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Research Article

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Mineralocorticoid Modulation of Apical and Basolateral Membrane $H^+/OH^-/HCO_3^-$ Transport Processes in the Rabbit Inner Stripe of Outer Medullary Collecting Duct

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Abstract

To examine the mechanism by which mineralocorticoids regulate HCO_3^- absorption in the rabbit inner stripe of the outer medullary collecting duct, we microfluorometrically measured intracellular pH (pH_i) in in vitro perfused tubules using 2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF) assaying the apical and basolateral membrane $H^+/OH^-/HCO_3^-$ transport processes in three groups of animals: those receiving chronic in vivo DOCA treatment (5 mg/kg per d × 2 wk); those with surgical adrenalectomy (ADX, [chronic × 2 wk]) on glucocorticoid replacement; and controls. Baseline pH_i was not different in the three groups. Cellular volume (vol/mm) was increased 38% in DOCA tubules versus controls, but unchanged in ADX tubules versus controls. Buffer capacities (B_T) were not different in the three groups. Apical membrane H^+ pump activity, assayed as the Na^+-independent pH_i recovery from an acid load (NH_3/NH_4^+ prepulse) and expressed as J_H ($dpH_i/dt \cdot vol/mm \cdot B_T$) was increased 76% in DOCA tubules versus controls, and decreased 56% in ADX tubules versus controls. Basolateral membrane Cl^-/HCO_3^- exchange activity assayed as the pH_i response to basolateral Cl^- addition was increased 73% in DOCA tubules versus controls, and decreased 44% in ADX tubules versus controls. When examined as a function of varying [Cl^-], the Vmax of Cl^-/HCO_3^- exchange activity was significantly increased in DOCA tubules (control, 72.7±15.7 pmol · mm^{-1} · min^{-1} vs DOCA, 132.3±22.5 pmol · mm^{-1} · min^{-1}, $P < 0.02$), while the $K_{1/2}$ for Cl^- was unchanged. Basolateral membrane Na^+/H^+ antiporter activity assayed as the Na^+-dependent pH_i recovery from an acid load was not changed in chronic DOCA tubules versus controls. In conclusion, the apical membrane H^+ pump and basolateral membrane Cl^-/HCO_3^- exchanger of the rabbit $OMCD_i$ are regulated in parallel without chronic alterations in pH_i under the conditions of mineralocorticoid excess and deficiency. The parallel changes in these transporters accounts for the alterations in $OMCD_i$ HCO_3^- absorption seen under these conditions. (*J. Clin. Invest.* 1992. 90:180–187.) Key words: proton pump • chloride-bicarbonate exchanger • intracellular pH • adrenalectomy • chronic desoxycorticosterone acetate

Introduction

Mineralocorticoids are known to be an important regulator of net acid excretion by the kidney (1, 2). This action is mediated in part by a direct effect of mineralocorticoids, independent of effects on Na^+ transport (3), on H^+ secretion in the inner stripe of the outer medullary collecting duct ($OMCD_i$).[1] Several mechanisms exist whereby mineralocorticoids could directly stimulate transepithelial H^+ secretion in the $OMCD_i$: (*a*) stimulating the apical membrane H^+ pump; (*b*) stimulating the basolateral membrane Cl^-/HCO_3^- exchanger; (*c*) inhibiting the basolateral membrane Na^+/H^+ antiporter; or (*d*) stimulating the apical and basolateral membrane $H^+/OH^-/HCO_3^-$ transporters in parallel. Several investigators have found that the in vivo mineralocorticoid status of the animal alters the level of NEM-sensitive H^+-ATPase activity measured in the $OMCD_i$ (4–7). In addition, in $OMCD_i$'s harvested from adrenalectomized animals, acute exposure of aldosterone stimulates NEM-sensitive H^+-ATPase activity by a process requiring protein synthesis (7). These enzymatic studies suggest that the primary effect of mineralocorticoids may be mediated by a direct effect on the apical membrane H^+ pump. Effects on the other $H^+/OH^-/HCO_3^-$ transport mechanisms have not been investigated.

The purpose of the present study was to examine the mechanism(s) by which mineralocorticoids modulate HCO_3^- absorption in the rabbit $OMCD_i$, using the fluorescent measurement of cell pH (pH_i). The results demonstrate that increases and decreases in mineralocorticoid activity modulate apical and basolateral membrane $H^+/OH^-/HCO_3^-$ transporters in parallel without chronically altering pH_i, accounting for changes in $OMCD_i$ HCO_3^- absorption in these conditions.

Methods

The technique of in vitro microperfusion of isolated rabbit $OMCD_i$ was used as previously described (8). Briefly, female New Zealand White rabbits weighing 1.5–2.0 kg were maintained on standard laboratory chow and tap water ad lib. $OMCD_i$ segments were identified and dissected free from 1-mm coronal slices at 4°C (pH 7.4, solution 1, Table I). To avoid the outer stripe, perfused segments were dissected from the

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1. *Abbreviations used in this paper:* ADX, adrenalectomy; BCECF, 2',7'-bis (carboxyethyl)-5(6)-carboxyfluorescein; $B_{non\text{-}CO_2HCO_3^-}$, non-$CO_2/HCO_3^-$, buffer capacity; B_T, total buffer capacity; DIDS, 4,4'-diisothiocyano-stilbene-2, 2'-disulfonate; DOCA, desoxycorticosterone acetate; $[NH_4^+]_o$, concentration of NH_4^+ in the bath; $OMCD_i$, outer medullary collecting duct; pH_i, intracellular pH.

Table I. Composition of Solutions

	1	2	3	4	5	6	7	8	9	10
Na^+	145	135	—	125	135	135	135	135	135	—
K^+	5	5	5	5	5	5	5	5	5	5
Mg^{2+}	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Ca^{2+}	1.6	1.6	1.6	1.6	7.0	1.6	6.9	6.7	5.0	1.6
Choline	—	—	120	—	—	—	—	—	—	110
NH_4^+	—	—	—	10	—	—	—	—	—	10
N-methyl D-glucosamine	—	—	15	—	—	—	—	—	—	15
Cl^-	125.2	125.2	125.2	125.2	—	103.2	10	20	40	125.2
HCO_3^-	25	—	—	—	25	25	25	25	25	—
HPO_4^{2-}	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
SO_4^{2-}	—	—	—	—	1.0	1.0	1.0	1.0	1.0	—
Gluconate	—	—	—	—	114	—	103.8	93.4	70	—
Glucose	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3
Alanine	5	5	5	5	5	5	5	5	5	5
Hepes	—	25	25	25	25	25	25	25	25	25

All units are millimolar. All solutions were titrated to 290 mOsM with addition of raffinose. Solutions 3 and 10 were titrated to pH 7.40 with 1 N *N*-methyl-D-glucosamine hydroxide. Solutions 1, 2, and 4–9 were titrated to pH 7.40 with 1 N NaOH. All HCO_3^- containing solutions were equilibrated with 94% O_2/6% CO_2; other solutions were equilibrated with 100% O_2.

inner half of the inner stripe. Tubules were transferred into a bath chamber with a vol of ~90 μl, constructed of black lucite to minimize light reflection. The peritubular fluid was continuously exchanged at ~10 ml/min by hydrostatic pressure, allowing a complete fluid exchange within 1 s. Tubular lumens were perfused at flow rates of 25–50 nl/min. Bath pH was monitored continuously by placing a commercial flexible pH electrode into the bath (MI-5089; Microelectrodes, Inc., Londonderry, NH). Bath solutions were prewarmed at 37°C, continuously equilibrated with either 94% O_2/6% CO_2 or 100% O_2, and passed to the bath chamber through CO_2 impermeable tubing (Clarkson Controls and Equipment Co., Detroit, MI). Bath temperature of 37±0.3°C was maintained by a specially designed water-jacketed glass coiled line.

To minimize motion, the distal end of tubule was sucked gently into a collection pipette. In addition, the average length of the tubule exposed to the bath fluid was limited to ~250–500 μm. The tubules were loaded with the acetoxymethyl derivative of 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF-AM) (Molecular Probes, Inc., Eugene, OR), 8 μM, from the bath using solution 1 (Table I). Loading was continued until signal to background fluorescence at 450 nm excitation was ≥ 10:1, usually requiring 10–15 min. Tubules were then washed with solution 1, (Table I) at pH 7.4 for a minimum of 10 min, followed by the control solution of each experiment.

Luminal and peritubular solutions used in these studies are listed in Table I. CO_2/HCO_3^--free solutions were bubbled with 100% oxygen passed through a 3-N KOH trap. Desoxycorticosterone acetate (DOCA), 4,4′-diisothiocyano-stilbene-2,2′-disulfonate (DIDS), nigericin, amiloride hydrochloride, and all solution salts were purchased from Sigma Chemical Co. (St. Louis, MO).

Cell pH (pH_i) measurement. BCECF fluorescent emission was measured at 530 nm with an inverted epifluorescent microscope (Nikon Diaphot; Nikon Inc., Garden City, NY) attached to a dual excitation microspectrofluorimeter (SPEX CM-1; SPEX Inds., Inc., Edison, NJ) which allows rapid alternation between two excitation wavelengths (500 and 450 nm), as previously described (9). Fluorescence was measured using a 20× objective, on an area of the tubule ~125 μm in length that included the entire width of the tubule. No attempts were made to make measurements on single cells. Although immunocytochemical studies based on the staining with antibodies against the H^+-ATPase and band 3 Cl^-/HCO_3^- exchanger have found evidence for two cell types in the inner stripe segment (10), ultrastructural (11), morphologic (12), and electrophysiologic (13, 14) studies strongly support one cell type in the inner half of the $OMCD_i$ where the present studies were performed. Wiener and Hamm (15) were able to divide $OMCD_i$ cells into two groups based on ease of BCECF loading, but found both cell types possessed the same apical and basolateral membrane $H^+/OH^-/HCO_3^-$ transporters. Thus, these studies suggest that the $OMCD_i$ is composed of one cell type with variable degrees of differentiation. Generally, the measured segment started ~50–100 μm from the perfusion pipette. Background fluorescence at each of the excitation wavelengths was measured on the tubule before loading with BCECF, and the results were subtracted from the measured fluorescence during the experiment. A fluorescent ratio was then calculated as fluorescence with 500 nm excitation divided by that with 450 nm excitation. The initial rate of change in the fluorescence excitation ratio was defined by the slope of a line drawn tangent to the initial deflection ([d(F500/F450)/dt]).

BCECF fluorescence excitation ratios were calibrated intracellularly using the method of Thomas et al. (16). Tubules were bathed and perfused with well-buffered solutions (25 mM Hepes, 60 mM phosphate, and appropriate [HCO_3^-]) of varying pH containing 7 μM nigericin (a K^+/H^+ antiporter) and 120 mM K^+ (9). Because of the small amount of variability between tubules, a calibration was generated in each of three experimental groups of animals (chronic DOCA, adrenalectomy, and control) and was used to convert F500/F450 fluorescent ratios to pH units in each experimental group.

Buffer capacity. The buffer capacity was determined using the technique of either rapid NH_3/NH_4^+ addition or withdrawal, as described by Roos and Boron (17). The non-CO_2/HCO_3^- buffer capacity $B_{non\text{-}CO_2/HCO_3^-}$ (mmol · liter^{-1} · pH unit^{-1}) is given by the formula:

$$B_{non\text{-}CO_2/HCO_3^-} = [NH_4^+]_i/\Delta pH_i \quad (1)$$

where ΔpH_i is the measured pH_i change. $[NH_4^+]_i$ is calculated from the formula:

$$[NH_4^+]_i = [NH_4^+]_o\,(pH_o - pH_i) \quad (2)$$

where $[NH_4^+]_o$ is the concentration of NH_4^+ in the bath; pH_o is the pH of the peritubular solution; and pH_i is either the cell pH after NH_3/NH_4^+ addition, or the cell pH just before NH_3/NH_4^+ withdrawal. In

studies performed in the absence of CO_2/HCO_3^-, $B_{non\text{-}CO_2/HCO_3^-}$ represents the total buffer capacity of the cell (B_T). In studies performed in the presence of CO_2/HCO_3^-, the CO_2-buffering capacity, B_{CO_2} (mmol · liter^{-1} · pH unit^{-1}) is calculated by the formula:

$$B_{CO_2} = 2.3[HCO_3^-]_i \quad (3)$$

where $[HCO_3^-]_i$ is given by the formula:

$$[HCO_3^-]_i = \alpha \cdot pCO_2 \cdot 10(pHi - pK) \quad (4)$$

where α is the solubility of CO_2 in water, pH_i is the cell pH at the start of the experimental manuever, and a pK of 6.1 was used. In the presence of CO_2/HCO_3^-, the total buffer capacity of the cell is equal to:

$$B_T = B_{non\text{-}CO_2/HCO_3^-} + B_{CO_2/HCO_3^-} \quad (5)$$

Calculation of proton fluxes. Transporter activities were expressed as proton fluxes (J_H, pmol · mm^{-1} · min^{-1}) induced by the experimental maneuvers, calculated using the formula:

$$J_H = dpH_i/dt \cdot vol/mm \cdot B_T \quad (6)$$

where vol/mm is the cellular volume of the tubules expressed per millimeter length. Cellular volumes were determined for each tubule by optical measurement of internal and external diameters of each tubule before experimentation. When transporter activity was measured under acidic pH_i conditions (H^+ pump and Na^+/H^+ antiporter), the buffer capacity was determined by NH_3/NH_4^+ withdrawal. In these studies measuring Na^+/H^+ antiporter and H^+ pump activity, the buffer capacity was determined on the same tubule. When transporter activity was measured under alkaline pH_i conditions (Cl^-/HCO_3^- exchanger), $B_{non\text{-}CO_2/HCO_3^-}$ was calculated from NH_3/NH_4^+ addition made during the Na^+/H^+ antiporter and H^+ pump studies, plus the B_{CO_2/HCO_3^-} determined for each Cl^-/HCO_3^- exchanger tubule.

Experimental groups. Three experimental groups of animals were used. The first group consisted of rabbits maintained on standard laboratory chow (Teklad, Madison, WI) and tap water ad lib. (control). There were two control groups of animals, one for each experimental group. The second group of rabbits was maintained on standard laboratory chow and tap water ad lib., and received 5 mg/kg body wt DOCA (dissolved in sesame oil) intramuscularly every day for 14 d (DOCA). The third group of rabbits was surgically adrenalectomized and maintained on standard laboratory chow and 0.9% normal saline drinking water ad lib. (ADX). Preoperatively and on the first postoperative day, ADX animals received 50 μg of dexamethasone (dexamethasone sodium phosphate; Elkins-Sinn, Cherry Hill, NJ) subcutaneously. Thereafter, the rabbits were maintained on glucocorticoid replacement therapy (dexamethasone 10 μg/kg s.c.) from the second postoperative day until death (12–15 d). This dose of glucocorticoid minimizes mineralocorticoid activity, while enhancing food and water intake as well as the general well-being of the animals (18–20).

Statistics. Results are reported as mean±standard error. Data are analyzed using the two-tailed Student's *t* test for paired and unpaired data, as appropriate.

Results

Chronic in vivo DOCA administration

The first set of studies examined the effects of chronic in vivo DOCA administration on the apical and basolateral membrane $OMCD_i$ $H^+/OH^-/HCO_3^-$ transport processes. Shown in Table II is a comparison of baseline pH_i, cell volume, and buffer capacities obtained in $OMCD_i$'s harvested from chronic DOCA and control rabbits. Baseline pH_i values obtained in the presence of 25 mM HCO_3^- and 40 mm Hg pCO_2 (pH 7.4, solution 1, Table I) in 21 DOCA tubules was 6.93±0.02. This value was not significantly different from the baseline pH_i values determined under similar conditions in 23 control tubules (6.92±0.03, P = NS). However, chronic DOCA treatment led to a 38% increase in cell volume (control, n = 17, 6.51±0.29 · 10^{-10} liter/mm; chronic DOCA, n = 33, 8.97±0.25 · 10^{-10} liter/mm, $P < 0.05$). There was no significant difference in buffer capacities between the two groups determined by either NH_3/NH_4^+ addition or withdrawal (Table II).

Table II. Baseline Cell pH, Cell Volume, and Buffer Capacities in Chronic DOCA Tubules

	Control	Chronic DOCA
Baseline cell pH	6.92±0.03	6.93±0.02
	n = 23	n = 21
Cell vol (10^{-10} liter/mm)	6.51±0.29	8.97±0.25§
	n = 17	n = 33
Buffer capacity (mmol · liter^{-1} · pH unit^{-1})		
(H^+ pump and Na^+/H^+ antiporter studies)*	33.16±3.80	34.37±4.21
	n = 17	n = 22
(Cl^-/HCO_3^- exchanger studies)‡	39.02±2.50	40.16±2.58
	n = 18	n = 20

Values are means±SEM. n = number of tubules. * Buffer capacity was determined by NH_3/NH_4^+ withdrawal. ‡ Buffer capacity was the mean $B_{non\text{-}CO_2/HCO_3^-}$ value obtained from NH_3/NH_4^+ addition in the H^+ pump and Na^+/H^+ antiporter studies plus the B_{CO_2/HCO_3^-} for each tubule in the Cl^-/HCO_3^- exchanger studies. § $P < 0.05$ chronic DOCA versus controls.

Apical membrane H^+ pump activity. Apical membrane H^+ pump activity was assessed as the Na^+-independent pH_i recovery from an acid load performed in the absence of ambient CO_2/HCO_3^-. In previous studies, we have found Na^+-independent pH_i recovery from an acid load to be NEM-sensitive and SCH 28080-insensitive, likely reflecting the presence of a simple vacuolar H^+ pump (21). In addition, we have shown that a sustained increase in cell calcium induced by peritubular Na^+ removal results in inactivation of the H^+ pump (22). To lessen the influence of this variable, we examined H^+ pump activity in the presence of peritubular Na^+, with Na^+-dependent pH_i recovery inhibited by 1 mM peritubular amiloride. 1 mM peritubular amiloride completely blocks Na^+-dependent pH_i recovery in this segment (21). Shown in Fig. 1 is a typical experimental tracing. 12–15 min before the experiment, tubules were bathed in a 135-mM Na^+, CO_2/HCO_3^--free solution (pH 7.4, solution 2, Table I) and luminally perfused with a Na^+-free, CO_2/HCO_3^--free solution (pH 7.4, solution 3, Table I). 1 mM amiloride was then added to the peritubular solution and continued throughout the remainder of the experiment. After 5 min exposure to this solution, the peritubular solution was changed to an identical solution containing 10 mM NH_4Cl (pH 7.4, solution 4, Table I), and then 4–5 min later it was changed back to an identical solution without NH_4Cl (solution 2). The initial rate of pH_i recovery from the induced acid load (Fig. 1) was used as an index of apical membrane H^+ pump activity. Shown in Fig. 2 are the results of these studies. In 11 chronic DOCA tubules, J_H was increased 76% when compared to seven control tubules (control, n = 7, 2.23±0.48 pmol · mm · $^{-1}$min^{-1}; DOCA, n = 11, 3.93±0.51 pmol ·

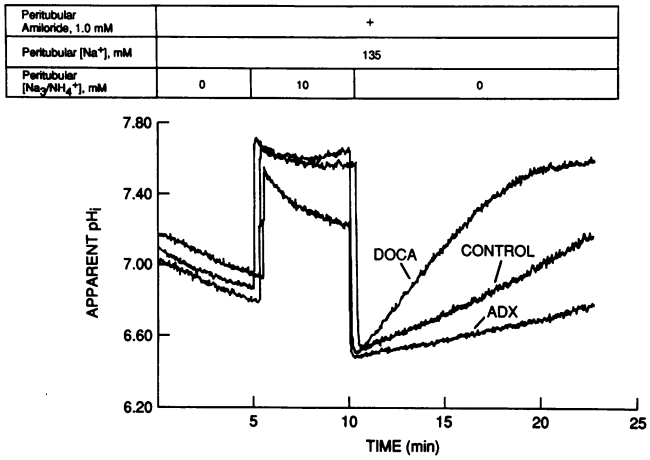

Figure 1. Assay for apical membrane H^+ pump activity. Na^+-independent cell pH recovery after an acid load in the presence of peritubular Na^+ plus amiloride. Before the experimental tracing shown above, tubules were initially bathed for 15 min with a CO_2/HCO_3^--free solution (pH 7.4, solution 2, Table I) and luminally perfused with a Na^+-free, CO_2/HCO_3^--free solution (pH 7.4, solution 3, Table I). At the beginning of the experimental tracing above, 1 mM amiloride was then added to the peritubular solution and continued throughout the remainder of the experiment. After 5 min exposure to this solution, the peritubular solution was changed to an identical solution containing 10 mM NH_4Cl (pH 7.4, solution 4, Table I) for 4–5 min before changing back to an identical solution without NH_4Cl (solution 3).

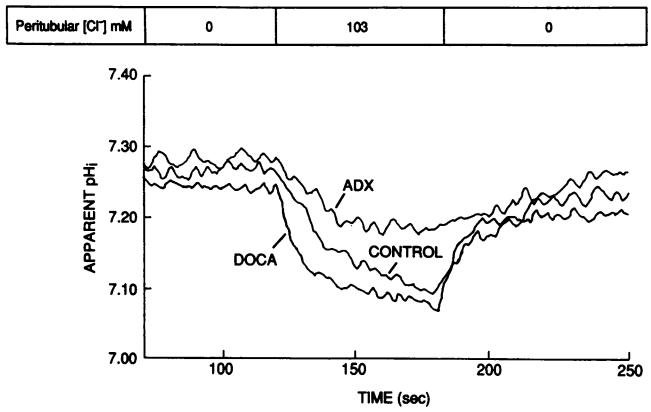

Figure 3. Assay for basolateral membrane Cl^-/HCO_3^- exchange activity: peritubular Cl^- addition-induced acidification. Tubules were initially bathed with a control solution containing 125.2 mM Cl^- and 25 mM HCO_3^- (pH 7.4, solution 1, Table I) and luminally perfused with a Cl^--free, 25 mM HCO_3^- solution (pH 7.4, solution 5, Table I). The peritubular solution was then changed to a Cl^--free, 25 mM HCO_3^- solution (solution 5) for 15 min and continued for the first 1–2 min of the experimental tracing above. Cl^- was then added to the peritubular solution (pH 7.4, solution 6, Table I) for 1 min before switching back the Cl^--free solution (solution 5).

mm · $^{-1}$min^{-1}, $P < 0.05$). Thus, chronic in vivo DOCA treatment leads to a stimulation of the apical membrane H^+ pump.

Basolateral membrane Cl^-/HCO_3^- exchange activity. Basolateral membrane Cl^-/HCO_3^- exchange activity was assessed as the effect of peritubular Cl^- addition on pH_i. Shown in Fig. 3 is a typical experimental tracing. Tubules were bathed and luminally perfused with a Cl^--free solution containing 25 mM HCO_3^- (pH 7.4, solution 5, Table I) for 15 min before experimentation. The peritubular solution was then changed to an identical solution containing 103 mM Cl^- (pH 7.4, solution 6, Table I) for 1 min before changing back to the Cl^--free solution (solution 5). The initial rate of cell acidification after peritubular Cl^- addition (Fig. 3) was used as an index of basolateral membrane Cl^-/HCO_3^- exchange activity. Shown in Fig. 4 are the results of these studies. In 20 chronic DOCA tubules, J_H was increased 73% when compared to 18 control tubules (control, 43.45±6.34 pmol · mm^{-1} · min^{-1}; DOCA, 75.24±10.84 pmol · mm^{-1} · min^{-1}, $P < 0.02$). Thus, chronic in vivo DOCA treatment leads to a stimulation of the basolateral membrane Cl^-/HCO_3^- exchanger.

To assure that the assay was measuring a DOCA-induced increase in basolateral membrane Cl^-/HCO_3^- exchange activity, similar experiments were performed in chronic DOCA tubules in the absence and presence of the disulfonic stilbene inhibitor of Cl^-/HCO_3^- exchange activity, DIDS. After initially determining the rate of peritubular Cl^--induced cell acidification in the absence of peritubular DIDS, the peritubular solution was changed to a Cl^--free solution containing 100 μM peritubular DIDS for 6–8 min. The rate of peritubular Cl^--induced cell acidification was then reexamined. In seven chronic DOCA tubules, the rate of cell acidification induced by peritubular Cl^- addition was inhibited 96% in the presence of peritu-

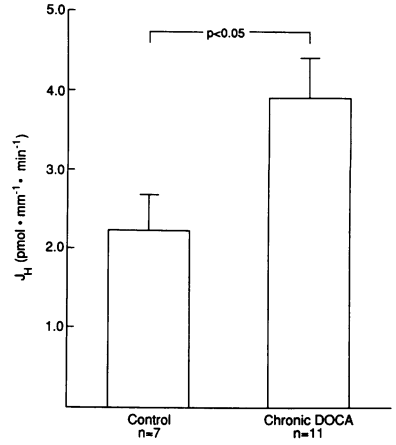

Figure 2. Effect of chronic DOCA treatment on apical membrane H^+ pump activity. Mean J_H±SEM for control and chronic DOCA tubules is shown.

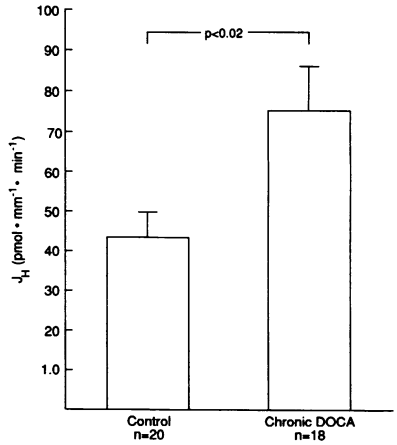

Figure 4. Effect of chronic DOCA treatment on basolateral membrane Cl^-/HCO_3^- exchange activity. Mean J_H±SEM of control and chronic DOCA tubules is shown.

bular DIDS (baseline 69.18±23.84 pmol·mm^{-1}·min^{-1}; DIDS 2.69±1.24 pmol·mm^{-1}·min^{-1}, $P < 0.05$). The 96% inhibition of peritubular Cl^--induced acidification by peritubular DIDS was similar to the 97% inhibition found in control tubules (n = 3, baseline 34.47±0.51 pmol·mm^{-1}·min^{-1}; DIDS 1.06±1.31 pmol·mm^{-1}·min^{-1}, $P < 0.01$). These studies indicate that the cell acidification that occurred upon peritubular Cl^- addition in the DOCA tubules was predominantly due to an increase in basolateral membrane Cl^-/HCO_3^- exchange activity.

To further examine the nature of this DOCA-induced increase in basolateral membrane Cl^-/HCO_3^- exchange activity, the apparent $K_{1/2}$ for peritubular Cl^- of the exchanger was determined in similar studies. Tubules were again bathed and luminally perfused in a Cl^--free solution containing 25 mM HCO_3^- (solution 5) for 15 min before experimentation. Peritubular additions of 10, 20, 40 and 103 mM Cl^- (solutions 7, 8, 9, and 6, respectively) were examined. The order of the Cl^- additions was varied from tubule to tubule. The kinetics of this transporter were determined from the rate of change of J_H in response to peritubular Cl^- addition. When more than one measurement was made with the same Cl^- concentration on a tubule, these were averaged to provide a result for that tubule. The initial acidification rate (J_H) in eight DOCA-treated tubules with addition of 10 mM Cl^- was 15.48±1.99 pmol·mm^{-1}·min^{-1}; 20 mM, 27.70±3.21 pmol·mm^{-1}·min^{-1}; 40 mM, 44.17±3.77 pmol·mm^{-1}·min^{-1}; and 103 mM, 80.65±9.19 pmol·mm^{-1}·min^{-1}. The initial acidification rate (J_H) in 11 control tubules with addition of 10 mM Cl^- was 10.22±2.28 pmol·mm^{-1}·min^{-1}; 20 mM, 17.05±3.5 pmol·mm^{-1}·min^{-1}; 40 mM, 26.78±4.34 pmol·mm^{-1}·min^{-1}; and 103 mM, 47.28±7.41 pmol·mm^{-1}·min^{-1}. Shown in Fig. 5 is the Eadie-Hofstee transformation of this data. There was no significant difference between the two groups in the $K_{1/2}$ values (control, 67.72±16.00 mM; DOCA 75.29±12.85 mM, P = NS), whereas a significant difference in the Vmax values was seen (control 72.69±15.73 pmol·mm^{-1}·min^{-1}; DOCA 132.31±22.49 pmol·mm^{-1}·min^{-1}, $P < 0.02$). Thus, chronic DOCA treatment leads to no change in the $K_{1/2}$, but increases the Vmax of the basolateral membrane Cl^-/HCO_3^- exchanger.

Basolateral membrane Na^+/H^+ antiporter activity. Basolateral membrane Na^+/H^+ antiporter activity was assessed as the Na^+-dependent pH_i recovery from an acid load. Shown in Fig. 6 is a typical experimental tracing. Tubules were initially bathed in a HCO_3^--free solution containing 135 mM Na^+ (pH 7.4, solution 2, Table I) and luminally perfused with a Na^+-free, HCO_3^--free solution (pH 7.4, solution 3, Table I) for 15 min before experimentation. The peritubular solution was then changed to a Na^+-free solution containing 10 mM NH_4Cl (pH 7.4, solution 10, Table I) for 4–5 min. The peritubular solution was then changed to an identical solution without the NH_4Cl (pH 7.4, solution 3, Table I) for 1 min before changing to an identical solution containing 135 mM Na^+ (solution 2). The initial rate of pH_i recovery from the induced acid load in the presence of Na^+ was used as an index of basolateral membrane Na^+/H^+ antiporter activity (Fig. 6). In addition, the initial rate of pH_i recovery for 1 min in the absence of Na^+ (Fig. 6) is an index of Na^+-independent H^+ extrusion (apical membrane H^+ pump activity). In 11 DOCA-treated tubules, the initial Na^+-independent pH_i recovery rate (J_H) from an acid load was 71% increased in DOCA-treated tubules when compared to 10 control tubules (control 1.08±0.20 pmol·mm^{-1}·min^{-1}; DOCA 1.85±0.26 pmol·mm^{-1}·min^{-1}, $P < 0.05$), thus confirming our previous findings on the apical membrane H^+ pump above. Previously, we have found that the lower rate of apical membrane H^+ pump activity determined by this method is due to inhibition of the H^+ pump by a peritubular Na^+ removal-induced sustained increase in cell calcium (22).

However, as shown in Fig. 7, Na^+-dependent pH_i recovery from an acid load was not significantly different between the two groups (control 28.66±6.73 pmol·mm^{-1}·min^{-1}; DOCA 40.98±6.31 pmol·mm^{-1}·min^{-1}, P = NS). For chronic DOCA treatment to enhance transepithelial HCO_3^- absorption, one would expect to see an inhibition in basolateral membrane Na^+/H^+ antiporter activity, but in fact if anything, there was a tendency for an increase in Na^+/H^+ antiporter activity. Thus, it is probable that changes in Na^+/H^+ antiporter activity

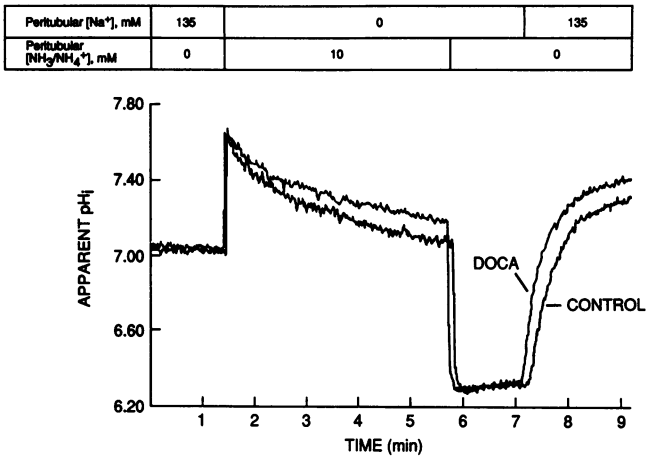

Figure 6. Assay for basolateral membrane Na^+/H^+ antiporter activity: Na^+-dependent pH_i recovery from an acid load. Tubules were initially bathed with a control solution containing 145 mM Na^+ and 25 mM HCO_3^- (pH 7.4, solution 1, Table I) and luminally perfused with a Na^+-free, CO_2/HCO_3^--free solution (pH 7.4, solution 3, Table I). The peritubular solution was then changed to CO_2/HCO_3^--free solution containing 135 mM Na^+ (pH 7.4, solution 2, Table I) for 15 min and continued for the first 1–2 min of the experimental tracing above. The peritubular solution was then changed to a Na^+-free, CO_2/HCO_3^--free solution containing 10 mM NH_4Cl (pH 7.4, solution 10, Table I). After 3–4 min, the peritubular solution was changed to an identical solution without NH_4Cl (pH 7.4, solution 3, Table I). After 1 min, the peritubular solution was changed to an identical solution containing 135 mM Na^+.

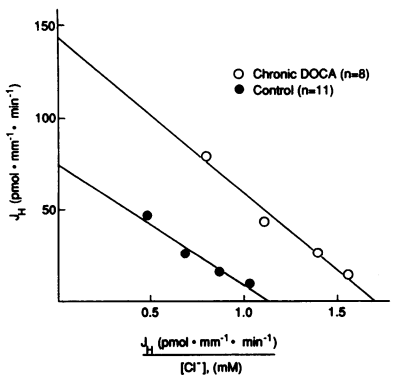

Figure 5. Effect of chronic DOCA treatment on the acidification rate as a function of peritubular [Cl^-]: Eadie-Hofstee transformation.

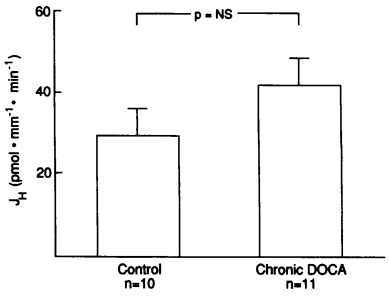

Figure 7. Effect of chronic DOCA treatment on basolateral membrane Na^+/H^+ antiporter activity. Mean J_H±SEM for control and chronic DOCA tubules is shown.

do not contribute to the increased $OMCD_i$ transepithelial HCO_3^- absorption seen with chronic DOCA treatment.

Chronic adrenalectomy

To determine whether mineralocorticoid deficiency also results in parallel modulation of apical and basolateral membrane $H^+/OH^-/HCO_3^-$ transporters, we next examined the effects of chronic ADX. Shown in Table III is a comparison of baseline pH_i, cell volume, and buffer capacities obtained in $OMCD_i$'s harvested from ADX and control animals. Baseline pH_i values obtained in the presence of 25 mM HCO_3^- and 40 mm Hg pCO_2 (pH 7.4, solution 1, Table I) in 37 tubules from ADX rabbits was 6.98±0.02, which was not significantly different from the baseline pH_i values obtained in 18 tubules from control rabbits (7.02±0.03, P = NS). In addition, ADX had no effect on cellular volume (control, 6.45±0.22 · 10^{-10} liter/mm, n = 17; ADX 6.55±.18 · 10^{-10} liter/mm, n = 41, P = NS). There was no significant difference in buffer capacities between the two groups determined by either NH_3/NH_4^+ addition or withdrawal (Table III).

Apical membrane H^+ pump activity. Apical membrane H^+ pump activity was assessed as the Na^+-independent pH_i recovery from an acid load. Shown in Fig. 1 is a typical experimental tracing. Shown in Fig. 8 are the results of this study. ADX led to a 56% reduction in apical membrane H^+ pump activity when compared to control tubules (control, n = 6, 1.47±0.21 pmol · mm^{-1} · min^{-1}; ADX, n = 9, 0.65±0.07 pmol · mm^{-1} · min^{-1}, $P < 0.01$).

Table III. Baseline Cell pH and Cellular Volume in Chronic ADX Tubules

	Control	Chronic ADX
Baseline cell pH	7.02±0.03	6.98±0.02
	n = 18	n = 37
Cell vol (10^{-10} liter/mm)	6.45±0.22	6.55±0.18
	n = 17	n = 41
Buffer capacity (mmol · liter^{-1} · pH unit^{-1})		
(H^+ pump studies)*	25.07±2.09	26.23±1.95
	n = 7	n = 9
(Cl^-/HCO_3^- exchanger studies)‡	29.80±1.40	29.50±1.29
	n = 7	n = 26

Values are means±SEM. n = number of tubules. * Buffer capacity was determined by NH_3/NH_4^+ withdrawal. ‡ Buffer capacity was the sum of the mean $B_{non\text{-}CO_2/HCO_3^-}$ value obtained from NH_3/NH_4^+ addition in the H^+ pump studies plus the B_{CO_2/HCO_3^-} for each tubule in the Cl^-/HCO_3^- exchanger studies.

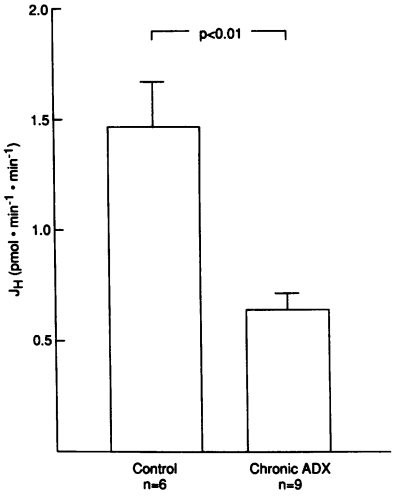

Figure 8. Effect of chronic adrenalectomy on apical membrane H^+ pump activity. Mean J_H±SEM for control and chronic adrenalectomy tubules is shown.

Basolateral membrane Cl^-/HCO_3^- exchange activity. Basolateral membrane Cl^-/HCO_3^- exchange activity was assessed as the effect of peritubular Cl^- addition on pH_i, as shown in Fig. 3. Shown in Fig. 9 are the results of these studies. ADX led to a 44% reduction in basolateral membrane Cl^-/HCO_3^- exchange activity when compared to control tubules (control, n = 7, 43.95±6.78 pmol · mm^{-1} · min^{-1}; ADX, n = 26, 24.60±3.83 pmol · mm^{-1} · min^{-1}, $P < 0.05$).

Discussion

Mineralocorticoid hormone receptors are present in all cells of the $OMCD_i$ (23). There is no net transport of Na^+ or K^+ in this segment under normal conditions and chronic DOCA treatment has no effect on the transport of these two ions in this segment (24). Although mineralocorticoid hormones can influence acidification through such factors as volume status, potassium balance, ammoniagenesis, and distal delivery of nonbicarbonate buffer, Hulter et al. (1, 2) found that mineralocorticoids can independently modulate distal nephron acidification. In addition, Stone et al. (3) found that chronic minera-

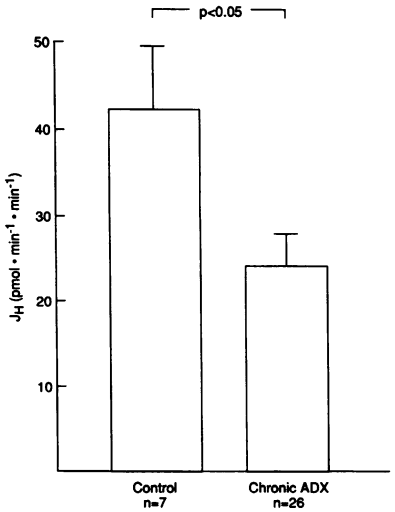

Figure 9. Effect of chronic adrenalectomy on basolateral membrane Cl^-/HCO_3^- exchange activity. Mean J_H±SEM for control and chronic adrenalectomy tubules is shown.

locorticoid treatment of rabbits in vivo increased, and chronic adrenalectomy decreased net H^+ secretion in the $OMCD_i$ perfused in vitro.

Apical membrane H^+ pump. We found a 71–76% stimulation of apical membrane H^+ pump activity by two different apical membrane H^+ pump assays after chronic DOCA treatment, and a 56% decrease in H^+ pump activity after adrenalectomy. The DOCA results are of a similar magnitude to the increase in net transepithelial HCO_3^- absorption found by Stone et al. (3) in $OMCD_i$'s dissected from rabbits chronically treated with DOCA. In addition, our ADX results are of a similar magnitude to the 40% reduction in net transepithelial HCO_3^- absorption we found previously (8). Of note, the measured H^+ flux (J_H) across the apical membrane in our studies was less than rates of net transepithelial H^+ secretion found in control and DOCA tubules perfused in vitro (3, 8). The difference may be a result of the nominal presence of ambient CO_2/HCO_3^- in our studies that was removed to decrease any contribution to pH_i by the Cl^-/HCO_3^- exchanger (9). Jacobsen (25) has found that decreasing peritubular CO_2 from 40 mmHg to 10–14 mmHg results in a 52% reduction in net transepithelial H^+ secretion. Thus, in our experiments J_H may have been less than predicted from flux studies due to the nominal presence of CO_2/HCO_3^-.

Enzymatic studies of rat medullary collecting ducts by Mujais (4) and rabbit medullary collecting ducts by Garg and Narang (5) found a twofold increase in NEM-sensitive ATPase activity after chronic aldosterone treatment of either normal rats or adrenalectomized rabbits, respectively. In addition, Khadouri et al. (7) found almost a 65% reduction in medullary collecting duct NEM-sensitive ATPase activity in adrenalectomized rats. These results agree with our transport measurements.

With chronic DOCA treatment, enhanced cortical collecting duct K^+ secretion may lead to a state of relative K^+ deficiency. Recently, Wingo (26) found in K^+-deficient rabbits that 100 μM luminal omeprazole inhibited both net HCO_3^- and K^+ absorption in the $OMCD_i$ suggesting the presence of an H^+-K^+-ATPase on the apical membrane under these conditions. Previously, we have found that chronic DOCA treatment does not significantly alter rabbit muscle K^+ content (unpublished observations) and recently, Garg (27) found no increase in H^+-K^+-ATPase activity in the rabbit medullary collecting duct after chronic DOCA treatment. This is in contrast to the marked elevation of H^+-K^+-ATPase activity they found in the medullary collecting duct under conditions of K^+ deficiency (28). Thus, Na^+-independent pH_i recovery from an induced acid load under conditions of mineralocorticoid excess most likely represents a vacuolar H^+ pump.

Basolateral membrane Cl^-/HCO_3^--exchanger. In our studies, we found that chronic DOCA treatment led to a 73% stimulation of basolateral membrane Cl^-/HCO_3^- exchange activity, while adrenalectomy led to a 44% decrease in basolateral membrane Cl^-/HCO_3^- exchange activity. In further studies, we found that chronic DOCA treatment led to no change in the apparent $K_{1/2}$ for Cl^-, while the Vmax was increased. These results are suggestive that chronic DOCA treatment increases the number of active Cl^-/HCO_3^- exchangers present.

Therefore, for both the apical membrane H^+ pump and basolateral membrane Cl^-/HCO_3^- exchanger, mineralocorticoid excess and deficiency increases and decreases, respectively, the activity of these two $H^+/OH^-/HCO_3^-$ transporters in the $OMCD_i$. These effects occurred to a degree comparable to the changes found in $OMCD_i$ net transepithelial HCO_3^- absorption under similar conditions of mineralocorticoid excess and deficiency.

Aldosterone mechanism of action. One possible explanation for our results is an aldosterone-induced increase in H^+ pump and Cl^-/HCO_3^- exchanger number. Aldosterone has been shown to increase Na^+-K^+-ATPase pump number in the cortical collecting duct (20, 29–31) by: (*a*) acutely stimulating exocytosis of Na^+-K^+-ATPase pumps from a latent pool of pumps already present within the cell (20, 31); (*b*) stimulating Na^+-K^+-ATPase mRNA and protein biosynthesis (32); and, (*c*) stimulating Na^+-K^+-ATPase α and B subunit gene transcription (33). Thus, it is possible that aldosterone has a similar mechanism of action in the $OMCD_i$ to increase H^+ pump and Cl^-/HCO_3^- exchanger number.

Khadouri et al. (7) found NEM-sensitive H^+-ATPase activity from ADX rats to increase threefold after acute (2 h) aldosterone exposure. In addition, the aldosterone-induced increase in NEM-sensitive ATPase activity was prevented by inhibitors of mRNA and protein synthesis, actinomycin D and cycloheximide, respectively. The early sensitivity to cycloheximide and actinomycin D suggests a requirement for protein synthesis, but the synthesized protein need not be the H^+ pump, but may rather be a regulatory protein.

One interesting observation in our studies was the disparity in alterations of cell volume and $H^+/OH^-/HCO_3^-$ transport activity seen with chronic DOCA treatment and ADX. Previously, in the proximal tubule, conditions associated with hypertrophy (chronic metabolic acidosis, chronic K^+ deficiency, and chronic hyperfiltration) have been found to lead to a parallel increase in apical and basolateral membrane $H^+/OH^-/HCO_3^-$ transport processes (34). In our studies, chronic DOCA treatment resulted in similar findings with an increase in cell volume and a parallel increase in apical membrane H^+ pump and basolateral membrane Cl^-/HCO_3^- activities. However, with ADX, decreases in $H^+/OH^-/HCO_3^-$ transport activity were found without detectable changes in cell volume. These observations suggest a possible disparity between the modulation of $H^+/OH^-/HCO_3^-$ transport processes and cell volume under conditions where $H^+/OH^-/HCO_3^-$ transport processes are decreased in parallel. The mechanism(s) of a DOCA-induced increase in cell volume and $H^+/OH^-/HCO_3^-$ transport processes and a ADX-induced decrease in $H^+/OH^-/HCO_3^-$ transport processes without a change in cell volume remain to be elucidated.

Parallel modulation of apical and basolateral membrane $H^+/OH^-/HCO_3^-$ transporters. Parallel modulation of apical and basolateral membrane $H^+/OH^-/HCO_3^-$ transporters in response to a variety of conditions has been extensively documented in the proximal tubule. Parallel adaptive increases in apical membrane Na^+/H^+ antiporter activity and basolateral membrane $Na^+/3HCO_3^-$ cotransporter activity have been found under the chronic conditions of metabolic acidosis (35, 36) and alkalosis (35), respiratory acidosis (37, 38) and alkalosis (37), hyperfiltration (39), and K^+ depletion (40). In addition, acute exposure of the in vitro microperfused proximal tubule to angiotensin II has been found to increase in parallel the activities of these two transporters (41). Under the conditions of chronic mineralocorticoid excess or deficiency, we found no difference in baseline pH_i measured under identical conditions between experimental and control tubules. Chronic DOCA treatment caused a parallel increase in apical mem-

brane H^+ pump activity (76%) and basolateral membrane Cl^-/HCO_3^- exchange activity (73%). Similarly, chronic adrenalectomy resulted in a parallel decrease in apical membrane H^+ pump activity (56%) and basolateral membrane Cl^-/HCO_3^- exchange activity (44%). Thus, parallel regulation of both apical and basolateral membrane $H^+/OH^-/HCO_3^-$ transporters may be a common mechanism to alter net transepithelial transport of HCO_3^- across the nephron without deleterious effects of prolonged alterations in pH_i.

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