only if they secreted bicarbonate during the control periods. As reported previously (1) net bicarbonate secretion occurred most regularly in animals treated with $NaHCO_3$. Therefore, of the 32 tubules studies only 8 were obtained from untreated animals. 19 tubules were obtained from animals given 20 meq/kg $NaHCO_3$ by gavage on the preceding day and 5 from animals whose drinking water had been replaced by 0.075 M $NaHCO_3$ for 4 days.

RESULTS

Control measurements. The mean rate of net total CO_2 secretion in all tubules studied with the control solutions was -0.94 ± 0.16 pmol $cm^{-1}s^{-1}$ ($n = 32$). The mean transepithelial potential difference was -17 ± 2.5 mV ($n = 29$).

Effect of sodium. When sodium in the bath and perfusate was replaced by choline, secretion of total CO_2 and the voltage decreased significantly (Tables I and II and Fig. 1). In five of seven tubules the direction of transport of total CO_2 changed from net secretion to absorption (Fig. 1).

Ouabain. 10 μM ouabain added to the bath caused a small decrease in net total CO_2 secretion in four of five tubules (Fig. 2), but had no significant effect on total CO_2 secretion, taking all of the tubules into consideration (Table I). The ouabain caused the voltage to reverse polarity from 20 mV (lumen negative) in the controls to 9 mV (lumen positive) with the drug (Table II).

[1] In the chloride-free solutions $CaCl_2$ was replaced either by $Ca(No_3)_2$ or by Ca citrate (and in the latter case a part of the Na citrate was replaced by Na methylsulfate so that total concentration of citrate did not change.

TABLE II
Transepithelial Voltages across Cortical Collecting Tubules

Experiment	Potential difference*		Change	No. of tubules
	Control	Experimental		
	mV			*n*
No sodium	−25±7	−4±3	21±4‡	6
Ouabain, 10 μM, in bath	−20±8	+9±5	28±9‡	5
Amiloride, 10 μM, in perfusate	−10±4	+1±1	10±4‡	6
No chloride	−13±4	−7±2	6±3	6
Acetazolamide, 0.1 mM, in perfusate and bath	−15±4	−20±6	−5±2	5

* Mean values±1 SEM.
‡ $P < 0.05$.

Amiloride. 10 μM amiloride added to the perfusate caused the rate of net total CO_2 secretion to increase significantly (Fig. 3 and Table I), and the voltage to decrease (Table II).

Effect of chloride. Bicarbonate is secreted by turtle bladders in exchange for chloride (2). To test whether chloride is necessary for bicarbonate secretion in collecting tubules, chloride in the perfusate and bath was completely replaced by nitrate or methylsulfate. Neither net secretion of total CO_2 nor the voltage changed significantly when the chloride was replaced. (Fig. 4 and Tables I and II). Therefore, exchange with chloride is not a specific requirement for bicarbonate secretion by collecting tubules.

Acetazolamide. Acetazolamide 0.1 mM in the perfusate and bath significantly inhibited net secretion of total CO_2 (Fig. 5 and Table I) but did not affect the voltage (Table II).

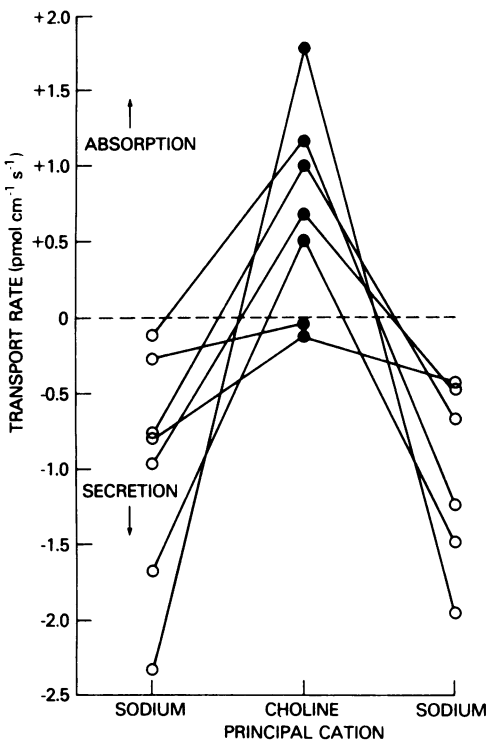

FIGURE 1 Effect of sodium replacement in the perfusate and bath by choline on the rate of total CO_2 transport by cortical collecting tubules.

DISCUSSION

Comparison to bicarbonate secretion by turtle urinary bladder. Both rabbit collecting tubules and turtle urinary bladders secrete bicarbonate. The mechanism may well be the same in the two tissues, because when the same drugs were tested on the two tissues the

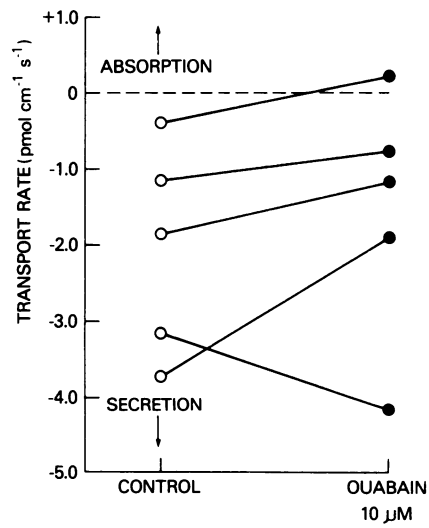

FIGURE 2 Effect of ouabain (10 μM) in the bath on the rate of total CO_2 transport by cortical collecting tubules.

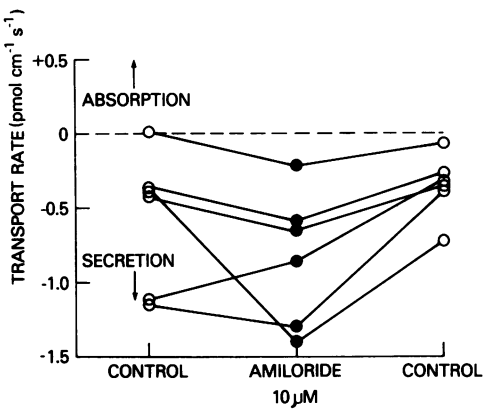

FIGURE 3 Effect of amiloride (10 μM) in the perfusate on the rate of total CO_2 transport by cortical collecting tubules.

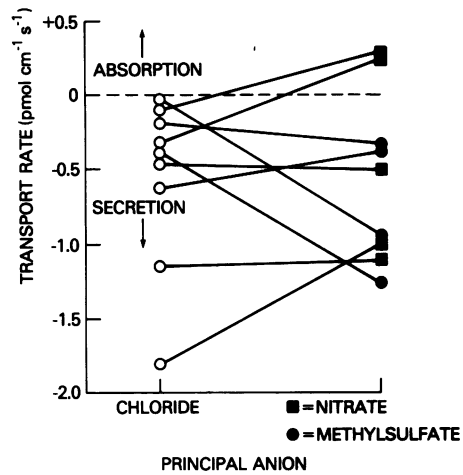

FIGURE 4 Effect of chloride replacement in the perfusate and bath by nitrate or methylsulfate on the rate of total CO_2 transport by cortical collecting tubules.

results were in agreement.[2] Acetazolamide inhibited bicarbonate secretion in both tissues and ouabain had no effect (2). The results of additional experiments that were performed on collecting tubules (but not previously on turtle bladders) however, raise doubts whether the mechanism invoked for the turtle bladder applies to collecting tubules.

The mechanism proposed for turtle bladders is that bicarbonate is secreted specifically in exchange for chloride that is absorbed and the exchange is electrically neutral (2). The evidence supporting this conclusion is as follows: (*a*) replacement of chloride in the mucosal and serosal baths by sulfate inhibits bicarbonate secretion, and (*b*) removal of bicarbonate from the serosal bath decreases chloride flux from mucosa to serosa.

In contrast, chloride was not specifically required for bicarbonate secretion by collecting tubules. Bicarbonate secretion was not affected when all of the chloride was replaced by nitrate or methylsulfate. Although this result does not rule out exchange of bicarbonate for chloride when the latter is present, it does rule out a specific requirement for chloride. It has not been reported whether nitrate or methylsulfate support bicarbonate secretion in turtle bladders as they do in the collecting tubules. Along the same lines as the present studies, rabbit antral (13) and ileal (14, 15) mucosa which also secrete bicarbonate in vitro continue to do so when chloride is totally replaced in the mucosal and serosal baths by isethionate (13) or sulfate (15).

Sodium is not believed to be directly involved in the secretion of bicarbonate by turtle bladders because ouabain, which inhibits sodium transport, did not inhibit bicarbonate secretion (2). In agreement with this finding, ouabain also did not inhibit bicarbonate secretion in cortical collecting tubules. In contrast, however, replacement of sodium by choline did inhibit bicarbonate secretion. Choline in place of sodium reversibly transformed net bicarbonate secretion into net bicarbonate absorption in the collecting tubules. Therefore, either the secretion of bicarbonate by col-

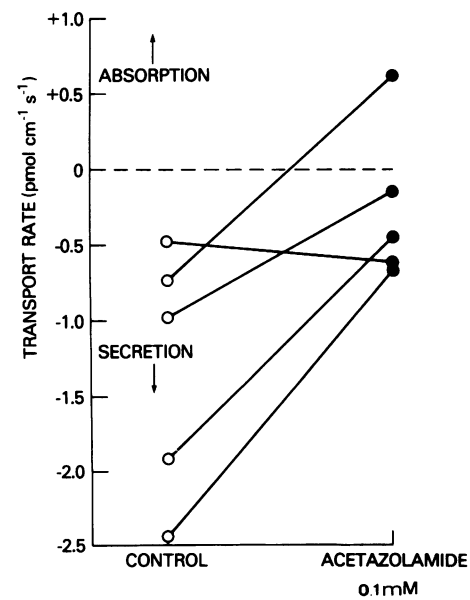

FIGURE 5 Effect of acetazolamine (0.1 mM) in the perfusate and bath on the rate of total CO_2 transport by cortical collecting tubules.

[2] Note, however, that details of the experimental conditions differed between the two sets of studies. Leslie et al. (2) short circuited their turtle bladders and used the pH stat technique to maintain a low pH in the mucosal solution. We are unable to apply either of these techniques to the isolated perfused tubules.

lecting tubules requires sodium or choline inhibits the process. It has not been reported whether the replacement of sodium by choline affects bicarbonate secretion by turtle bladders as well.

Comparison of the requirements for bicarbonate secretion to those for bicarbonate absorption by collecting tubules. In Fig. 6 the effects of various experimental conditions on net bicarbonate secretion by cortical collecting tubules are compared to the effects on net bicarbonate absorption which were measured previously (16). The tubules are grouped according to whether they were secreting bicarbonate in the initial control periods or were absorbing it. The results are arranged so that for both sets of tubules secretion (net bath-to-lumen transport) is to the left and absorption (net lumen-to-bath transport) to the right. Three of the maneuvers caused the same change in net transport regardless of its initial direction. Replacement of sodium by choline changed bicarbonate transport toward absorption regardless of whether there was initially net secretion or absorption, amiloride changed bicarbonate transport toward secretion, and ouabain had no significant effect in either set of tubules. Acetazolamide, on the other hand, inhibited both net bicarbonate absorption and secretion.

To analyze these results it would be helpful to know whether absorption and secretion of bicarbonate are two processes that occur simultaneously or whether there is only one process whose direction varies. Leslie et al. (2) have proposed that there are two separate processes in the turtle bladder: acidification by hydrogen ion secretion[3] and bicarbonate secretion. When bicarbonate is present in the mucosal fluid, hydrogen ion secretion should result in its absorption (18). The evidence that they adduced for separate processes was as follows: (*a*) whereas acidification caused a voltage oriented positive towards the mucosa, bicarbonate secretion was nonelectrogenic, (*b*) replacement of chloride by sulfate inhibited bicarbonate secretion, but not acidification, and (*c*) when acidification was inhibited by lowering the pH of the mucosal fluid, bicarbonate secretion continued.

We were unable to distinguish with the methods used in the present study whether one or two processes were present in collecting tubules since what was measured (net bicarbonate transport) could have been affected by bicarbonate absorption (directly or via hydrogen ion secretion) as well as by bicarbonate secretion. The results can be rationalized on either basis. There could be one process (either hydrogen ion or bicarbonate transport) whose direction and magnitude was determined by the preceding state of the animal, resulting either in net bicarbonate secretion or absorption under the control conditions in vitro (1). This process might have been modulated towards bicarbonate secretion (as by amiloride), towards net absorption (as by removal of sodium), or completely inhibited (as by acetazolamide). On the other hand there could be two processes, assumed for the sake of argument to be secretion of hydrogen ions and independent secretion of bicarbonate, as proposed by Leslie et al. (2) for the turtle bladder. Then the rate and direction of bicarbonate transport under control conditions would be determined by the balance of the two processes and the changes that were observed could have been caused by alterations in either process. For example, choline substituted for sodium shifted transport toward net bicarbonate absorption. This could have resulted either from enhanced secretion of hydrogen ions or decreased secretion of bicarbonate, or both. Amiloride shifted transport toward net bicarbonate secretion. This could have resulted from enhanced bicarbonate secretion, decreased hydrogen ion secretion, or both. Acetazolamide by this line of reasoning must have inhibited both processes.

If there were two processes, they could be distinguished by inhibiting one selectively while the other remained unaffected. This was accomplished in the turtle bladder by lowering the mucosal pH to the point of zero net acidification and demonstrating continued bicarbonate secretion by the pH stat technique or by measurement of evolved $^{14}CO_2$ (2). The comparable manipulations were impractical, however, for us to perform with collecting tubules.

Role of the transepithelial voltage. Acidification in turtle (19) and toad (20) urinary bladders is an electrogenic process that is significantly affected by the transepithelial voltage. The voltage across mammalian distal convoluted tubules is believed to be an important driving force for acidification in that tissue as well (21, 22).

Under control conditions the mean transepithelial voltage across collecting tubules secreting bicarbonate in the present studies was -17.1 ± 2.5 mV ($n = 29$) which is significantly less than the voltage previously measured under identical conditions (16) across tubules that were absorbing bicarbonate, -34.9 ± 4.9 mV ($n = 29$). The lower voltage might theoretically have been caused by decreased sodium absorption, increased potassium secretion, or increased chloride conductance. In the absence of appropriate measurements we cannot determine which of these, if any was responsible. It is unlikely, however, that the lower voltage is a result of the observed differences in

[3] This point is disputed. Brodsky and Schilb (17) contend that acidification is the result of direct bicarbonate absorption not linked to hydrogen ion secretion. We do not have a basis for distinguishing between the disputed alternatives in collecting tubules and recognize that the process involved might be either hydrogen ion secretion or direct bicarbonate absorption.

bicarbonate transport per se, because enhanced bicarbonate absorption (or hydrogen ion secretion) should have caused the voltage to become more positive in the lumen rather than more negative, as was observed.

Putting aside the cause of the change in voltage, the question remains whether the voltage affected bicarbonate (or hydrogen ion) transport as previously proposed for distal tubules. Judging from the results, it seems unlikely that the voltage had any important effect, since changes in bicarbonate secretion and absorption under various conditions did not correlate with the changes in voltage. For example, the transepithelial voltage decreased when sodium was replaced by choline, and when amiloride or ouabain was added, yet net bicarbonate secretion increased (amiloride), decreased (choline), or remained unchanged (ouabain) (Fig. 6). Acetazolamide, on the other hand, had no significant effect on the voltage, but inhibited bicarbonate transport whether there was initial net secretion or absorption in the control periods.

Amiloride enhanced net secretion (or inhibited absorption) of bicarbonate which is consistent with its known renal action to inhibit urinary acidification (23, 24). The mechanism of action of amiloride on bicarbonate transport is unclear. The drug is a potent inhibitor of sodium and potassium transport by rabbit collecting tubules (6). The inhibition of sodium transport by amiloride apparently is not directly related to the effect on bicarbonate, however, because sodium transport was also inhibited by ouabain (25) but that drug did not enhance net bicarbonate secretion (Fig. 6).

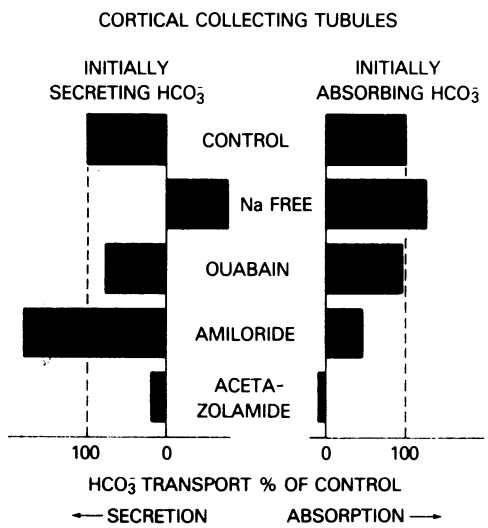

FIGURE 6 Effects of cations and drugs on bicarbonate transport by cortical collecting tubules. Tubules that were secreting bicarbonate in the control periods (present study) are compared to tubules that were absorbing bicarbonate (16). "Sodium-free" indicates that sodium was replaced by choline in the perfusate and bath. The concentrations of ouabain, amiloride and acetazolamide were 10 μM in the bath, 10 μM in the perfusate, and 0.1 mM in the perfusate and bath, respectively.

Acetazolamide inhibited transport of bicarbonate regardless of whether the bicarbonate was initially secreted or absorbed (Fig. 6). The drug is a carbonic anhydrase inhibitor. Although we cannot rule out nonspecific effects, it is simplest to postulate that all of the effects on bicarbonate transport were the result of inhibition of carbonic anhydrase. An important role for the enzyme in urinary acidification is generally recognized (17), but it is not clear what role it might play in bicarbonate secretion.

Relation of bicarbonate secretion to elevated partial pressure of carbon dioxide (P_{CO_2}) in urine. A number of mechanisms were previously proposed (17) to account for the fact that urinary P_{CO_2} may exceed that in plasma when the rate of bicarbonate excretion is elevated (26). In addition to the previous explanations, Arruda et al. (27) recently concluded that the urinary P_{CO_2} may increase pari passu with urinary bicarbonate concentration. They found that the elevated P_{CO_2} of highly alkaline urine was reproduced by adding bicarbonate to solutions in vitro. Bicarbonate secretion by cortical collecting tubules elevates the urinary bicarbonate concentration and therefore could elevate urinary P_{CO_2} by the same mechanism. Consider the well-known relation between bicarbonate and carbon dioxide: $HCO_3^- + H^+ \rightleftarrows H_2CO_3 \rightleftarrows CO_2 + H_2O$. Secretion of bicarbonate into the tubule lumen drives the reaction to the right, increasing the P_{CO_2} of the urine. This provides an additional explanation for the high P_{CO_2} of alkaline urine in addition to those previously proposed (17, 26) and is consistent with the conclusion of Arruda et al. (27).

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