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Research Article

Ouabain and lithium decrease acidification in open-circuited bladders by eliminating the electrical gradient favoring acidification. The effect of ouabain and lithium on acidification in cortical and medullary collecting tubules derived from starved New Zealand white rabbits was studied by using the techniques of isolated nephron microperfusion and microcalorimetric determination of total CO2 flux. Bath and perfusion solutions were symmetric throughout all studies, and solutions contained 25 meq of bicarbonate and were bubbled with 93.3% O2/6.7% CO2 gas mixtures. In cortical collecting tubules, ouabain (10(-8) M) addition to bath resulted in a decrease in both potential difference (PD), from -16.4 to -2.2 mV (P less than 0.001), and total CO2 flux (JTCO2), from +6.0 to 1.5 pmol/mm per min (P less than 0.005). In medullary collecting tubules neither PD nor JTCO2 changed with the addition of ouabain in either 10(-8) or 10(-4) M concentration. Replacement of 40 mM NaCl with 40 mM LiCl in both perfusate and bath in cortical collecting tubules resulted in decreases in both PD, from -11.6 to 0.4 mV (P less than 0.005), and JTCO2, from +10.8 to +4.2 pmol/mm per min (P less than 0.025). This substitution had no effect on medullary collecting tubules. When control flux rates were plotted against animal bladder urine pH, both medullary and cortical tubules showed good inverse correlation between these […]

Characterization of Acidification in the Cortical and Medullary Collecting Tubule of the Rabbit

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ABSTRACT Ouabain and lithium decrease acidification in open-circuited bladders by eliminating the electrical gradient favoring acidification. The effect of ouabain and lithium on acidification in cortical and medullary collecting tubules derived from starved New Zealand white rabbits was studied by using the techniques of isolated nephron microperfusion and microcalorimetric determination of total CO_2 flux. Bath and perfusion solutions were symmetric throughout all studies, and solutions contained 25 meq of bicarbonate and were bubbled with 93.3% O_2/6.7% CO_2 gas mixtures. In cortical collecting tubules, ouabain (10^{-8} M) addition to bath resulted in a decrease in both potential difference (PD), from −16.4 to −2.2 mV ($P < 0.001$), and total CO_2 flux (J_{TCO_2}), from +6.0 to 1.5 pmol/mm per min ($P < 0.005$). In medullary collecting tubules neither PD nor J_{TCO_2} changed with the addition of ouabain in either 10^{-8} or 10^{-4} M concentration. Replacement of 40 mM NaCl with 40 mM LiCl in both perfusate and bath in cortical collecting tubules resulted in decreases in both PD, from −11.6 to 0.4 mV ($P < 0.005$), and J_{TCO_2}, from +10.8 to +4.2 pmol/mm per min ($P < 0.025$). This substitution had no effect on medullary collecting tubules. When control flux rates were plotted against animal bladder urine pH, both medullary and cortical tubules showed good inverse correlation between these variables, with higher values of flux rate for the medullary tubules. The data support a role for transepithelial PD in acidification in the cortical collecting tubule and also suggest that both cortical and medullary segments of the collecting tubule participate when urinary acidification is increased during starvation in the rabbit.

INTRODUCTION

One of the difficulties in understanding acidification by the collecting tubule is that the data obtained from a variety of techniques and models are contradictory. Older clearance studies suggested that adequate distal sodium delivery was required for normal distal acidification (1). With the development of the turtle bladder model, it became apparent that sodium itself was not specifically required for acidification; but acidification was found to depend, at least in part, upon the presence of a lumen negative potential difference (2–8). Ouabain, lithium, and amiloride, which abolished in part or in toto the lumen negative potential by their effects upon sodium transport, were found to inhibit bladder acidification in the open-circuited state (8–10). In the short-circuited condition, these agents had no effect (9, 10). Thus, at least some portion of acidification is voltage dependent.

The application of the details of the voltage-influenced model of the turtle bladder to the clearance data previously mentioned led to the suggestion that distal renal acidification might be influenced by transepithelial potential, especially in view of the markedly lumen negative potential present in the cortical collecting tubule (11, 12). When directly tested in the cortical collecting tubule, though, amiloride and ouabain gave disparate results. While amiloride markedly diminished both transepithelial potential and total CO_2 flux, ouabain was found to inhibit only transepithelial potential with no detectable effect on total CO_2 flux. In addition, choline substitution for sodium did not influence acidification in the direction predicted (13). Thus, the evidence did not support the model of an active electrogenic proton pump in parallel to active sodium reabsorption which the data from the turtle bladder suggested.

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Further studies performed with clearance techniques and clinical data continued to suggest, however, that transepithelial potential might influence acidification in the mammalian collecting tubule. Ouabain, amiloride, and lithium were all found to inhibit distal acidification as assessed by the urine-blood P_{CO_2} difference in animals (14, 15), and lithium and amiloride were found to cause an acidification defect in patients and animals (10, 16, 17).

New data in collecting tubules have suggested the presence of two functionally distinct segments in the cortex and medulla (18–20). These segments differ in the nature of the transepithelial potential, the cortical tubule having a lumen negative potential and the medullary having a more positive potential (18–20), and in their ability to transport total CO_2, the medullary tubule being capable of higher rates of transport (18). In further investigation, no significant transepithelial active sodium transport was found to occur in the medullary segment (20). Acidification in this segment, therefore, would not be expected to respond to agents that act by decreasing active sodium transport. The just published report of Stone et al. (20a) shows this is the case when amiloride or tetramethyammonium is used.

Because of the possibility that the original study of the effect of ouabain on acidification might have been influenced by the presence of medullary tubule tissue, we decided to reinvestigate this issue. In addition we decided to evaluate the effect of ouabain on acidification in the medullary tubule since this was not known. Also, the effect of lithium substitution on acidification in these segments was chosen for study because of lithium's effect in the turtle bladder and the known effect of lithium on acidification in both the whole animal and the clinical setting. The data obtained showed an effect of both ouabain and lithium to reduce transepithelial potential and total CO_2 flux (J_{TCO_2}),[1] in keeping with the original models of acidification developed in the turtle bladder. In addition, data were obtained suggesting that cortical collecting tubules contribute significantly to overall acidification.

METHODS

Female New Zealand white rabbits were obtained at 1.5–3.0 kg size (Lesser's Rabbitry, Union Grove, WI) and allowed 1 wk to acclimatize to the biological resource laboratory with free access to water and standard rabbit chow, and then starved for 1–3 d while being allowed continued access to water. Three rabbits were injected with 1.25 mg/kg deoxycorticosterone pivalate in oil 1 wk before study to induce higher potential differences in the collecting tubule (21). After the period of starvation, rabbits were killed by decapitation and the kidneys were harvested. Immediately before decapitation, ear vein blood was obtained for pH measurement; and, after decapitation, a sample of bladder urine was obtained anaerobically for pH determination. 1–2-mm thick coronal sections were taken from the center of the kidneys and immediately placed in dissection medium (the standard bath below plus 5% vol/vol fetal calf serum), placed on a brass block containing a commercial blue-ice preparation, and kept at 4°C. Collecting tubules were dissected freehand using number 5 Dumont forceps (Roboz Surgical, Washington, DC) under a dissection microscope. Care was taken during dissection that cortical tubules were obtained from as near the surface of the kidney as possible. Medullary tubules were originally obtained by dissecting in the normal manner (from the papilla upwards to the surface) but were later obtained by dissecting in a papillary direction beginning at the outer medullary stripe. No attempt was made to distinguish between inner and outer medullary collecting tubules.

Once obtained, the tubules were transferred to the experimental bath chamber using a common transfer pipette. Tubules were perfused in the manner of Burg et al. (22) and Burg (23) with small modifications as noted in the following description. The tubule-holding pipette was maneuvered near the proximal end of the tubule in the bath and slight suction applied to pick up the tubule. The perfusion pipette was then advanced into the lumen of the tuble and further suction applied to the holding pipette to firmly hold the tubule. No guard pipette was used. Fixed Sylgard 184 (Dow Corning Corp., Midland, MI) was applied to the holding pipette during construction, and a small amount of free Sylgard applied to the holding pipette before the experiments. Once this end of the tubule was in place the collection pipette was approximated to the free end of the tubule and suction gently applied to draw the tubule into the pipette. Again, the collecting pipette was constructed with a constriction at the very tip and Sylgard was fixed at the tip during construction. A small amount of free Sylgard was added here before the study to assure a tight seal. After the tubule was affixed at both ends, the collection pipette was cleaned with chloroform and acetone and filled with water-equilibrated mineral oil. At this point the bath heating system was turned on, the pump feeding bath fluid to the chamber was activated, and the vacuum line removing bath overflow was set in place. Additionally, a gas line was brought into place which bubbled with bath fluid as it entered the bath chamber. The gas mixture used here was the same as that used in preparation of the bath and perfusion fluids, 6.7% CO_2 and 93.3% O_2. The time was then noted and a 50–60-min equilibration period was begun. After the equilibration period was finished, three to five fluid samples were obtained and the total CO_2 in the samples determined. 15–40-nl constant-volume pipettes were used for the collections. Perfusion rates were kept between 3 and 8 nl/ml per minute. After the samples for the control period were completed, one of two protocols was followed. Either ouabain at 10^{-4} or 10^{-8} M concentration was added to the bath, or a solution with 40 mM LiCl replacing 40 mM NaCl was exchanged for the control perfusate and bath. 30 min were allowed for equilibration and the three to five timed collections of fluid were made with the volume pipette. Throughout the course of the experiment, transepithelial potential difference was monitored with a WPI model F-223A digital electrometer (WPI, New Haven, CT), and note made of the potential difference during each period. The perfusion pipette was used as the probe electrode and was connected to one half of a calomel cell with a 0.9%-NaCl-in-agar bridge. The bath was used as ground and was similarly connected to the other half of the calomel system by an identical bridge.

[1] *Abbreviation used in this paper:* J_{TCO_2}, total CO_2 flux.

Bath and perfusate used in any period were identical; no correction for liquid junction potential was made. When electrical drift occurred during the course of the experiment, both control and experimental potential were corrected for the drift.

The solutions used in the protocols are shown in Table I. All fluids were bubbled with 6.7% CO_2 and 93.3% O_2, both before the experiment and in the experimental bath, in which a flow through system was used. The total CO_2 of the bath and perfusion fluids were measured daily at the completion of the experiment.

Total CO_2 of the perfused and collected fluids were determined by microcalorimetry using 15–35-nl samples measured with a picapnotherm (Microanalytic Instrumentation Inc., Bethesda, MD) (24). Samples were compared with values obtained for 25 mM sodium carbonate standards using the same volume pipette and corrected for blank or water injections. Standards were run before, during, and after the experimental periods.

These experiments were performed at 39°C after 1 h equilibration and with identical bath and perfusate solutions in all periods. Under these conditions volume flux is negligible and total CO_2 flux may be calculated at J_{TCO_2} net = V_1 (total CO_2 collected − total CO_2 perfused) /L, where V_1 is rate of fluid collection determined by timing collections of fixed volume, and L is tubule length.

Total CO_2 flux and potential difference pre- and postdrug administration were compared by paired t test. All data are given as mean±SE.

RESULTS

Group I. 12 cortical collecting tubules were studied to determine the effects of the addition of ouabain to the bath on transepithelial potential difference and total CO_2 flux. As noted in Methods, these animals and all studied in the following groups were starved for 1–3 d before study to induce higher rates of total CO_2 reabsorption (25). In the control period the transepithelial potential in these tubules was −16.0±4.0 mV and after the addition of ouabain to the bath (10^{-8} M) the potential declined to −2.2±2.0 mV (Table II, Fig. 1). The mean paired difference was 14.2±2.4 ($P < 0.001$). Similarly, in the control period the net total CO_2 flux was measured to be 6.0±4.5 pmol/mm per min and after the administration of ouabain this rate fell to 1.5±1.6 pmol/mm per min. The mean paired difference here was 4.6±.01 ($P < 0.005$) (Table II, Fig. 2).

TABLE I
Composition of Solutions

	Control	Lithium substitution solution
	mmol/liter	
NaCl	105	65
$NaHCO_3$	25	25
K_2PO_4	2.5	2.5
CaCl	1.0	1.0
$MgSO_4$	1.2	1.2
Na Acetate	10	10
Glucose	5.5	5.5
Alanine	6	6
LiCl	—	40

Group II. A total of 11 medullary collecting tubules were studied, six at the ouabain dose (10^{-8} M) used in the cortical collecting tubules, and five at a higher dose (10^{-4} M). In the six tubules treated with low dose ouabain, the control potential difference was +9.6±5.6 mV and was not changed at +10.5±5.6 mV after ouabain (mean paired difference 0.5±0.7, NS). In the five tubules treated with high dose ouabain, the control potential difference was +4.9±1.9 mV and was unchanged, +5.3±3.8 mV, after ouabain (mean paired difference 2.4±2.0, NS). The net total CO_2 flux was 21.7±1.2 pmol/mm per min in control and 22.0±1.9 pmol/mm per min after low dose ouabain, (mean paired difference 0.3±1.5, NS); in tubules exposed to high dose ouabain, the control total CO_2 flux was 24.9±6.4 pmol/mm per min and after ouabain the rate was not altered at 24.7±3.1 pmol/mm per min (mean paired difference 0.6±4.3, NS). These data are presented in Table II and Figs. 3 and 4.

Group III. In this series of studies, the effect of lithium substitution for sodium, 40 mmol of lithium chloride was substituted for an equal amount of sodium chloride in both perfusion and bath solutions. In the 11 cortical collecting tubules studied, the control potential difference was −11.6±4.2 mV. After the substitution was performed, the potential difference was 0.4±1.4 mV (mean paired difference 12.0±4.7, $P < 0.05$) (Table II, Fig. 5). In this same group of tubules the control rate of total CO_2 flux was +10.8±1.8 pmol/mm per min and, after the substitution of lithium for sodium, the rate decreased to +4.2±2.8 pmol/mm per min (mean paired difference 6.6±2.3, $P < 0.025$) (Table II, Fig. 6).

Group IV. This series examined the effect of lithium substitution for sodium on potential difference and total CO_2 flux rate in medullary-derived tubules. In these seven tubules the control potential difference was 0.0±2.4 mV and was −0.1±2.3 mV after the substitution (mean paired difference 0.1±1.8, NS) (Table II, Fig. 7). Control total CO_2 flux in these tubules was 26.5±4.6 pmol/mm per min and did not change, 25.3±2.7 pmol/mm per min after the fluid substitution was performed (mean paired difference 1.1±1.9, NS). These data are shown in Table II and Fig. 8.

In reviewing the data from the series above, it was noted that the control value for total CO_2 flux varied considerably between the experimental groups. At first the reason for this was not clear, but review of the data on the pH of the final urine obtained at the time the animals were killed showed that the pH of the urine varied between groups as well. In the cortical tubule

TABLE II

Effect of Lithium and Ouabain on Total CO_2 Flux and Potential Difference

	Group	n	Flow rate	PD	ΔPD	P	J_{TCO_2}	Δ J_{TCO_2}	P
			nl/min	*mV*			*pmol/mm/min*		
I	Cortical	12							
	Control		4.18±0.5	−16±4.0	14.2±2.4	<0.001	+6.0±4.6	4.6±1.01	<0.005
	Ouabain 10^{-8} M		4.54±0.56	−2.2±2.0			+1.5±1.6		
IIa	Medullary	6							
	Control		3.9±0.56	9.6±5.6	0.5±0.7	NS	+21.7±1.2	0.3±1.46	NS
	Ouabain 10^{-8} M		3.95±0.58	+10.5±5.6			+22.0±1.9		
IIb	Medullary	5							
	Control		4.44±1.07	+4.9±1.9	+2.4±2.0	NS	+24.9±6.4	0.6±4.31	NS
	Ouabain 10^{-4} M		4.16±0.54	+5.3±3.8			+24.7±3.1		
III	Cortical	11							
	Control		3.26±0.35	−11.6±0.4	12.0±4.7	<0.005	+10.8±1.8	6.6±2.29	<0.025
	Lithium		3.19±0.36	+0.4±1.4			+4.2±2.8		
IV	Medullary	7							
	Control		4.87±0.60	0.0±2.4	0.1±4.6	NS	26.5±4.6	1.2±1.93	NS
	Lithium		5.38±0.53	−0.1±2.3			25.3±2.7		

Numbers given are mean±SE. PD, potential difference.

groups, the rate of total CO_2 flux in group I control period was 6.0±4.5 pmol/mm per min and the final urine pH in these animals was 6.51 in the eight rabbits in which the urine was tested. In the lithium-treated group of cortical tubules the control rate of total CO_2 flux was 10.8±1.8 pmol/mm per min as previously noted, and the final urine pH in the group was 5.46. In the medullary segment studies the control rates of net total CO_2 flux were 21.7±1.2, 24.9±6.4, and 30.7±4.6 pmol/mm per min in the low dose ouabain, high dose ouabain, and lithium groups, respectively. The final urine pH in these groups of animals was found to be 6.19, 5.63, 5.50, again in the same order. Since the pH tended to be lower as the rates measured for total CO_2 flux were seen to increase, we decided to analyze the data formally to determine if a true correlation was present. Cortical and medullary data were kept separate, and the tubules were grouped by the final urine pH exhibited by the animals. Grouping was done at 0.5 pH-unit intervals between pH 4.5 and 7.5. The grouped data were plotted as illustrated in Fig. 9. The line determined by linear regression for the grouped cortical tubule data gives a slope of −4.224, an x intercept of 8.03, and a correlation coefficient of 0.921. In the medullary tubules, the x intercept was found to be 9.53, the slope was −6.901, and the correlation coefficient was 0.9143.

When the data after the administration of the agents in this study were analyzed in the same manner, the medullary tubules postinhibitor show a slope of −4.527, an x intercept of 11.38, and a correlation coefficient of 0.7174, little change from the control values. Because of the influence of the flux rate obtained in cortical tubules at pH between 5.0 and 5.5 in the postinhibitor period, the correlation for the line obtained with all the data is poor (0.268). The rate obtained at the pH in question, however, is greatly influenced by a single tubule. Note that the addition of ouabain or lithium inhibits total CO_2 flux at every level of urine pH. It also appears that the percentage inhibition is greater at lower rates and higher urine pH levels, though the absolute decrease in flux rate is greater at higher rates of net flux and lower urine pH values (Fig. 9).

DISCUSSION

While a coherent model of acidification has been developed for the turtle bladder, a similar model has not been fitted to the mammalian collecting tubule because of inconsistencies present in the data available up to now. In the turtle bladder, it seems relatively certain that acidification proceeds via an active electrogenic proton pump that exists in parallel with an active sodium reabsorptive mechanism that provides a lumen negative potential difference favoring acidification (6, 26). Acidification is not strictly dependent upon the presence of sodium, but it is influenced by the presence of the lumen negative potential. If sodium transport is

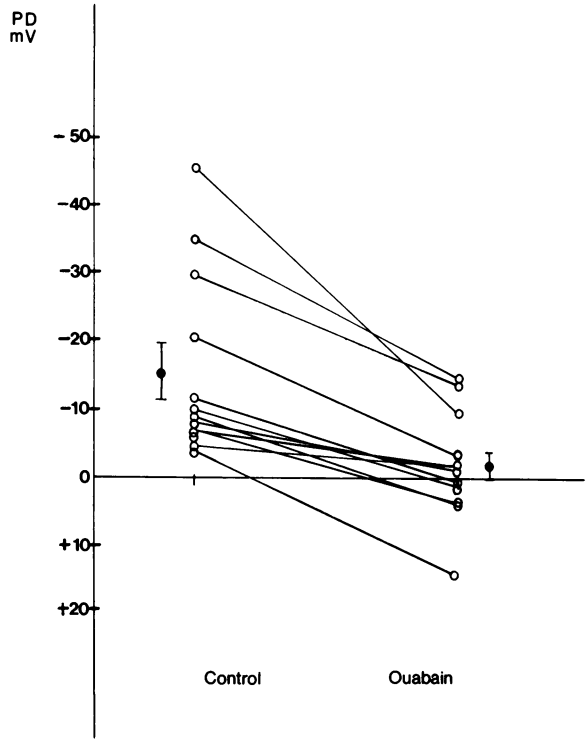

FIGURE 1 Effect of ouabain (10^{-8} M) added to bath after control potential difference (PD) was determined in cortical collecting tubules. PD control, −16.0±4.0 mV; postouabain PD, −2.2±2.0 mV. n = 12. Means and SE are indicated.

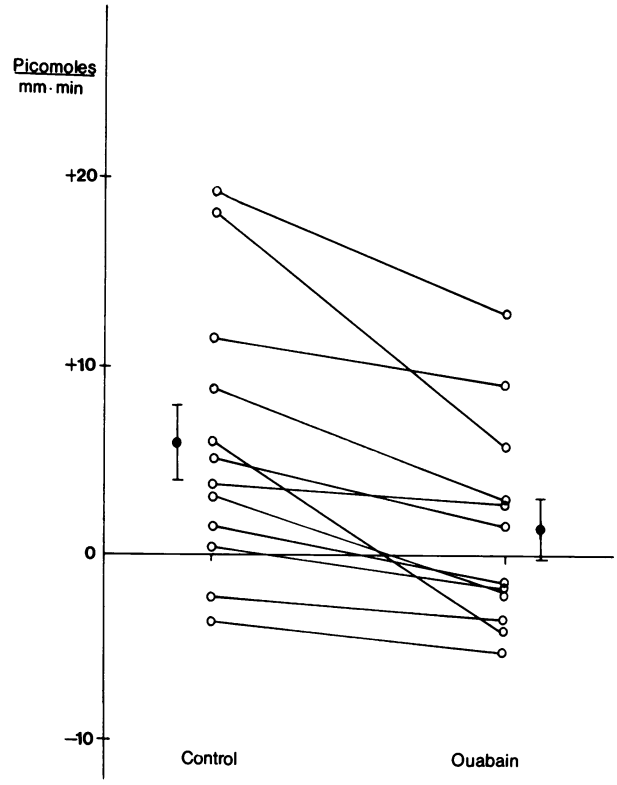

FIGURE 2 Effect of ouabain (10^{-8} M) added to bath after control J_{TCO_2} was determined in cortical collecting tubules. Control J_{TCO_2} was 6.0±4.5 pmol/mm per min. Postouabain was 1.5±1.6 pmol/mm per min. n = 12. Means and SE are indicated. $P < 0.005$.

inhibited while the bladder is in the open circuited state, there is a decline in the rate of acidification (8–10), but if sodium transport is inhibited and the transepithelial potential is kept constant by voltage clamp, no effect is seen (9, 10). Thus, a relationship exists that has been described as voltage dependence of acidification (10). Acidification is not totally abolished by maneuvers altering the potential difference across the membrane unless a very large potential is imposed in opposition, so merely inhibiting sodium transport should decrease acidification but not abolish it (3, 5, 7). Similarly, enhancement of the transepithelial potential should enhance the rate of acidification, and this has also been shown to be true (7). This model has been developed over the course of years and has been found to be internally consistent and predictive.

The data obtained from whole animal studies using the urine minus blood P_{CO_2} difference as a measure of distal acidification have been found to be suggestive of a similar mechanism for acidification in the mammalian distal tubule. Here the administration of ouabain, amiloride, or lithium, all of which apparently inhibit acidification in the turtle bladder solely by means of their effect upon sodium transport and transepithelial potential, inhibit the development of a normal urine minus blood P_{CO_2} difference during the administration of bicarbonate (10, 14, 15). While the interpretation of these data requires that one accept the proposal that urine minus blood P_{CO_2} in these circumstances is due to the secretion of protons, the data do suggest that sodium transport is influential in the process that is taking place. Although these data do not allow a definite and unqualified model of acidification to be made, they do agree with the previously stated model from the turtle bladder. In addition, animal and clinical data show that lithium administration will result in distal tubular acidosis, as will the administration of amiloride (10, 16, 17).

The data obtained from the isolated, perfused collecting tubule do not at present agree with the information gleaned from other sources. McKinney and Burg (13), using microcalorimetric analysis for total CO_2 and measurement of transepithelial potential, found that amiloride inhibited both transepithelial potential and the rate of total CO_2 flux. After tubules were exposed to ouabain at 10^{-4} M concentration, no change in total

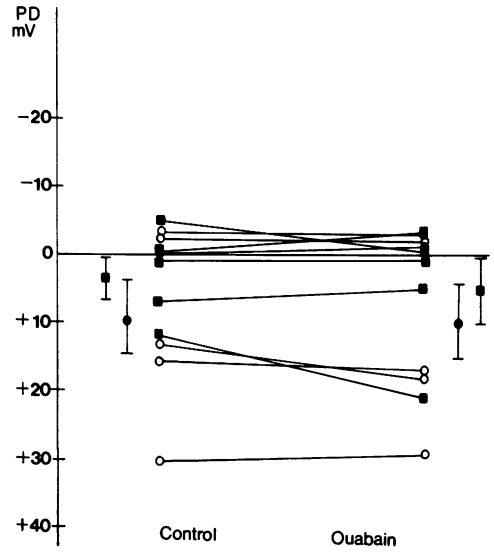

FIGURE 3 Effect of ouabain on potential difference (PD) in medullary collecting tubules. Ouabain was added to bath at 10^{-8} M (O, ●) in 6 and 10^{-4} M (■) in five medullary collecting tubules. In low dose studies the PD was +9.6±5.6 mV in control and +10.5±5.6 mV (NS) after ouabain. In high dose studies PD was +4.9±1.9 mV in control and +5.3±3.0 after ouabain. Means and SE of both groups are given.

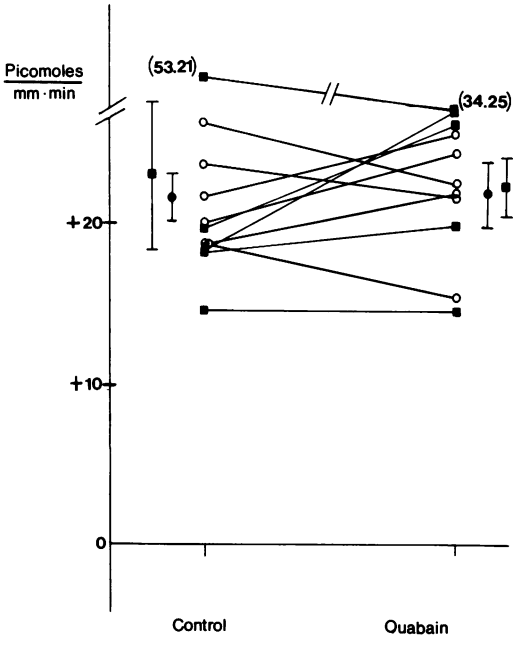

FIGURE 4 The effect of bath ouabain 10^{-8} M (O, ●) and 10^{-4} M (■) on J_{TCO_2} was evaluated in medullary collecting tubules. In low dose experiments (n = 6) control J_{TCO_2} was +21.7±1.2 pmol/mm/min and J_{TCO_2} was 22.0±1.9 pmol/mm per min after ouabain. In five tubules exposed to high dose ouabain, J_{TCO_2} was 24.9±6.4 pmol/mm per min preouabain and 24.7±3.1 pmol/mm per min postouabain. Neither group change was significant.

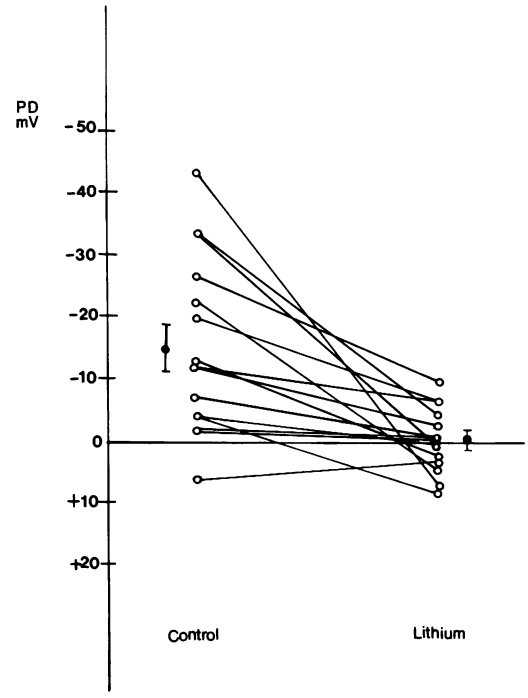

FIGURE 5 Effect of lithium substitution on potential difference (PD) in cortical collecting tubules. Changes in PD after replacement of 40 mM of NaCl with 40 mM LiCl in perfusate and bath solutions was studied in a total of 15 cortical collecting tubules. (four of these had no simultaneous measurement of J_{TCO_2}). In 15 tubules the control PD was −15.4±3.6 mV and was −0.92±1.3 after the substitution. Mean plus SE given (for data limited to the 11 tubules in which J_{TCO_2} was measured simultaneously see Table II, Group III). $P < 0.005$.

CO_2 flux was found despite the presence of a marked change in the transepithelial potential. In addition, they were unable to show a decrease in acidification after sodium was replaced by choline, again despite a decrease in potential difference (13). In fact, acidification increased significantly. Because of these discrepancies and because of the fact that no clear relationship existed between native potential difference and the rate of acidification, McKinney and Burg (13) could not support the use of the turtle model in the rabbit tubule.

Two problems with interpretation of these data exist. First, the sodium substitution data are problematic because of work performed in the turtle bladder by Arruda et al. (9) that demonstrate that choline stimulates acidification and depresses bicarbonate secretion by a mechanism independent from voltage effects, presumably by a metabolic effect. Second, the experimental group in which ouabain was applied to tubules was very small, consisting of five tubules. Of these tubules, only one did not decrease its rate of acidification after ouabain. Since the time when these studies were performed, information has been obtained showing that

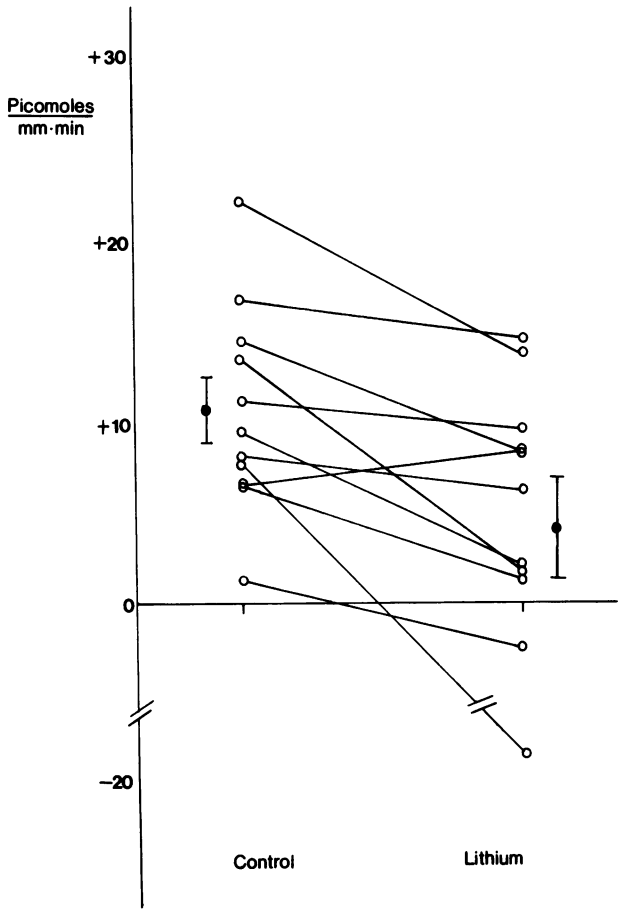

FIGURE 6 The effect on J_{TCO_2} of the replacement of 40 mM NaCl in perfusate and bath solution by LiCl in cortical collecting tubules is shown. In control the mean J_{TCO_2} is 10.8±1.8 pmol/mm per min and after substitution it decreased to +4.2±2.8 pmol/mm per min. Means±SE are shown. $P < 0.025$.

there are at least two functionally distinct segments of the collecting tubule, the cortical and medullary segments, respectively. Lombard et al. (18) have shown that acidification may occur at higher rates in the medullary segment than in the cortical segment, and that the potential in the medullary segment is lumen positive. These findings are supported by the recent work of Stokes (20) who showed no significant active flux of sodium in the medullary segment and a lumen positive potential. Since the medullary cortical differences had not yet been defined and the tubules in question were quite long, it seems possible that some significant amount of medullary tubule may have been present in the one tubule that did not respond to ouabain.

Recently, using antimony electrodes to determine the lumenal pH, Koeppen and Helman (25) showed that the minimal pH achieved in the cortical collecting

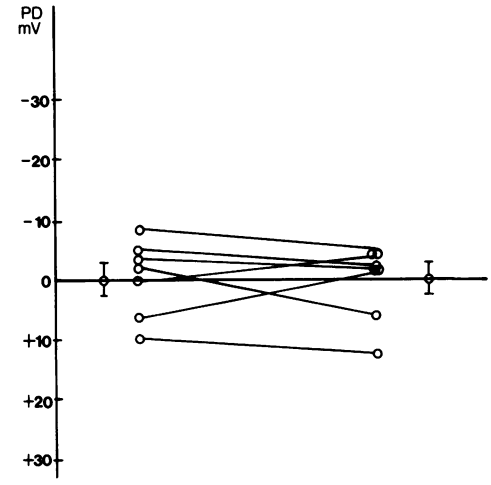

FIGURE 7 Effects of substitution of 40 mM LiCl for 40 mM NaCl in perfusate and bath on potential difference (PD) in seven medullary collecting tubules is shown. Control PD was 0.0±2.4 mV and PD was −0.1±2.3 mV after fluid exchanges. No difference was found. Means and SE are shown.

tubules of the starved rabbit is altered by the addition of ouabain to the bath. When the transepithelial potential declined, so did the deviation of the lumenal pH from the equilibrium pH. Thus, by this technique, acidification was a function of transepithelial potential and was inhibitable by ouabain.

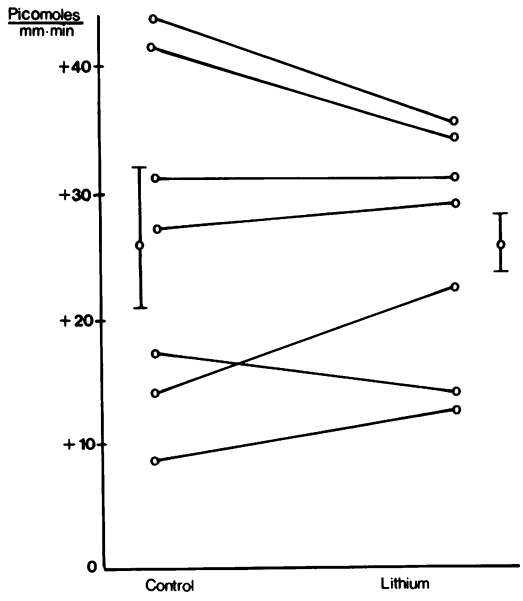

FIGURE 8 Effects of substitution of 40 mM LiCl for 40 mM NaCl in perfusate and bath on J_{TCO_2} in seven medullary collecting tubules is shown. Control J_{TCO_2} was +26.5±4.6 pmol/mm per min and J_{TCO_2} was unchanged +25.3±2.7 pmol/mm per min after fluid substitution. Means and SE are given.

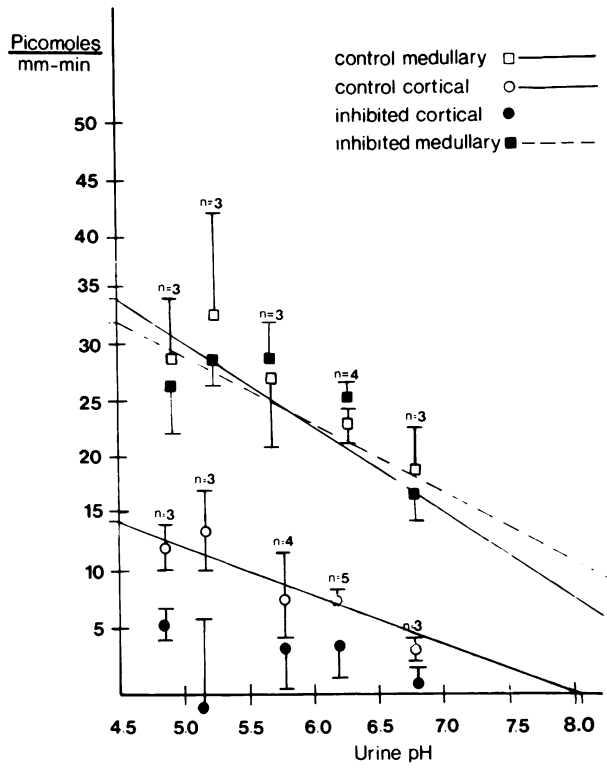

FIGURE 9 J_{TCO_2} pre- and postinhibitory measures were plotted vs. animal bladder urine pH in animals free of calcium carbonate debris (one excluded). Data were grouped by 0.5 pH-unit increments and mean J_{TCO_2} and pH were plotted. Means, SE and n for each group are given. In medullary collecting tubules, control J_{TCO_2} vs. urine pH plotting (□, solid line) gave a slope of −6.901, intercept 9.53, and correlation coefficient of 0.9143. The J_{TCO_2} obtained from these same medullary tubules after inhibitors (Li or ouabain) were applied is shown (■, dotted line). The slope for this line is −4.527, the x intercept is 11.38, and the correlation is 0.7174. In cortical collecting tubules, plotting control J_{TCO_2} vs. bladder pH (○, solid line) gave a slope of −4.224, an intercept of 8.03, and a correlation of 0.921. Cortical tubules postinhibition had low correlation (0.227) when plotted vs. urine pH. Ungrouped data from noninhibited cortical tubules give a slope of −4.03, x intercept of 7.905, and a correlation of 0.428, corresponding to $P < 0.075$. Ungrouped medullary data give a slope of −5.839, x intercept of 10.19, a correlation of −0.3636, and $P \simeq 0.15$.

In view of this discrepant data, we decided to reinvestigate the issue of the effect of ouabain on acidification in the cortical collecting tubule. Because lithium is a cause of distal renal acidosis, and the effect of lithium on acidification in the turtle bladder had been previously assessed, we decided to measure the effects of this agent on acidification as well. Additionally, the effect of these agents on acidification in a segment in which sodium transport did not result in a lumen negative potential, the medullary collecting tubule, was also studied. As indicated in Figs. 1 and 2, ouabain decreased both the transepithelial potential and the total CO_2 flux in the cortical collecting tubule. This effect was found at a dose of 10^{-8} M ouabain. Since the purpose of adding ouabain was to achieve a diminution in the lumen negative potential, and this was achieved at this low dose of ouabain, this dose was felt to be appropriate in these studies. When ouabain was applied to medullary tubules, shown in Figs. 3 and 4, neither potential nor rate of total CO_2 flux was altered. Here, ouabain was tested at two doses to rule out tubule insensitivity to the agent. Similarly, when lithium was substituted for sodium in the cortical collecting tubules both the potential difference and the rate of total CO_2 transport decreased and, again, this finding was not duplicated in the medullary tubule. These results are what one would predict given application of the turtle bladder model of acidification to the known facts concerning the collecting tubule segments. In those tubules in which a lumen negative potential is generated by sodium transport, total CO_2 flux is altered when the potential is altered. In the medullary segments, where no significant active transepithelial sodium transport or lumen negative potential is present, the agents inhibitory to sodium transport have no effect.

The results obtained in this study are thus in agreement with the data obtained in the turtle bladder, and are also in agreement with the results obtained by Koeppen and Helman (25) in their study in the cortical collecting tubule. Furthermore, the absence of any effect in the medullary collecting tubule is predictable from the previous study of Stokes (20), the data of Lombard et al. (18), and the prediction that the disparity with the earlier work of McKinney and Burg (13) is explainable as stated above. The data from the cortical collecting tubule are thus compatible with the data obtained from the other techniques and fit with a model of electrogenic acidification that is influenced by the presence of a lumenal potential generated by sodium transport. It should be stressed, however, that the data are compatible but cannot be taken as proof that such a mechanism occurs. To prove such a mechanism exists, it would be necessary to successfully voltage clamp a tubule and measure the effects of imposed transepithelial potential directly.

In addition to the findings with regard to the effect of inhibiting transepithelial potential on total CO_2 flux, information collected with regard to the urine pH in these animals allows several observations to be made concerning renal adaptation. When the rate of total CO_2 flux is plotted against the urine pH, an inverse relationship is suggested for both cortical and medullary tubules (Fig. 9). When the slopes of the two lines are compared, the line for the cortical tubules is found to be less steep. More important, it is noted that, at a high

urine pH, the cortical tubule apparently contributes little to acidification, but as the urine pH is progressively more acidic the rate in the cortical tubule becomes significant when compared to the medullary rate. While it might be argued that inhibition of the cortical collecting tubule might have little effect on overall acidification when the rabbit's urine pH is relatively high, it is clear that inhibition of this segment might have important consequences when urine pH is in the lower range. Because of the influence of one tubule at the 5.0–5.5 pH interval, linear regression does not show a clear relationship between urine pH and J_{TCO_2} after inhibition of sodium transport in the cortical tubule. It is likely that $+J_{TCO_2}$ net increases with lower urine pH (and presumably increased adaptation) even after inhibition of sodium transport but the current data do not allow this conclusion to be reached with certainty. Such a slope would be consistent with the proposition that the transepithelial potential is not the sole mechanism adjusting the rate of total CO_2 flux.

One other point of interest arises from the plot in Fig. 9. In comparison to the recent publication of Lombard et al. (18), it is apparent that the rates of total CO_2 flux obtained in our study are higher than those obtained by the Dallas group, despite the fact that Lombard reports similar low urine pH levels. The rate of cortical collecting tubule total CO_2 transport remained near zero in the Lombard study and medullary tubules transported total CO_2 at a rate near 10 pmol/mm per min (18). While our mean rates of transport are higher for both cortical and medullary segments, it can be noted from Fig. 9 that, when cortical transport rates are near zero, the corresponding medullary rate is in the range of 10 pmol/mm per min, agreeing with the rate pair seen in the previous study of Lombard et al. (18). In our starved rabbits, this rate is seen at a urine pH near 7.0 rather than at the lower pH (5.0) reported in the Lombard study. Since the models are different (starvation vs. NH_4Cl loading), the urine pH values may represent different absolute amounts of acid secretion because of differences in buffer capacity.

The cortical rates of total CO_2 flux found in our study do not differ greatly from those of McKinney and Burg (13). Exact reasons for the differing rates obtained in various laboratories are difficult to assess, but the data from whole animal studies (10, 15) suggest that the portion of distal acidification influenced by agents which inhibit sodium transport and thus potential difference is considerable. For this reason, we feel that a significant rate of total CO_2 transport likely occurs somewhere along the distal nephron where sodium transport also occurs. In addition, Koeppen and Helman (25) have shown that the cortical collecting tubule can lower luminal pH, which would be consistent with total CO_2 transport occurring here.

In conclusion, we feel that we have demonstrated several significant points with regard to acidification by the collecting tubule. First, the rate of total CO_2 flux in the cortical collecting tubule falls in parallel with a fall in lumen negative transepithelial potential, which is consistent with data from whole animal studies and from the turtle bladder. Second, the medullary collecting tubule is not affected by the two agents, suggesting that these agents work in the cortical tubule through their effect on the potential and not through nonspecific mechanisms. Since these agents affect acidification in the turtle bladder solely through changes in voltage, it seems likely that these agents operate through a similar mechanism in the tubule. Definite demonstration that the change on voltages cause change in J_{TCO_2} requires voltage clamping of the tubule, which is as yet technically practical. Third, the cortical collecting tubule is capable of significant rates of acidification in animals which are producing urine with low pH. And, finally, both cortical and medullary collecting tubules show evidence for adaptation as urinary pH decreases during starvation in the rabbit.

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