Medullary Collecting Duct Acidification

Effects of Potassium, HCO_3 Concentration, and pCO_2

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Abstract. The medullary collecting duct (MCD) from renal outer medulla possesses significant HCO_3 absorptive capacity. In vitro microperfusion studies have shown that HCO_3 absorption in this segment is carbonic anhydrase dependent, affected by peritubular and luminal chloride concentrations, is independent of the presence of Na or the presence of Na transport, and is stimulated by mineralocorticoid hormone. The present in vitro microperfusion studies defined regulatory influences on MCD acidification as assessed by acute changes in the extracellular K and HCO_3 concentrations and pCO_2. These studies showed that acute changes in the peritubular K concentration to either 0 mM K or 50 mM K have no significant effect on HCO_3 absorption in MCD. Intracellular voltage recordings showed that elevation of peritubular K concentration from 5 to 50 mM produced only a 2.8 mV depolarization of the basolateral cell membrane of MCD cells. In addition, acute reduction of peritubular K from 5 to 0 mM had no significant effect on intracellular voltage. Studies that were designed to assess the effects of HCO_3 concentration and pCO_2 on acidification showed that acute reduction of peritubular HCO_3 concentration from 25 to 5 mM (pH change from 7.4 to 6.8) increased lumen-positive voltage from 30.2±3.8 to 40.0±4.4 mV, and simultaneously increased net HCO_3 absorption from 15.6±1.9 to 22.9±2.9 pmol·mm^{-1}·min^{-1}. Elevation of peritubular HCO_3 concentration from 25 to 50 mM (pH change from 7.4 to 7.8) significantly decreased lumen-positive voltage from 33.8±2.4 to 26.7±1.5 mV and simultaneously decreased net HCO_3 absorption from 17.9±1.2 to 12.8±1.3 pmol·mm^{-1}·min^{-1}. In addition, acute reduction of peritubular pCO_2 from 40 to <14 mmHg (final pH 7.8–7.9) significantly decreased lumen-positive voltage from 31±4.4 to 15.7±1.0 mV. Coincidentally, HCO_3 absorption decreased significantly from 11.0±3.7 to 5.3±0.7 pmol·mm^{-1}·min^{-1}. We conclude that: (*a*) alteration of peritubular K concentration from 0 to 50 mM in vitro does not affect HCO_3 absorption in the MCD, and that this lack of effect appears to be related to a low basolateral cell membrane K conductance; (*b*) net HCO_3 absorption and the associated lumen-positive voltage can be modulated by in vitro changes in peritubular HCO_3 and pCO_2 (or pH); and (*c*) the MCD perfused in vitro appears to be a good model for studying the mechanisms and regulation of distal nephron acidification.

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Introduction

We, and more recently others, have demonstrated via in vitro microperfusion studies that the medullary collecting duct (MCD)[1] from renal outer medulla possesses significant HCO_3 absorptive capacity (1–4). Indeed, when the rabbit MCD from inner stripe of outer medulla is perfused and bathed in vitro with symmetrical Ringer's HCO_3 solutions, transepithelial voltage is always oriented lumen positive and net HCO_3 absorption occurs at a rate of 10–20 pmol·mm^{-1}·min^{-1} (1–4). Recent studies using in vitro microperfusion of rat MCD demonstrate virtually identical features, except that the rate of acidification is even greater with net HCO_3 absorptive rates of 20–40 pmol·mm^{-1}·min^{-1} (5). Certain characteristics of the MCD acidification process have been elucidated. First, proton secretion (HCO_3 absorption) occurs against an electrical and chemical gradient and thus appears to be active (1). Second, the net acidification process as well as the lumen-positive transepithelial voltage is obliterated by exposure of the in vitro

1. *Abbreviations used in this paper:* CCT, cortical collecting tubule(s); MCD, medullary collecting duct(s); J_V, net volume flux; V_T, transepithelial voltage.

perfused MCD to carbonic anhydrase inhibitors (1). Third, there appears to be an important role for the anion chloride in net acidification by the MCD, since total removal of peritubular chloride results in ablation of net HCO_3 absorption and removal of luminal chloride results in augmentation of net HCO_3 absorption (2). Fourth, proton secretion in this nephron segment is molecularly independent of sodium, in that it is not affected by peritubular ouabain, luminal amiloride, or total replacement of luminal and peritubular sodium (3, 4). Finally, proton secretion appears to be under control, in part, by adrenal corticosteroid hormones, since adrenalectomy results in significant inhibition and DOCA administration results in a significant increase in net HCO_3 absorption, and since in vitro addition of aldosterone to rabbit MCD harvested from adrenalectomized animals results in significant stimulation of net HCO_3 absorption. In summary, then, we have a distal nephron segment with significant proton secretory capacity that is carbonic anhydrase-dependent, affected by peritubular and luminal chloride concentrations, is independent of the presence of sodium or the presence of sodium transport, and is stimulated by mineralocorticoid hormone. Other than the mineralocorticoid dependence of proton secretion in the MCD (3) we know very little about regulation of proton secretion in this nephron segment. Specifically, there is no information available on the effects of extracellular K concentration, as well as extracellular pH, pCO_2, and HCO_3 concentration on the acidification process in the MCD. Because in both the rabbit and the rat this appears to be the distal nephron segment with the highest capacity for proton secretion, it is important to define those factors that regulate the acidification process.

The studies presented here represent initial attempts to define regulatory influences on MCD acidification by acute changes in the extracellular environment in vitro. They demonstrate that acidification in this segment is unaffected by alteration of peritubular K concentration from 0 to 50 mM. In addition, these studies show that net acidification in this nephron segment is sensitive to the peritubular HCO_3 concentration and pCO_2 (or pH).

Methods

Outer MCD segments were dissected and perfused in vitro by techniques previously described (6). Briefly, female New Zealand white rabbits that weighed 1.5–2.5 kg were killed by decapitation. One kidney was removed and a 1-mm coronal slice was placed in chilled bath medium at pH 7.4. Segments were dissected free-hand with the aid of a dissecting microscope and sharpened forceps. The freed segments were transferred to a thermostatically controlled lucite perfusion chamber and perfused between concentric glass pipettes at 38°±1°C. Several solutions were used as perfusate and bath in these studies. The major ionic composition of the various solutions are outlined in Table I. The control perfusate was an artificial plasma ultrafiltrate with the following composition in millimolar: NaCl 105; KCl 5; $NaHCO_3$ 25; Na acetate 10; $NaHPO_4$ 2.5; CaCl 1.8; $MgSO_4$ 1; glucose 8.3; alanine 5. The control bath was identical to the perfusate except for the addition of 5% by volume fetal calf serum. The bath was exchanged at a rate of 0.6 ml/min. Unless stated otherwise, all solutions were bubbled with 95% O_2/5% CO_2 gas mixture to the point of equilibration at 37°C and drawn anaerobically into plastic syringes and refrigerated until use. Previous studies using identical methods have documented that solutions handled in such a manner, irrespective of the [HCO_3] or pCO_2, result in bath pH that remains constant and at the desired level (7).

Strict attention was given to the origin of all segments studied. Outer MCD segments were dissected in such a manner that the proximal portion originated at the junction of the outer and inner stripe of the outer medulla, and the distal portion ended at the junction of the inner stripe of outer medulla with the inner medulla.

After initiation of perfusion, tubules were allowed to equilibrate for 60 min at 38°C in the control bath. Transepithelial voltage (V_T) was monitored throughout all experiments. Net volume absorption was determined by the use of exhaustively dialyzed ^{3}H-inulin as volume marker. Net volume flux (J_V) was determined from the changes in the ^{3}H-inulin activity between perfused and collected fluid via equations previously described (1). Net total CO_2 flux ($JHCO_3$) was calculated from the total CO_2 content of perfused and collected fluid measured by microcalorimetry (8) and calculated by the following equation: $JHCO_3 = [V_I(C_I - C_O)]/[L]$, where V_I is the perfusion rate, C_I is the perfusate total CO_2, C_O is the collected fluid total CO_2, and L is the tubule length. The equation assumes a negligible change in HCO_3 concentration due to water movement, which was substantiated by measuring J_V and discarding experiments in which the $J_V \geqq \pm 0.05$ $nl \cdot mm^{-1} \cdot min^{-1}$. Total CO_2 measurements were made by collecting into 30–60-nl pipettes that were then used to inject samples into the phosphoric acid chamber of the picapnotherm. Measurements were made immediately after collection, and reproducibility was $\sim \pm 2.5\%$ when a single sample was measured multiple times. Note that in all studies reported, tubule length ranged from 1.0 to 1.8 mm and perfusion rates ranged between 1.8 and 3.0 $nl \cdot min^{-1}$. Thus, in all reports of significant difference between control and experimental periods, the change in collected fluid total CO_2 was over 10%, which was a difference that was easily detectable by the methods used. In some experiments, net Cl transport was determined by measurement of Cl concentration in perfused and collected fluid utilizing the electrometric titration technique (9). Chloride flux was calculated in a manner identical to that of net HCO_3 flux, which was described above.

In some tubules, both transepithelial voltage and intracellular voltage were monitored simultaneously. Intracellular voltage was measured by puncturing individual cells with 1 M KCl-filled Ling electrodes using methods identical to those published previously (10). Ling electrodes were pulled with an ultrafine micropipette puller (Frederick Haer & Co., Brunswick, ME). Electrode resistances were measured to be between 100 and 200 megaohms. All intracellular voltages were referenced to the bath and were measured using a high input impedance electrometer (FD223, W-P Instruments, Inc., New Haven, CT). Tubular cells were impaled by use of a hydraulic drive micromanipulator (David Kopf Instruments, model 1207B, Tujunga, CA). Stable intracellular voltage recordings were obtained and maintained for as long as 90 min. Criteria for adequate impalements were the same as those described as well as graphically presented previously (11).

Briefly, four types of recordings were obtained. The first type, an initial rapid negative deflection followed by a steady decay in voltage over 1–3 min, was seen intermittently but no more than 20% of the time. Such a tracing was uniformly associated with visible evidence of swelling of the impaled cell, and all such punctures were ignored. The second type of puncture consisted of an initial negative deflection with

a steady increase in the negativity to a stable value after a few minutes. Such punctures were considered acceptable and constituted about one-third of the successful impalements. The third type of tracing consisted of an initial negative deflection, with an almost immediate fall in the magnitude of this negative voltage followed by a gradual 1–3-min increase in intracellular negativity, to a plateau level more negative than the initial spike. Such punctures constituted about two-thirds of the successful impalements. Finally, the fourth type of tracing was identical to the second and third form described above, but the plateau voltage was maintained for too short a time to complete the appropriate protocols, i.e., stable voltage for <10 min. This short-lived but technically successful impalement occurred with almost half of all impalement attempts. Data obtained with successful impalements was only acceptable if the recorded voltage, upon removal of the microelectrode from the cell, was < ±5 mV different from the prepuncture voltage. (This occurred in all but one impalement.) The frequency of successful impalements using such methods and criteria are a function mostly of experience. In MCD cells, the success rate is roughly 20–25%, while in our hands the success rate in cortical collecting tubules is closer to 50%, and that in proximal tubules is even higher (these success rates refer to percentage of tubules studied).

Several groups of studies were performed. Group I studies examined the effects of alterations in peritubular K concentration on net HCO_3 absorption, transepithelial voltage, and intracellular voltage. After control measurements were made, the bath was changed to an identical one except for alteration in K concentration. Bath K concentration changes were of two types. In some tubules the bath K concentration was reduced to 0 by replacing 5 mM of KCl with 5 mM NaCl. In these experiments, fetal calf serum was omitted from the bath to ensure that [K] was unmeasurable by standard flame photometry. In other tubules, bath K concentration was increased to 50 mM by replacing 45 mM of bath NaCl with KCl (see Table I). An additional group of tubules was studied with simultaneous changes in both luminal and peritubular K concentration. In these experiments, control perfusate and bath were replaced by a high K low Cl solution. The K concentration in perfusate and bath was elevated to 75 mM by replacement of 70 mM NaCl with 65 mM K gluconate and 5 mM KCl (see Table I). In these experiments, both net HCO_3 absorption and net Cl transport were measured.

The second major group of studies investigated the effects of alteration in peritubular HCO_3 concentration and pCO_2 on V_T and net HCO_3 absorption. After control measurements were obtained, bath solution was changed to one that contained either 5 mM HCO_3 (acute metabolic acidosis) or one that contained 50 mM HCO_3 (acute metabolic alkalosis). When HCO_3 concentration was reduced to 5 mM it was done by replacing 20 mM $NaHCO_3$ with 20 mM NaCl, and when bath HCO_3 was elevated it was done by replacing 25 mM NaCl with 25 mM $NaHCO_3$ (Table I). Both solutions were equilibrated with 95% O_2/5% CO_2, so that the final pHs were 6.8 and 7.7, respectively. In a final group of tubules, after control collections were obtained, the response to low ambient pCO_2 was determined. Bath was changed to one that was identical to control, except that the experimental bath was equilibrated with 98% O_2/2% CO_2. The pCO_2 and the pH of this hypocapneic bath were 10–14 mmHg and 7.8–7.9, respectively.

Table I. Solutions Used in Studies on Rabbit MCD*

	Normal	Zero K	High K	High K/ Low Cl	Low HCO_3	High HCO_3
Na	145	150	100	75	145	145
K	5	0	50	75	5	5
Cl	115	115	115	50	135	90
HCO_3	25	25	25	25	5	50
Gluconate	0	0	0	65	0	0
pH‡	7.40	7.40	7.40	7.40	6.80	7.70

* In mM.

‡ pH determined at 37°C after equilibration with 95% O_2/5% CO_2.

In all experiments where perfusate and bath were asymmetrical with respect to ionic composition, the observed V_T and, when applicable, intracellular voltages were corrected for calculated liquid junction potentials as previously described (12). Statistical analysis was performed using the paired t test, and $P \leq 0.05$ was considered to indicate a significant difference.

Results

Effects of acute changes in peritubular K concentration (Table II). In six tubules, transepithelial voltage and net HCO_3 absorption were measured during control circumstances (bath K concentration 5 mM) and after an increase in bath K concentration to 50 mM. Control V_T was 20.6±3.7 mV lumen positive. After an increase in bath K concentration to 50 mM, there was a slight but significant ($P < 0.05$) increase in lumen-positive voltage to 24.3±3.5 mV. Presumably, this slight increase in lumen-positive voltage is secondary to paracellular diffusion of K, since it has been previously demonstrated that the MCD of the rabbit has a higher permeability to K than Na. In contrast to the effect on V_T, elevation of peritubular K concentration to 50 mM had no significance on net HCO_3 absorption in these tubules. Net HCO_3 absorption during control circumstances was 12.2±1.86 pmol · mm^{-1} · min^{-1}, and was unchanged at 12.2±2.0 pmol · mm^{-1} · min^{-1} when bath K concentration was 50 mM.

Just as with elevation of peritubular K, acute removal of peritubular K had no effect on MCD transport. Net HCO_3 absorption during control conditions was 16.0±1.46 pmol · mm^{-1} · min^{-1}, and was unchanged when bath K was completely removed at 14.9±1.70 pmol · mm^{-1} · min^{-1}. Similarly, there was no significant effect on transepithelial voltage, with control V_T being 27.4±2.6 mV and V_T with 0 K bath being 27.9±2.7 mV.

In our previous studies, we observed that when MCD were perfused in vitro with symmetrical Ringer's HCO_3 solutions, net HCO_3 absorption was matched virtually 1:1 by net Cl secretion. The route of this net Cl secretion is unknown, but the high lumen-positive voltage could facilitate paracellular movement of Cl from bath to lumen, assuming that the paracellular shunt pathway Cl permeability is sufficiently high. Similarly, the high lumen-positive voltage would be expected to provide a significant driving force for net movement of K as well as Na (in the absorptive direction). However, previous studies (13) have demonstrated that the rabbit MCD, when perfused in vitro with symmetrical Ringer's HCO_3 solutions, does not transport significant amounts of Na or K. Thus, the

Table II. Effects of Bath [K], [HCO_3], and pCO_2 on Net HCO_3 Absorption and Voltage in MCD

Protocol	N	V_T in mV		$JHCO_3$ in pmol·mm^{-1}·min^{-1}	
		Control	Experimental	Control	Experimental
Bath [K] (50 mM)	6	20.6±3.7	24.3±3.5*	12.2±1.0	12.2±2.0
Bath [K] (0 mM)	5	27.4±2.6	27.9±2.7	16.0±1.5	14.9±1.7
Bath [HCO_3] (5 mM)	6	30.2±3.8	40.0±4.4‡	15.6±1.9	22.9±2.9‡
Bath [HCO_3] (50 mM)	6	33.8±2.4	26.7±1.5*	17.9±1.1	12.8±1.3‡
Bath pCO_2 (10–14 mmHg)	6	31.1±4.4	15.7±1.0‡	11.0±3.7	5.3±0.6‡

* $P < 0.05$, control vs. experimental. ‡ $P < 0.01$, control vs. experimental. $JHCO_3$, net total CO_2 flux.

finding of equal amounts of HCO_3 absorption and Cl secretion in our previous studies raises the possibility that there is some more direct linkage between proton secretion and Cl secretion than just electrogenic proton secretion shunted by paracellular Cl movement. To address this issue, we perfused and bathed a group of five MCD with solutions designed to favor shunting of the electrogenic proton secretion by K rather than Cl. Tubules were perfused and bathed with the high K, low Cl solution (see Table I). Net HCO_3 absorption and net Cl secretion were measured in these tubules. The results of these studies are presented graphically in Fig. 1, where net Cl secretion in pmol·mm^{-1}·min^{-1} is plotted against net HCO_3 absorption in the same units. For comparison, included in Fig. 1 are our previous data in MCD perfused and bathed with symmetrical Ringer's HCO_3 solutions. All paired collections for Cl and HCO_3 flux in tubules perfused and bathed with high K, low Cl solution demonstrated significantly greater HCO_3 absorption than Cl secretion. In no circumstance were the Cl and HCO_3 fluxes equal. These experiments demonstrated

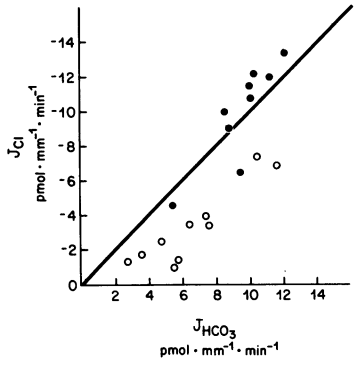

Figure 1. Net Cl secretion plotted against net HCO_3 absorption (both in pmol·mm^{-1}·min^{-1}) in tubules perfused and bathed with high K, low Cl solutions is illustrated by the open circles. In all experiments, net HCO_3 absorption exceeded net Cl secretion. For comparison is illustrated the relationship between Cl secretion and HCO_3 absorption when tubules are perfused and bathed with Ringer's HCO_3 solutions containing 5 mM K and 115 mM Cl (closed circles). See text for discussion.

that net HCO_3 absorption in MCD is not obligatorily coupled to Cl secretion. Although K fluxes were not measured in these experiments, presumably the difference between HCO_3 absorption and Cl secretion was represented mostly by net K absorption. In comparing the net HCO_3 absorptive rates in the present studies with those of our previous studies (2), there was a tendency for net HCO_3 flux to be slightly lower when MCD are perfused with the high K, low Cl solution. Since these experiments are unpaired, it is premature to make any conclusions from this data. However, should these differences prove to be real, they may be secondary to partial reduction in proton secretion by the lower peritubular Cl concentration used in these experiments. Irrespective of the magnitude of the net HCO_3 absorption in these studies, and whether or not there is a significant difference between the rates of HCO_3 absorption in these experiments as compared with our previous experiments using Ringer's HCO_3 solutions as bath and perfusate, the purpose of this group of experiments was to determine whether the relative rates of HCO_3 absorption and Cl secretion can differ in MCD.

Intracellular voltage recordings. Since elevation of bath [K] to 50 mM and reduction of bath [K] to 0 mM produced no major effects on acidification in MCD, we were interested in determining the response of intracellular voltage to these two maneuvers. We thus performed individual cell punctures of isolated perfused MCD. In these experiments, we measured the control intracellular voltage with reference to the bath and also measured the response of intracellular voltage to elevation of peritubular K concentration from 5 to 50 mM and to reduction of peritubular K concentration from 5 to 0 mM. In 14 punctures of 9 tubules, control intracellular voltage averaged −36.1±2.84 mV with respect to the bath. In these same tubules, transepithelial voltage was +29.0±1.3. Increasing the bath K concentration from 5 to 50 mM resulted in a small but significant ($P < 0.01$) depolarization of the basolateral cell membrane to −33.3±2.91 mV. This small depolarization is in contrast to the magnitude of basolateral cell membrane voltage depolarization that was observed by others and ourselves in proximal tubules and cortical collecting tubules (CCT) (10, 11, 14). Indeed, this very small depolarization in response to a log increase in peritubular K concentrations suggests that

the basolateral cell membrane of the MCD has a relatively low K conductance.

Removal of K from the bath (11 punctures, 6 tubules) produced no significant change in basolateral cell membrane or transepithelial voltage, with the former being -40.1 ± 1.79 mV under control conditions and -40.5 ± 1.83 mV at zero K bath, and the latter being $+30.3\pm1.37$ and 31.0 ± 1.34 at 5 and 0 mM bath K, respectively. If we can assume that removal of bath K inhibits Na-K-ATPase, this observation is compatible with the recent demonstration that ouabain in the peritubular fluid does not affect HCO_3 transport in the MCD (4).

Effects of peritubular HCO_3 concentration and pCO_2 on MCD (table II). In six MCD the effects of acute reduction of peritubular HCO_3 concentration from 25 to 5 mM (pH 7.4–6.8) was determined. Reduction of bath HCO_3 concentration resulted in a significant increase in lumen-positive voltage from 30.2 ± 3.8 to 40.0 ± 4.4 mV, $P < 0.01$. Simultaneously, net HCO_3 absorption increased significantly from 15.6 ± 1.9 to 22.9 ± 2.9 pmol $\cdot$ mm^{-1} $\cdot$ min^{-1}, $P < 0.01$. In two of these tubules, the peritubular HCO_3 replacement was not with Cl but with gluconate. Thus, peritubular Cl concentration remained the same as during control conditions. Results of these two tubules were the same as the remaining four and the data for all six tubules was pooled.

In another group of six tubules, the effects of an acute increase in peritubular HCO_3 concentration from 25 mM to 50 mM (pH 7.4–7.7) were determined. Elevation of peritubular HCO_3 concentration produced a significant decrease in lumen-positive voltage from 33.8 ± 2.4 to 26.7 ± 1.5 ($P < 0.05$). Simultaneously, net HCO_3 absorption decreased from 17.9 ± 1.1 to 12.8 ± 1.3 pmol $\cdot$ mm^{-1} $\cdot$ min^{-1} ($P < 0.01$).

The final group of experiments determined the effect of acute reduction in peritubular pCO_2 on acidification in six MCD. In these experiments, after control collections were made at a pCO_2 of 40 mmHg, the bath was changed to one that had been equilibrated to 2% CO_2. The measured pCO_2 in all experiments was always between 10 and 14 mmHg, and bath pH ranged between 7.80 and 7.90. This acute reduction in peritubular pCO_2 and increase in pH produced a major decrease in a lumen-positive voltage from 31 ± 4.4 to 15.7 ± 1.0. Coincident with this reduction in lumen-positive voltage was a major decrease in net HCO_3 absorption from 11.0 ± 3.7 to 5.3 ± 0.6 pmol $\cdot$ mm^{-1} $\cdot$ min^{-1}.

Discussion

The medullary collecting duct from the inner stripe of outer medulla has been identified as a major capacity segment for distal nephron hydrogen ion secretion. This was initially demonstrated in in vitro studies of rabbit tubules (1–4) and has subsequently been documented by similar studies on rat tubules that were perfused in vitro (5). Note that in all of these in vitro microperfusion studies, H^+ secretion was assessed by net HCO_3 absorption, since the studies used 25 mM HCO_3 in perfusion solutions. While 25 mM HCO_3 may not be the usual concentration for this buffer in luminal fluid at the level of the MCD in rabbit or rat, use of such a HCO_3 concentration allows for easy measurement of the proton secretory capacity of this segment. Because of the potential importance of the MCD to net distal nephron acidification, it is important to determine which factors modulate proton secretion in this segment. As mentioned previously, we have provided evidence that hydrogen ion secretion in this segment is responsive to mineralocorticoid hormones (3). In addition, we have demonstrated that acidification can occur independent of the Na ion and is dependent upon the presence of peritubular Cl (2, 3). However, there are several possible modulators of acidification in this segment that have not been examined. The present studies were designed to elucidate some of the regulatory mechanisms.

By virtue of its anatomic location in the kidney, the MCD is expected to be exposed to major changes in the peritubular K concentration that may reflect not only changes in K balance but also changes in the hydration status. For this reason, we tested the effects of acute alterations in peritubular K concentration on acidification in this segment. Our studies clearly demonstrate that changes in the K concentration from 0 to 50 mM have no significant effect on net HCO_3 absorption by the MCD. It should be noted that these studies do not address the question of whether chronic changes in K balance affect MCD acidification. Indeed, such studies will require studying MCD harvested from animals who have been maintained on low and high K-containing diets.

Because we failed to find a significant effect of acute alterations in peritubular K concentration on acidification in this segment, we further investigated the effects of these K concentration changes on intracellular voltage. There were several reasons for performing these electrophysiologic measurements. Based on our earlier studies (2), we believe that the MCD cell is characterized, in part, by an apical cell membrane electrogenic proton secretory mechanism coupled with a basolateral cell membrane neutral Cl-HCO_3 exchanger that allows for base exit from cell to peritubular fluid. The luminal cell membrane electrogenic proton pump should be affected by the electrical potential across the apical cell membrane, while the basolateral cell membrane Cl-HCO_3 exchanger should be insensitive to the voltage across the basolateral cell membrane but sensitive to the relative concentrations of Cl and HCO_3 across this membrane. Since elevation of peritubular K concentration to 50 mM in proximal convoluted and proximal straight tubule cells as well as CCT cells results in major depolarization of intracellular voltage (10, 11, 14), we would expect to observe an effect on net HCO_3 absorption if the basolateral cell membrane of the MCD cell also possessed high K conductance. Thus, if elevation of the peritubular K concentration to 50 mM significantly depolarizes the cell, one would expect an increase in proton secretion due to improvement of the electrical gradient against which the proton pump

must work. We did not see such an increase in proton secretion (HCO_3 absorption). Thus, we tested the effect of a log increase in the peritubular K concentration on intracellular voltage. Indeed, our studies clearly show that this maneuver results in minimal depolarization of the basolateral cell membrane voltage. Recently presented studies by Koeppen (15) are in agreement.

Our studies also show that reduction of peritubular K to 0 mM does not affect transepithelial voltage, net HCO_3 absorption, or intracellular voltage. We have previously demonstrated that acidification can persist unchanged in the MCD when all luminal and peritubular fluid Na is removed (3). In addition, Laski and Kurtzman (4) have recently demonstrated that ouabain added to the peritubular side of the rabbit MCD perfused in vitro has no significant effect on transepithelial voltage nor on net acidification. Our results with zero K bath are compatible with the observation that acidification in MCD is not affected by inhibition of basolateral membrane Na-K-ATPase.

A second purpose of our studies was to evaluate the stoichiometry of net HCO_3 absorption and net Cl secretion. As mentioned previously, when MCD of rabbit are perfused in vitro with symmetrical Ringer's HCO_3 solutions, net HCO_3 absorption is matched by net Cl secretion. From the magnitude of the transepithelial voltage and the previous observations by Stokes (13) demonstrating significant Na and K permeabilities in this segment, one would expect that HCO_3 absorption might, in part, be matched by voltage-driven passive movements of Na or K. Because of the demonstration by Stokes (13) that the MCD is more permeable to K, and because our previous studies (2) examined the MCD with solutions in which the Cl concentration was >20-fold that of the K concentration, we wanted to perform experiments in which the tubules were perfused and bathed in solutions that would allow significant voltage-driven net K transport. It is clear from our experiments using the high K, low Cl perfusate and bath that net HCO_3 absorption and net Cl secretion are not obligatorily coupled 1:1. Since it is not the purpose of these studies to define physiologic relevance of voltage-driven K transport in this segment, but rather to determine whether proton secretion (HCO_3 absorption) and Cl secretion are tightly linked, we do not want to speculate on the physiologic role of the MCD with respect to K transport. Since HCO_3 absorption in our studies always exceeded net Cl secretion, it is clear that bulk electroneutrality was maintained by the movement of a cation from lumen to bath. Which cation moved and the route of its movement are not important for the present studies.

In comparing the data from the present studies and those reported by us earlier, it is important to point out that no contradictions exist. When MCD are perfused and bathed with Ringer's HCO_3, the equality of net HCO_3 absorption and net Cl secretion is compatible not only with transcellular Cl movement, which is somehow coupled to proton secretion, but also with paracellular movement of Cl which is driven by the lumen-positive voltage. Such paracellular Cl movement, since it appears to account for the entire bulk electroneutrality of proton secretion, would presumably occur through an anion selective paracellular pathway. Using electrophysiologic techniques we have demonstrated that, in the MCD, passive Cl permeability exceeds passive Na permeability (2). However, the difference between these two ionic permeabilities is not sufficient to account for the totally preferential Cl movement. Recent demonstration that the basolateral cell membrane of MCD cells is highly Cl conductive (15) makes our earlier electrophysiologic measurements of passive Cl permeability unreliable. Isotopic permeability coefficients, utilizing ^{36}Cl and excluding significant exchange diffusion of Cl, will be necessary.

The present studies cannot be used to quantitatively or qualitatively address the route(s) of transepithelial Cl movement. Rather, they help to clarify our earlier observations, in that they exclude 1:1 coupled, i.e., HCl secretion. Some or all of the Cl may be secreted via transcellular or paracellular routes. Determination of apical cell membrane Cl conductance and the electrochemical gradient for Cl across the apical cell membrane will be helpful in addressing this issue further.

In our first studies on the rabbit MCD perfused in vitro, we tested whether manipulation of the acid base status of the rabbit from which the MCD was harvested had any significant effect on expression of in vitro acidification (1). The rationale for such studies stemmed from the observations by McKinney and Burg (16, 17) in which the in vitro CCT of the rabbit either reabsorbed or secreted HCO_3, depending upon the acid base status of the animal from which the CCT was harvested. In our earlier studies, we were unable to find an effect of either ammonium chloride or $NaHCO_3$ pretreatment of the rabbits on MCD acidification as studied in vitro (1). At that time, we were concerned that this observation might in part be explained by blunting of in vitro expression of in vivo acidification rates, due to the fact that all in vitro studies were performed at normal pH. In other words, we believed it was possible that the MCD in vivo responded appropriately to acid base stimuli, but that this in vivo response was rapidly lost in vitro when tubules were exposed to a peritubular HCO_3 concentration of 25 mM and a pCO_2 of 40 mmHg with a pH of 7.4. For this reason we tested the effects of in vitro changes in acid base status on acidification by the MCD.

When peritubular fluid was acutely changed to one simulating acute metabolic acidosis, i.e., 5 mM as opposed to 25 mM peritubular HCO_3 concentration, net acidification was significantly increased, as was lumen-positive transepithelial voltage. In contrast, when peritubular fluid was acutely changed to one which simulated acute metabolic alkalosis with a peritubular HCO_3 concentration of 50 mM and a peritubular pCO_2 of 40, net HCO_3 absorption was significantly suppressed, as was lumen-positive transepithelial voltage. In both these groups of experiments it is impossible to determine whether the effect observed was due to a change in the concentration of HCO_3 or a change in pH. Since our earlier studies are

compatible with a basolateral cell membrane Cl-HCO_3 exchanger (2), it is possible that the major mechanism behind our observations is the effect of the chemical concentration of peritubular HCO_3 on the Cl-HCO_3 exchanger. While isohydric changes in the peritubular HCO_3 concentration (i.e., with appropriate changes in pCO_2 to maintain pH constant) would help to address this question, it is clear that a complete understanding of the mechanism behind our observations will require definition of the electrical and chemical driving forces for proton and base movement across both basolateral and apical cell membranes. This can only be accomplished by additional studies examining the effects of changes in peritubular HCO_3 concentration, both isohydric and nonisohydric, on intracellular voltage and pH. The importance of the present studies is just the documentation that rates of acidification in the MCD can be altered by changes in peritubular HCO_3 concentration or pH.

Further support for regulation of acidification by the MCD when studied in vitro comes from recent studies by Laski and Kurtzman (4). These investigators demonstrated a significant correlation between net acidification rates and in vivo urine pH of the rabbit from which the MCD was harvested. As opposed to our studies in the rabbit and studies by Atkins and Burg (5) in the rat, the recent studies by Laski and Kurtzman (4) used a different animal model (starvation as opposed to ammonium chloride loading).

As an additional attempt to demonstrate regulation of MCD acidification in vitro, we examined the effects of a reduction in peritubular pCO_2. When pCO_2 is reduced to a level between 10 and 14 mmHg and pH as a result rises to ~7.8, there is a drastic reduction in net acidification as well as the lumen-positive voltage in MCD. We did not test lesser decreases in pCO_2, nor did we examine the effects of an elevated pCO_2 on acidification in this segment. Such studies will be required to determine the physiologic significance of acute changes in pCO_2 on distal nephron acidification. However, the importance of our studies using this major decrease in pCO_2 relates to the additional demonstration that acidification in this segment is responsive to acid base changes. The mechanism behind the reduction in acidification observed in the present studies is unclear. It is possible that the reduction of pCO_2 to the 10–14 range significantly reduces substrate for buffering of the intracellular hydroxyl generated behind the proton pump, or that the effect is secondary to an intracellular pH-induced change on proton secretion at the apical cell membrane (i.e., production of a less favorable chemical gradient for proton secretion due to alkalinization of the cell). Note that the effects of pCO_2 and possibly also the effects of peritubular HCO_3 concentration changes mentioned above may also be expressed via alterations in the number of proton pumps at the apical cell membrane. Recent studies have suggested that acidification by urinary bladder epithelial cells may in part be regulated by recycling of proton pumps from the apical cell membrane to storage vesicles in the cell cytoplasm and vice versa (18). Thus, appropriate stimuli to increased acidification result in migration of these preformed pumps to the apical cell membrane with resultant fusion. In converse, circumstances which would inhibit acidification might result in endocytosis of apical cell membrane proton pumps. Such possibilities remain to be tested in the rabbit MCD.

It should be emphasized that the purpose of the present studies was to investigate if net acidification by MCD can be regulated by changes in peritubular [K] as well as peritubular acid base conditions. While significant changes in net acidification were observed with alteration of peritubular [HCO_3] and pCO_2, the present studies cannot define the mechanism(s) responsible for these changes. Nor can the present studies determine if the predominant factor regulating net acidification by this nephron segment is peritubular pH or peritubular [HCO_3] or even intracellular pH. These questions can best be addressed in future studies using isohydric changes in peritubular [HCO_3] and pCO_2, as well as appropriate measurements of intracellular voltage and, if possible, intracellular pH during manipulation of net acidification.

In summary, rabbit MCD were studied in vitro to examine the influence of acute changes in peritubular K concentration as well as acute changes in peritubular acid base status on acidification and transepithelial voltage in this nephron segment. In addition, studies were conducted to examine intracellular voltage and the effects of peritubular [K] on intracellular voltage. These studies demonstrate that (*a*) alteration of peritubular K from 0 to 50 mM in vitro does not affect net HCO_3 absorption in the MCD; (*b*) MCD cells, in contrast to proximal tubule and CCT cells, possess a low basolateral cell membrane K conductance; (*c*) net HCO_3 absorption and Cl secretion are not obligatorily coupled; and (*d*) net HCO_3 absorption and the associated lumen-positive voltage, which is thought to be due to electrogenic proton secretion, can be modulated acutely by in vitro changes in peritubular HCO_3 concentration and pCO_2. Thus, the MCD perfused in vitro appears to be a good model for studying the regulation of distal nephron acidification.

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References

1. Lombard, W. E., J. P. Kokko, and H. R. Jacobson. 1983. Bicarbonate transport in cortical and outer medullary collecting tubules. *Am. J. Physiol.* 244:F289–F296.

2. Stone, D. K., D. W. Seldin, J. P. Kokko, and H. R. Jacobson. 1983. Anion dependence of rabbit medullary collecting duct acidification. *J. Clin. Invest.* 71:1505–1508.

3. Stone, D. K., D. W. Seldin, J. P. Kokko, and H. R. Jacobson. 1983. Mineralocorticoid modulation of rabbit medullary collecting

duct acidification. A sodium-independent effect. *J. Clin. Invest.* 72:77–83.

4. Laski, M. E., and N. A. Kurtzman. 1983. Characterization of acidification in the cortical and medullary collecting tubule of the rabbit. *J. Clin. Invest.* 72:2050–2059.

5. Atkins, J. L., and M. B. Burg. 1983. Secretion and absorption of bicarbonate by rat collecting ducts. *Clin. Res.* 31:423A.

6. Burg, M., J. Grantham, M. Abramow, and J. Orloff. 1966. Preparation and study of fragments of single rabbit nephrons. *Am. J. Physiol.* 210:1293–1298.

7. Jacobson, H. R. 1981. Effects of CO_2 and acetazolamide on bicarbonate and fluid transport in rabbit proximal tubule. *Am. J. Physiol.* 240:F54–F62.

8. Vurek, G. G., D. G. Warnock, and R. Corsey. 1975. Measurement of picomole amounts of carbon dioxide by calorimetry. *Anal. Chem.* 47:765–767.

9. Ramsay, J. A., R. H. Brown, and P. C. Croghan. 1955. Electrometric titration of chloride in small volumes. *J. Exp. Biol.* 32:822–829.

10. Biagi, B., K. Takahiro, M. Sohtell, and G. Giebisch. 1981. Intracellular potentials in rabbit proximal tubule perfused in vitro. *Am. J. Physiol.* 240:F200–F210.

11. Koeppen, B. M., B. A. Biagi, and G. H. Giebisch. 1983. Intracellular microelectrode characterization of the rabbit cortical collecting duct. *Am. J. Physiol.* 244:F35–F47.

12. Jacobson, H. R., and J. P. Kokko. 1976. Intrinsic differences in various segments of the proximal convoluted tubule. *J. Clin. Invest.* 57:818–825.

13. Stokes, J. B. 1982. Sodium and potassium transport across the cortical and outer medullary collecting tubule of the rabbit: evidence for diffusion across the outer medullary portion. *Am. J. Physiol.* 242:F514–F520.

14. Molony, D. A., J. P. Kokko, D. Seldin, and H. R. Jacobson. 1984. Acid peritubular pH suppresses sodium reabsorption in cortical collecting tubules. *Kidney Int.* 25:279.

15. Koeppen, B. M. 1984. Conductive properties of rabbit outer medullary collecting duct. *Kidney Int.* 25:278.

16. McKinney, T. D., and M. B. Burg. 1978. Bicarbonate absorption by rabbit cortical collecting tubules in vitro. *Am. J. Physiol.* 234:F141–F145.

17. McKinney, T. D., and M. B. Burg. 1978. Bicarbonate secretion by rabbit cortical collecting tubules in vitro. *J. Clin. Invest.* 61:1421–1427.

18. Gluck, S., C. Cannon, and Q. Al-Awqati. 1982. Exocytosis regulates urinary acidification in turtle bladder by rapid insertion of H^+ pumps into the luminal membrane. *Proc. Natl. Acad. Sci. USA.* 79:4327–4331.