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D K Stone, D W Seldin, J P Kokko, H R Jacobson

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Research Article

Rabbit medullary collecting duct (MCD) from inner stripe of outer medulla has been identified as a major distal nephron acidification site. The isolated, perfused tubule technique was used to examine the roles of mineralocorticoid and glucocorticoid in regulation of MCD acidification. Surgical adrenalectomy reduced bicarbonate reabsorptive rate (JHCO3, pmol X mm-1 X min-1) from the normal of 9.79 +/- 1.21 to 0.67 +/- 1.1. Chronic administration of deoxycorticosterone acetate (DOCA) increased JHCO3 of MCD significantly to 18.02 +/- 1.62 whereas chronic dexamethasone administration did not affect JHCO3. The direct effects of aldosterone and dexamethasone upon MCD acidification were examined by perfusing tubules harvested from adrenalectomized rabbits in the presence of aldosterone or dexamethasone. Aldosterone, at 5 X 10(-8) M, increased JHCO3 significantly from 1.27 +/- 0.28 to 3.09 +/- 0.34. At 10(-6) M, aldosterone produced a greater increase in JHCO3 from 0.67 +/- 1.1 to 9.39 +/- 1.59. In vitro dexamethasone treatment had no effect on JHCO3. Studies examining the sodium dependence of aldosterone-stimulated acidification demonstrated that JHCO3 in tubules harvested from normal and deoxycorticosterone acetate-treated animals was unaffected by total replacement of sodium with tetramethylammonium. Likewise, luminal amiloride (5 X 10(-5) M) had no effect on JHCO3 in tubules harvested from adrenalectomized and normal animals. Moreover, the acute, in vitro stimulatory effect of aldosterone was seen to occur in […]

Mineralocorticoid Modulation of Rabbit Medullary Collecting Duct Acidification

A SODIUM-INDEPENDENT EFFECT

DENNIS K. STONE, DONALD W. SELDIN, JUHA P. KOKKO, and HARRY R. JACOBSON,
Department of Internal Medicine, University of Texas Health Science Center, Southwestern Medical School at Dallas, Texas 75235

ABSTRACT Rabbit medullary collecting duct (MCD) from inner stripe of outer medulla has been identified as a major distal nephron acidification site. The isolated, perfused tubule technique was used to examine the roles of mineralocorticoid and glucocorticoid in regulation of MCD acidification. Surgical adrenalectomy reduced bicarbonate reabsorptive rate ($JHCO_3$, $pmol \cdot mm^{-1} \cdot min^{-1}$) from the normal of 9.79±1.21 to 0.67±1.1. Chronic administration of deoxycorticosterone acetate (DOCA) increased $JHCO_3$ of MCD significantly to 18.02±1.62 whereas chronic dexamethasone administration did not affect $JHCO_3$. The direct effects of aldosterone and dexamethasone upon MCD acidification were examined by perfusing tubules harvested from adrenalectomized rabbits in the presence of aldosterone or dexamethasone. Aldosterone, at 5×10^{-8} M, increased $JHCO_3$ significantly from 1.27±0.28 to 3.09±0.34. At 10^{-6} M, aldosterone produced a greater increase in $JHCO_3$ from 0.67±1.1 to 9.39±1.59. In vitro dexamethasone treatment had no effect on $JHCO_3$. Studies examining the sodium dependence of aldosterone-stimulated acidification demonstrated that $JHCO_3$ in tubules harvested from normal and deoxycorticosterone acetate-treated animals was unaffected by total replacement of sodium with tetramethylammonium. Likewise, luminal amiloride (5×10^{-5} M) had no effect on $JHCO_3$ in tubules harvested from adrenalectomized and normal animals. Moreover, the acute, in vitro stimulatory effect of aldosterone was seen to occur in the presence of luminal amiloride. These studies define a mammalian distal nephron segment that possesses major acidifying capacity, which is modulated by mineralocorticoid but independent of luminal sodium.

This work was presented, in part, in 1981, before the American Society of Nephrology, Wash., DC, and before the National Meeting of the American Federation of Clinical Research, Wash., DC.

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INTRODUCTION

A role for the adrenal cortex in the regulation of acid-base homeostasis has been apparent from both clinical and experimental observations (1–9). Several extrarenal factors, such as changes in volume status, alteration of potassium balance, and changes in filtered nonbicarbonate buffer have been shown to affect the renal response to alterations in plasma levels of adrenal steroids. Some studies, however, have suggested a more direct intrarenal role for steroids in stimulating urine acidification and net acid excretion (4–8). Recent balance studies in the adrenalectomized rat (8) demonstrated separate roles for both mineralocorticoids and glucocorticoids in renal acid excretion. Mineralocorticoids were found to promote urine acidification, i.e., increase the maximal urine-to-blood pH gradient, while glucocorticoids promoted net acid excretion by increasing excretion of phosphate and ammonia buffers (8).

Observations such as those cited above, coupled with the demonstration that urinary acidification in anuran bladders proceeds by a mechanism that is molecularly independent of sodium yet stimulated by aldosterone (10) and dexamethasone (11), raise the possibility that a sodium-independent steroid responsive acidifying segment (or segments) exists in the mammalian kidney.

Using in vitro microperfusion, we have recently demonstrated that the medullary collecting duct

(MCD)[1] from inner stripe of outer medulla is a quantitatively major site of distal nephron acidification in the rabbit (12). Additional characterization of the rabbit MCD reveals that this nephron segment always bears a spontaneously lumen-positive transepithelial voltage when perfused and bathed in symmetrical HCO_3 Ringer's solutions (12, 13). Under similar in vitro circumstances the MCD has been shown to demonstrate little or no net transport of Na or K, even after long-term administration of deoxycorticosterone acetate (DOCA) to the rabbits from which tubules are harvested for in vitro study (13). These features of the MCD are in contrast to those of the cortical collecting tubule (CCT) which, when perfused in vitro under similar circumstances as the MCD, usually displays a spontaneously lumen-negative voltage, demonstrates quantitatively large amounts of net sodium reabsorption and potassium secretion, and possesses significantly less bicarbonate reabsorptive capacity than the MCD (12–18).

It is well established in the in vitro perfused CCT that the magnitude of the lumen-negative voltage as well as the net absorptive and secretory rates of Na and K, respectively, are dependent on the mineralocorticoid hormone status of the animal from which the tubules are harvested (15–17). More recent studies of rabbit CCT perfused in vitro (19) have demonstrated Na^+ (voltage)-dependent luminal fluid acidification that is stimulated by 14–21 d of DOCA (5 mg/d) administration. In addition, a component of DOCA-stimulated, sodium-independent acidification was also observed in the rabbit CCT (19). However, neither the magnitude nor the specificity of the steroid effect (mineralocorticoid vs. glucocorticoid) was determined. Because of its greater capacity for HCO_3 reabsorption and its apparent lack of Na reabsorption or K secretion, the rabbit MCD seemed to be an ideal segment for in vitro microperfusion studies designed to (*a*) define the roles of mineralocorticoid and glucocorticoid in distal nephron acidification and (*b*) define the sodium dependence or independence of these effects.

METHODS

In vitro microperfusion

The technique of in vitro microperfusion was utilized as previously described (20). MCD was dissected from inner stripe of outer renal medulla of female New Zealand White rabbits. "A" solution (see below) made hypertonic (450 mosmol) by addition of NaCl was utilized as a dissection medium to facilitate tubule harvest. Tubules thus dissected were transferred to a lucite-perfusion chamber and were cannulated and perfused utilizing concentric glass pipettes. Electrical and hydraulic insulation was effected by use of Sylgard 184 (Dow Corning Corp., Midland, MI). Standard sodium containing glass was utilized for all experiments except those in which zero-Na medium (see below) was used. In these cases, the perfusion pipettes were forged from a low-sodium glass that was incubated in concentrated nitric acid for 1 wk before use, and then washed thoroughly with acetone and glass-distilled H_2O. To test sodium leach from this glass, samples were pulverized, mixed with glass-distilled H_2O, repeatedly vortexed and incubated for 24 h. The supernatant, as assayed by atomic absorption, was Na free.

Standard A solution, utilized as bath and perfusate in most protocols, was comprised as follows (mM): NaCl 115; KCl 5; $NaHCO_3$ 25; NaH_2PO_4 2.3; Na acetate 10; $MgSO_4$ 1; glucose 8; alanine 5; $CaCl_2$ 1.8. All solutions were equilibrated at 37°C with 5% CO_2, n95% O_2 to achieve a final pH of 7.4, and P_{CO_2} of 40 mmHg. Filtered fetal calf serum (Gibco Laboratories, Grand island, NY) was added to bath 5% vol/vol. In zero sodium experiments, bath and perfusate were composed as follows (mM): tetramethyl/ammonium (TMA) Cl hydroxide 115 (Alfa Products, Danvers, MA); KCl 2.7; $TMAHCO_3$ 25 (made from tetramethylammonium hydroxide [Sigma Chemical Co., St. Louis, MO] and CO_2 gas); KH_2PO_4 2.3; Ca^{++} acetate 1.8; $MgSO_4$ 1, glucose 8, alanine 5. No fetal calf serum was utilized in zero Na experiments. Zero Na solution was assayed by atomic absorption and was found to have [Na] $< 1 \times 10^{-7}$ M. All experiments were conducted at 37°C with bath continuously changed at a rate of 0.5 ml/min.

Tritiated inulin (exhaustively dialyzed) was used as a volume marker. No tubule exhibiting net volume flux $J_v > 0.05$ nl/min was utilized. J_v was determined by the following formula: $J_v = (Vi) - (Vo)/L$, where J_v = rate of net volume flux in nanoliters per minute; Vi = perfusion rate in nanoliters per minute; Vo = collection rate in nanoliters per minute; L = tubular length in millimeters.

Transepithelial voltage (V_T) was measured by means of an agarose-Ringer's solution-calomel electrode series, as described previously (21). In zero Na experiments, zero Na solution was substituted for Ringer's solution in the agarose bridges. The bicarbonate reabsorptive rate, $JHCO_3$ was determined by microcalorimetry (22).

Adrenalectomy

Female New Zealand White rabbits were surgically adrenalectomized as previously described (23). No accessory adrenal tissue was seen postsurgically, and corticosterone levels assayed by radioimmunoassay 10 d after surgery averaged 21 ng/dl, as opposed to levels in normal rabbits previously determined to be 2,501±491 mg/dl (23). All adrenalectomized animals were studied 10 d to 4 wk postsurgery. Animals were maintained on normal chow with saline drinking water.

DOCA and dexamethasone treatment

Normal rabbits, utilized to study the in vivo effects of chronic mineralocorticoid or glucocorticoid excess, received either DOCA (Organon, Inc., Atlanta, GA) 5 mg i.m. twice daily for 14 d or dexamethasone (Elkins-Sinn, Inc., Cherry Hill, NJ) 5 mg i.m. twice daily for 14 d.

[1] *Abbreviations used in this paper:* CCT, cortical collecting tubule; DOCA, deoxycorticosterone acetate; $JHCO_3$, bicarbonate reabsorption rate; J_v, rate of net volume flux, nl/min; L, tubular length, mm; MCD, medullary collecting duct; TMA, tetramethylammonium; Vi, perfusion rate, nl/min; V_O, collection rate, nl/min; V_T, transepithelial voltage.

Protocols

In vivo effects of mineralocorticoid and glucocorticoid. Tubules were harvested from adrenalectomized, normal, DOCA-treated, or dexamethasone-treated animals and perfused in vitro with measurement of $JHCO_3$ and V_T.

In vitro effects of mineralocorticoid and glucocorticoid. Tubules were harvested from adrenalectomized animals and perfused in vitro. After 90 min of equilibration, $JHCO_3$ and V_T were measured. Aldosterone (5×10^{-8} M or 10^{-6} M) or dexamethasone (10^{-6} M) in ethanol (final bath [ethanol] = 0.025%) was added to bath and the tubules were incubated for 3 h, after which time, repeat $JHCO_3$ and V_T determinations were performed. As a time control, similar incubations were carried out in bath containing 0.025% ethanol without steroid hormones.

Sodium dependence of MCD acidification. Tubules harvested from normal and DOCA-treated rabbits were bathed and perfused in zero sodium medium with determination of $JHCO_3$ and V_T. The effects of amiloride upon acidification were determined by measurement of $JHCO_3$ and V_T before, and 30 min after, addition of 5×10^{-5} M amiloride to perfusate.

Finally the effect of amiloride upon aldosterone-stimulated $JHCO_3$ was examined by perfusing tubules from adrenalectomized rabbits with amiloride (5×10^{-5} M)-containing perfusate, and measuring $JHCO_3$ and V_T before and after addition of aldosterone to the bath.

RESULTS

In vivo effects of mineralocorticoids and glucocorticoids. The results of in vivo steroid manipulation upon $JHCO_3$ and V_T are illustrated in Fig. 1. In MCD from adrenalectomized animals, both $JHCO_3$ (0.67±1.1 pmol · mm^{-1} · min^{-1}) and V_T (2.7±0.5 mV) are seen to be significantly lower ($P < 0.01$) than values observed in tubules obtained from normal animals where $JHCO_3$ averaged 9.79±1.21 pmol · mm^{-1} · min^{-1} and V_T was 12.4±1.2 mV. In contrast, chronic DOCA administration imposed a significant ($P < 0.05$) increase in $JHCO_3$ (18.02±1.62 pmol · mm^{-1} · min^{-1}) compared with normals. Although V_T was higher (16.6±3.8 mV) in tubules from DOCA-treated animals as compared with normals, the change was not significant. Chronic dexamethasone treatment had no effect on either $JHCO_3$ (8.81±0.71 pmol · mm^{-1} · min^{-1}) or V_T (11±1.74 mV).

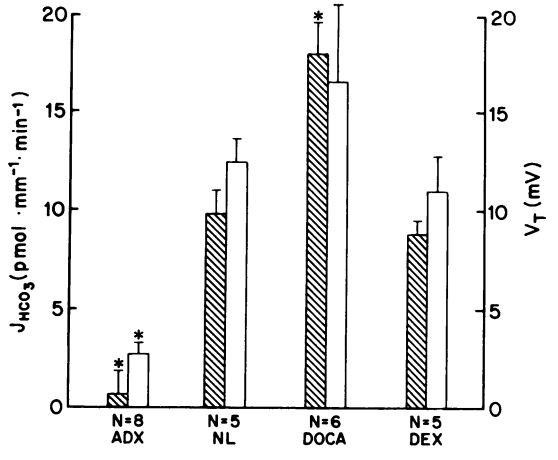

FIGURE 1 Effects of in vivo manipulations of mineralo- and glucocorticoid upon MCD $JHCO_3$ and V_T. Slashed bars (▨) represent $JHCO_3$; open bars (□) V_T. From left to right, $JHCO_3$ and V_T in tubules harvested from adrenalectomized animals (ADX), normal animals (NL), and animals that received 5 mg DOCA or dexamethasone (DEX) intramuscular twice daily for 14 d. *Refers to values significantly different from normal.

In vitro effects of aldosterone and dexamethasone. As illustrated in Fig. 2, both 5×10^{-8} M and 10^{-6} M bath aldosterone induced significant increases in $JHCO_3$ in experimental vs. control tubules. The aldosterone-stimulated increase is seen to be dose dependent, with 5×10^{-8} M aldosterone requiring a longer time (260 min) to achieve an increase in $JHCO_3$, which is lower than that achieved with 10^{-6} M aldosterone at 3 h. No increase in $JHCO_3$ was seen in either the time control group or in tubules incubated with 10^{-6} M dexamethasone.

In contrast to the effects of aldosterone on $JHCO_3$, acute changes were not evidenced in V_T. The V_T pre- and postincubation in tubules incubated with 5×10^{-8} M aldosterone (1.45±0.8 mV [control] vs. 1.0±0.6 mV [postaldosterone], P = NS) or 10^{-6} M aldosterone (2.7±0.5 mV prealdosterone; 2.9±0.5 mV after aldosterone) were not significantly different. Similarly, dexamethasone had no effect on V_T (2.28±0.6 mV, before dexamethasone; 1.0±0.4 mV after dexamethasone).

Na dependence of MCD acidification. Total bath and perfusate replacement of sodium with TMA had no effect upon either $JHCO_3$ or V_T in tubules harvested from normal and DOCA-treated animals, as shown by group comparison (Table I). Fig. 3 *A* illustrates the effects of 5×10^{-5} M luminal amiloride upon $JHCO_3$ in tubules from normal animals. Neither $JHCO_3$ (9.79±1.21 pmol · mm^{-1} · min^{-1}, preamiloride; 10.25±0.85 pmol · mm^{-1} · min^{-1} postamiloride P = NS) nor V_T (12.4±1.2 mV, preamiloride; 12.2±0.9 mV postamiloride, P = NS) were significantly affected. Likewise, addition of luminal amiloride had no effect on the acute, in vitro, stimulatory, effects of 10^{-6} M aldosterone (Fig. 3 *B*). Again, no change in V_T was seen despite the increase in $JHCO_3$ (V_T prealdosterone 1.1±0.6 mV; V_T postaldosterone 0.81±0.5 mV). By group comparison, neither $JHCO_3$ nor V_T of MCD from adrenalectomized rabbits was affected by the presence of luminal amiloride (Table I).

It is noteworthy that total sodium replacement, with the primary goal of maintenance of tubular integrity, was not easily achieved. Attempts were made to utilize choline and tetraethylammonium in substitution for

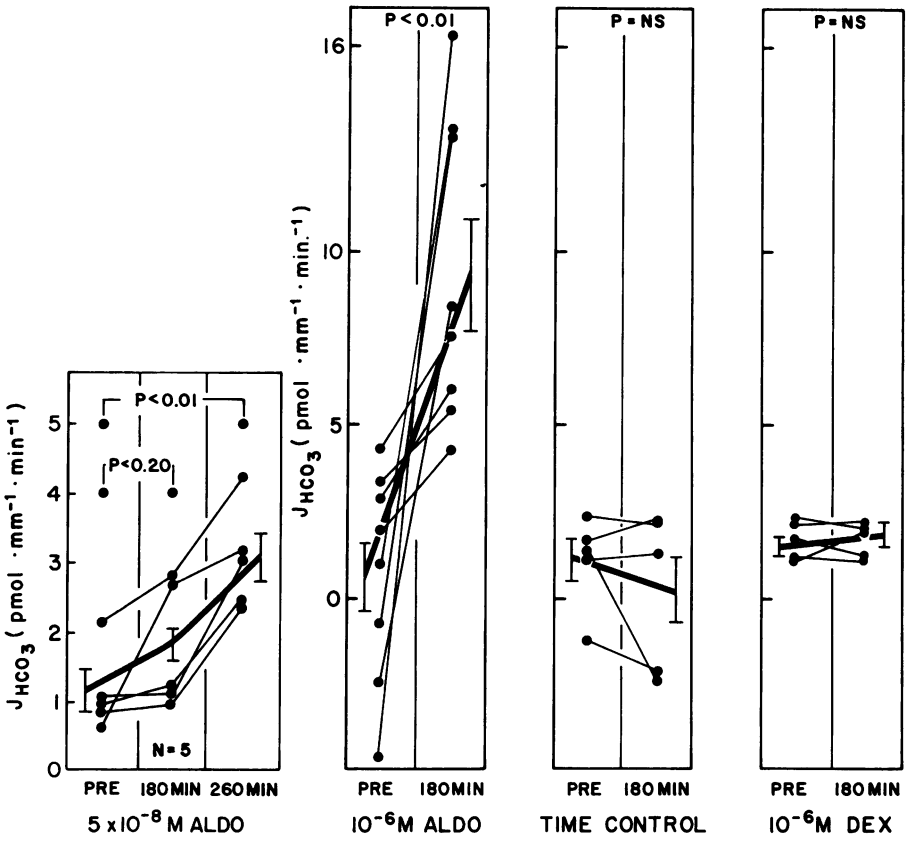

FIGURE 2 In vitro effects of aldosterone and dexamethasone on $JHCO_3$ in MCD harvested from adrenalectomized animals. Exhibited are $JHCO_3$ pre and 180 or 260 min after steroid addition.

sodium. Both cations induced swelling, granularity, and occasional desquamation of MCD cells. Although TMA did not cause morphologic changes during the study period, longer incubations did result in definite cellular deterioration, thus precluding use of TMA during the longer incubations required for the in vitro

TABLE I
Summary of the Effects of Amiloride and Sodium Removal on MCD $JHCO_3$ and V_T

Condition	n	$JHCO_3$	P	V_T	P
		pmol · mm^{-1} · min^{-1}		*mV*	
ADX	8	0.67±1.1		2.7±0.51	
ADX + AMIL	5	1.24±0.56	NS°	1.1±0.6	NS‡
Normal	5	9.79±1.21		12.4±1.2	
Normal post-AMIL		10.25±0.85	NS‡	12.2±0.9	NS‡
Normal TMA replacement of Na	5	9.20±0.94	NS‡	10.8±1.4	NS‡
DOCA	5	18.02±1.62	<0.05‡	16.6±3.8	NS‡
DOCA, TMA replacement of Na	5	16.61±1.10	NS§	13.8±1.2	NS§

ADX, adrenalectomized animals; AMIL, amiloride.
° Vs. ADX.
‡ Vs. normal.
§ Vs. DOCA.

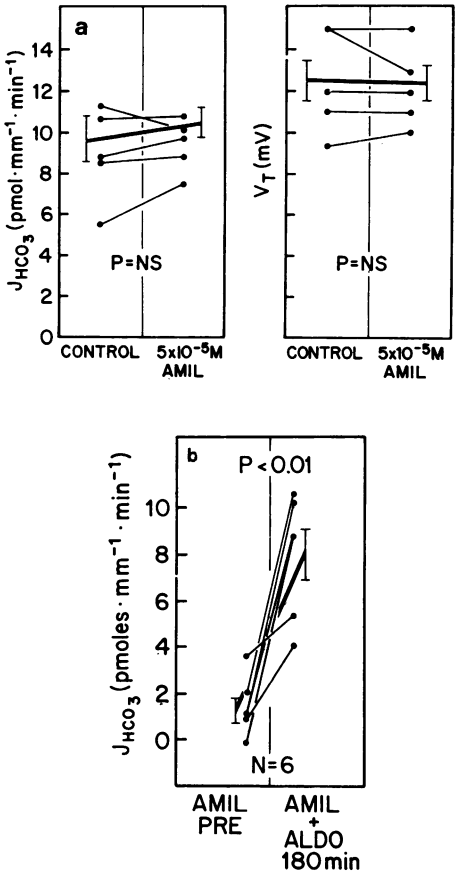

FIGURE 3 Effect of amiloride on $JHCO_3$ and V_T in MCD (*a*) $JHCO_3$ (*left*) and V_T (*right*) in normal MCD before and 30 min after addition of 5×10^{-5} M amiloride (AMIL) perfusate. (*b*) Effect of luminal amiloride (5×10^{-5} M) on aldosterone (ALDO) stimulation of $JHCO_3$ in tubules from adrenalectomized rabbits. Amiloride 5×10^{-5} M was present in perfusate throughout the experiments.

aldosterone effect. Similarly, it was not possible to study the effects of TMA vs. sodium in single tubules.

DISCUSSION

These studies demonstrate that MCD acidification is modulated by aldosterone through a sodium-independent mechanism. Adrenalectomy induces a sharp fall in $JHCO_3$ and lumen-positive voltage, a finding consistent with balance studies describing a reduced distal nephron acidifying capacity in the setting of mineralocorticoid deprivation (8). In contrast, chronic mineralocorticoid excess is shown to significantly increase the rate of MCD bicarbonate reabsorption, a finding in keeping with the demonstration of systemic metabolic alkalosis in the clinical setting of mineralocorticoid excess states (9). The present findings also reconcile the demonstration that MCD is a target site for aldosterone, as suggested in biochemical studies (24) and aldosterone receptor binding studies (25), with the finding that MCD has negligible mineralocorticoid inducible sodium reabsorptive capacity (13).

The observation that dexamethasone, either in vivo or in vitro, does not alter $JHCO_3$ in MCD is in keeping with the recent demonstration that glucocorticoids increase urinary acidification predominantly by means of events occurring proximal to the distal nephron, specifically, by increasing filtered phosphate delivery (8). The absence of a direct glucocorticoid effect in MCD is in distinction to the direct stimulatory effects of dexamethasone in turtle bladder acidification, where this glucocorticoid has been shown to be near equipotent with aldosterone in acutely stimulating acidification (10).

It is important to note that in contrast to CCT, where sodium reabsorption and the resultant production of a lumen-negative V_T have been shown to modulate, in part, the rate of acidification (19), $JHCO_3$ and V_T in MCD are unaffected by removal of sodium or addition of luminal amiloride (Fig. 4). Thus, sodium reabsorption via Na-H exchange or via amiloride-sensitive Na channels does not influence normal MCD acidification. It should be noted that our inability to perform long experiments in Na-free media prevents us from rigorously excluding Na-H exchange participating in the acute in vitro response of $JHCO_3$ to aldosterone. Although we used a relatively high concen-

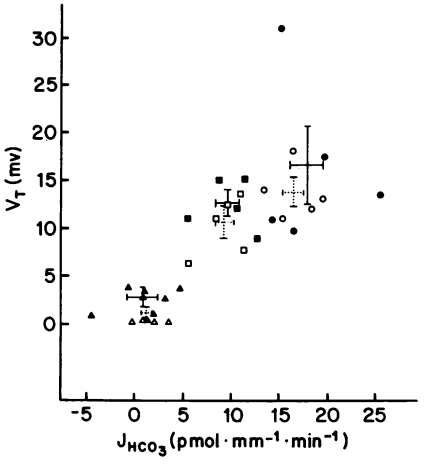

FIGURE 4 Sodium independence of aldosterone-modulated MCD acidification: $JHCO_3$ vs. V_T. ▲ = adrenalectomized animals (ADX); Δ = ADX + amiloride ($5 \times ^{-5}$ M); ■ = normal animals (NL) + 140 mM Na bath and perfusate; □ = NL + 140 mM TMA bath and perfusate; ● = DOCA + 140 mM Na bath and perfusate; ○ = DOCA + 140 mM TMA bath and perfusate. Solid bars depict the means and standard errors for the solid symbols, while hatched crossed bars depict the means and standard errors for the open symbols. In all circumstances neither V_T nor $JHCO_3$ was affected by Na replacement or amiloride.

tration of amiloride in these in vitro aldosterone experiments, the concentration was not sufficiently high to inhibit Na-H exchange with 150 mM luminal Na (26). Although we consider the induction of Na-H exchange by aldosterone to be unlikely, testing with high concentrations of luminal amiloride (1 mM) will be necessary.

Koeppen and Helman (19) have recently provided evidence for an electrogenic acidifying mechanism in rabbit CCT. That MCD acidification proceeds by an electrogenic mechanism is evidenced by (*a*) the parallel ablation of $JHCO_3$ and V_T with addition of acetazolamide to bath (12); (*b*) the demonstration by Stokes (13) that this segment possesses negligible net sodium or potassium reabsorptive capacity; (*c*) the demonstration that total chloride removal from lumen of MCD does not ablate lumen-positive voltage. Indeed, $JHCO_3$ increases (27); and (*d*) the present studies indicating that acidification occurs by means of a mechanism independent of luminal sodium. However, it is evident that the lumen-positive V_T of MCD is not solely a function of such an electrogenic acidifying process. The findings that in vitro aldosterone stimulates $JHCO_3$ but not V_T and that in vivo DOCA increases $JHCO_3$ without a parallel significant increase in V_T signify that an electrical shunt conductance, apparently variable, operates in parallel with the electrogenic acidifying mechanism to modulate the net V_T and, perhaps, indirectly to alter the rate of acidification by varying the electrical potential against which proton movement occurs. Such a conductance channel has been described to operate in parallel with the plasma membrane proton pump of Neurospora (28).

Recent studies on the turtle bladder have demonstrated the presence of an electrogenic proton-translocating ATPase in a luminal membrane fraction from turtle bladder epithelial cell homogenates (29). Although future studies will be required to document this, it is possible that luminal cell membrane of the rabbit medullary collecting duct also possesses such an acidifying mechanism.

In summary, the present in vitro microperfusion studies have identified the MCD as a distal nephron segment with the capacity for aldosterone-regulated acidification through a mechanism which is autonomous of sodium delivery or reabsorption.

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