

Mineralocorticoid–stimulated renal acidification: The critical role of dietary sodium

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Mineralocorticoid–stimulated renal acidification: The critical role of dietary sodium. Recent *in vitro* studies of isolated distal nephron segments have demonstrated that 1) mineralocorticoid hormone stimulates H^+ secretion by both Na^+ -dependent and Na^+ -independent mechanisms, and 2) the Na^+ -independent acidification mechanism has a greater capacity. These *in vitro* data suggest that mineralocorticoid administration *in vivo* might increase renal acid excretion when an augmentation in distal Na^+ reabsorption is precluded by rigid restriction of dietary Na^+ ; under these circumstances, virtually all Na^+ delivered to the distal nephron is reabsorbed in the basal state. In the present studies, prolonged (12 days) administration of DOC (15 mg/day) was undertaken in both Na^+ -fed and rigidly Na^+ -restricted dogs with chronic HCl acidosis. Na^+ -fed animals responded to DOC administration with a large increment in net acid excretion and complete correction of metabolic acidosis. Marked hypokalemia and significant kaliuresis also occurred. Na^+ -restricted dogs experienced no changes in renal acid excretion, systemic acid–base equilibrium, plasma $[K^+]$ or K^+ balance. These results suggest that both renal H^+ and K^+ excretory responses to prolonged mineralocorticoid hormone administration *in vivo* are critically dependent on the availability for reabsorption of surplus Na^+ within the distal nephron; this requirement is met when the diet, and hence the final urine, contains Na^+ but cannot be satisfied when dietary Na^+ is rigidly restricted.

Chronic administration of supraphysiologic amounts of mineralocorticoid hormone to animals fed a normal sodium diet results in transient sodium retention and a persistent stimulation of distal hydrogen ion secretion; as a result, renal net acid excretion increases transiently, new bicarbonate is generated and renal bicarbonate reabsorption increases [1–4].

Recent *in vitro* studies of isolated perfused distal nephron segments have suggested that there are at least two major mechanisms whereby mineralocorticoid hormone can stimulate renal acidification. First, mineralocorticoid hormone can augment the rate of sodium–dependent hydrogen ion secretion in rabbit cortical collecting tubules by enhancing the lumen–negative potential difference [5–7]. Second, mineralocorticoid hormone can augment the rate of sodium–independent hydrogen ion secretion by stimulating an active, electrogenic transport process in rabbit cortical collecting tubule and outer medullary collecting duct (MCD) [5, 8]. Stone et al concluded that the

MCD has “the capacity for aldosterone–regulated acidification through a mechanism which is autonomous of sodium delivery or reabsorption” [8]. There is evidence that the capacity of the sodium–independent medullary mechanism greatly exceeds that of the sodium–dependent cortical process [5, 8, 9]. The existence in the isolated turtle urinary bladder of a sodium–independent acidification mechanism that is stimulated by aldosterone also is well recognized [10].

These *in vitro* findings suggest that mineralocorticoids might stimulate renal acidification *in vivo* even in animals deprived of dietary sodium. Such animals would lack the capacity to increase distal sodium reabsorption because all sodium delivered to the distal nephron would be fully reabsorbed in the basal state. This constraint should, however, pose no obstacle to the sodium–independent acidification mechanism.

Thus, the question was posed: can mineralocorticoids stimulate renal acidification importantly in the absence of dietary sodium. Fragmentary data from studies performed in the 1950’s suggest not; DOC administered to three subjects ingesting a sodium–restricted diet induced negligible changes in urinary acid–base composition [3]. In view of the potential significance of the recent *in vitro* data, however, we thought a more rigorous examination of the issue was clearly warranted. We chose to assess the importance of the sodium–independent, mineralocorticoid–responsive acidification mechanism in the intact organism by comparing the effect of chronic mineralocorticoid administration in acidotic dogs receiving no dietary sodium to that observed in animals ingesting ample sodium. We chose to examine acidotic rather than normal animals in order to maximize the likelihood of detecting a difference in the steady–state increment in plasma bicarbonate induced by mineralocorticoids. As shown previously in studies of animals ingesting a sodium–rich diet, when daily endogenous acid load is normal, the mineralocorticoid–induced increment in net acid excretion is sufficient to generate only a small surplus of extracellular bicarbonate, which is sustained by the augmented tubular acidification. In contrast, when the daily acid load is increased by the chronic administration of hydrochloric acid, the augmented tubular acidification induced by mineralocorticoid hormone is sufficient to return the frankly depressed plasma bicarbonate to normal and to maintain it there in the presence of continued ingestion of the hydrochloric acid load [2].

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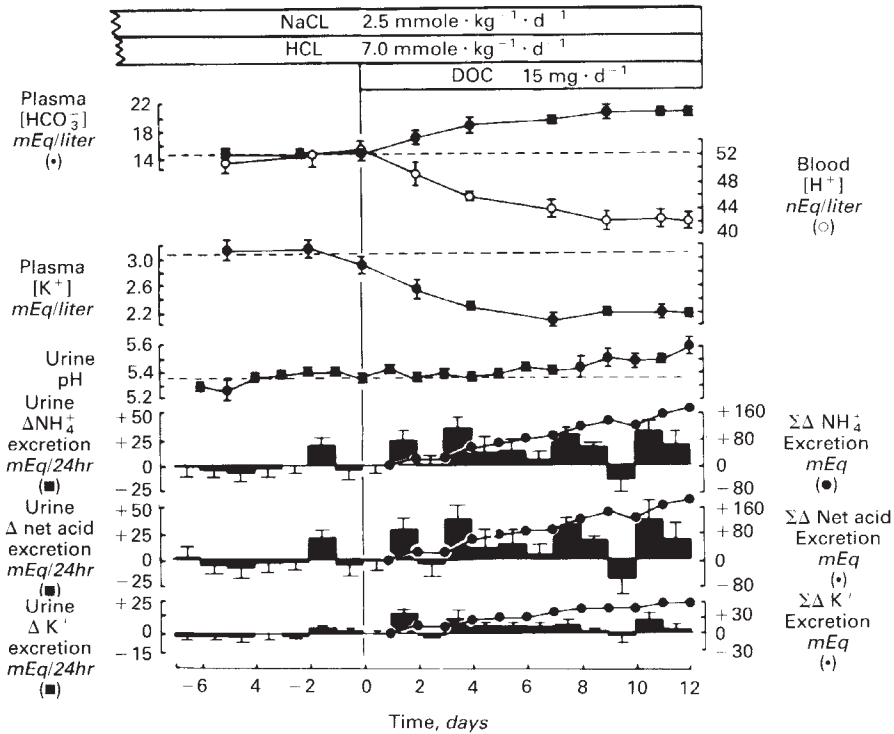


Fig. 1. Effect of DOC administration on plasma bicarbonate, hydrogen ion, and potassium concentrations, urine pH, and changes in urine ammonium, net acid, and potassium excretion in acidotic animals receiving a normal sodium intake (Group I). Cumulative changes ($\Sigma\Delta$) in urine acid and potassium excretion also are depicted. Data are plotted as mean \pm SEM ($N = 6$).

Methods

Studies on the effect of sodium intake on mineralocorticoid hormone-induced changes in acid-base equilibrium were carried out in 13 female mongrel dogs weighing between 10 and 21 kg. Animals were utilized for study only if average control plasma-bicarbonate concentration was between 19.0 and 24.0 mEq/liter. Throughout the study each dog was fed 30 g/kg/day of a synthetic diet, the composition of which has been previously described [11]. The intrinsic electrolyte content of the diet was less than 1.0 mEq of sodium, 0.3 mEq of chloride and 0.1 mEq of potassium per 100 g. All animals received 2.5 mEq/kg/day of potassium as neutral phosphate. Mineralocorticoid hormone was administered as DOC acetate in oil (Organon, West Orange, New Jersey), 7.5 mg intramuscularly twice a day. The sodium content of the diet was determined by the requirements of the individual protocols, as noted below. Supplemental electrolytes were added to the homogenized diet from stock solutions, the compositions of which were verified by direct analyses. The diet was homogenized with twice its weight of water and fed in one or two portions. Dogs that did not eat spontaneously were tube-fed and those that vomited at any time were not included.

Fasting arterial blood samples were drawn percutaneously from the femoral artery (9 a.m.) in heparinized glass syringes at 24 to 72 hr intervals. Urine was collected in metabolic cages over stainless steel surfaces into glass bottles containing mineral oil and thymol-chloroform preservative. Values for urinary excretion and balance data are reported as normalized to 20 kg body wt.

Group I: DOC administration to dogs with HCl-induced

chronic metabolic acidosis receiving a normal sodium diet ($N = 6$). Following a stable control period of four to eight days duration, HCl 7.0 mmole/kg was added to the daily diet for a 10 to 12 day period, an interval more than sufficient to produce a steady-state of metabolic acidosis [12]. DOC was administered for an additional 12 day period during continued HCl administration. NaCl, 2.5 mmole/kg, was added to the daily diet during all periods of study.

Group II: DOC administration to dogs with HCl-induced chronic metabolic acidosis receiving a sodium-restricted diet ($N = 7$). The protocol for this group was identical to that of Group I except that no NaCl was added to the diet.

The details of the balance technique, calculations and analytical methods have been described previously [13, 14]. Urinary net acid is calculated as ammonium plus titratable acidity minus bicarbonate. The anion gap in plasma is calculated as $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{HCO}_3^-)$ mEq/liter. Statistical significance was determined by Student's *t*-test [15].

Results

Group I

HCl feeding resulted in the anticipated steady-state of hyperchloremic acidosis (Fig. 1, Table 1). Administration of DOC resulted in significant changes in renal excretion and in systemic acid-base and electrolyte composition. Plasma bicarbonate concentration increased significantly and blood hydrogen ion concentration decreased significantly; indeed, complete correction of metabolic acidosis occurred. The correction of acidosis was attributable to a large cumulative increment in urinary net acid excretion ($+180$ mEq, $P < 0.05$), accounted for largely by a cumulative increment in ammonium excretion

Table 1. Effect of dietary Na⁺ intake on steady-state changes in plasma composition and body weight in response to DOC administration during chronic HCl acidosis (means ± SEM)

Period	H _a ⁺ nEq/liter	PaCO ₂ mm Hg	HCO ₃ ⁻ mEq/liter	ΔHCO ₃ ⁻ mEq/liter	Anion Gap mEq/liter	Cl ⁻ mEq/liter	Na ⁺ mEq/liter	K ⁺ mEq/liter	Creatinine mg/100 ml	Weight kg
Group I, normal Na⁺ diet (N = 6)										
Control	40.7	36	21.6		18	109	145	3.7	0.65	15.3
	±0.9	±0.8	±0.6		±1.1	±0.2	±0.7	±0.1	±0.01	±0.6
HCl	51.7 ^ψ	31 [†]	14.3 ^ψ	-7.3 ^ψ	15*	118 ^ψ	144*	3.0 ^ψ	0.68	15.3
Final 7 days	±1.3	±1.1	±0.7	±0.8	±0.5	±0.6	±0.4	±0.1	±0.00	±0.6
HCl and DOC	42.0 ^ψ	36 ^ψ	20.7 ^ψ	6.4 ^ψ	16	113 [†]	148 [†]	2.2 ^ψ	0.67	15.4
Days 8–12	±1.3	±1.1	±0.8	±0.7	±0.5	±1.0	±0.5	±0.1	±0.01	±0.7
Group II, Na⁺ restricted diet (N = 7)										
Control	39.6	37	22.4		18	107 ^π	144	3.8	0.64	11.6 [§]
	±0.7	±1.0	±0.3		±0.6	±0.6	±0.9	±0.1	±0.02	±1.0
HCl	50.9 ^ψ	32 [†]	15.0 ^ψ	-7.3 ^ψ	17*	116 ^{ψ§}	144	3.3 ^ψ	0.54* [§]	11.5 [§]
Final 7 days	±1.4	±1.5	±0.8	±0.7	±1.0	±0.7	±0.7	±0.1	±0.02	±1.1
HCl and DOC	51.7 ^π	33*	15.6 ^π	0.6 ^π	14* ^π	117 [§]	143 [†]	3.3 [†]	0.52	11.5 [§]
Days 8–12	±2.1	±1.3	±0.9	±0.5	±0.5	±1.2	±0.5	±0.1	±0.02	±1.1

Abbreviations are: H_a⁺, hydrogen ion activity in arterial blood; PaCO₂, CO₂ tension in arterial blood. *, † and ^ψ: significantly different from the previous period at the 0.05, 0.01, and 0.001 probability levels, respectively; §, ^π and ^π: significantly different from Group I at the 0.05, 0.01, and 0.001 probability levels, respectively.

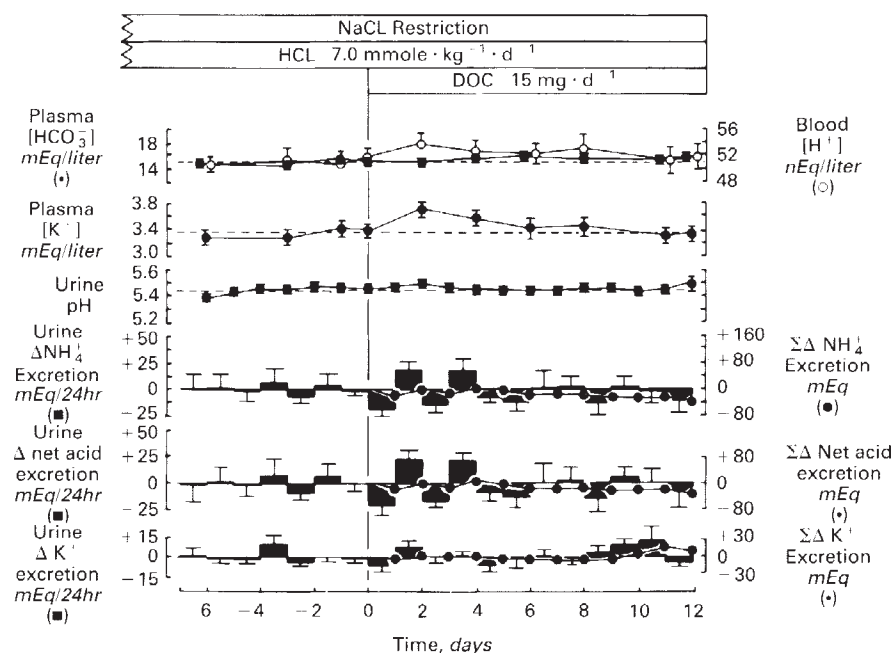


Fig. 2. Effect of DOC administration on plasma bicarbonate, hydrogen ion, and potassium concentrations, urine pH, and changes in urine ammonium, net acid and potassium excretion in acidotic animals receiving a sodium-restricted diet (Group II). Cumulative changes (ΣΔ) in urine acid and potassium excretion also are depicted. Data are plotted as mean ± SEM (N = 7).

(+168 mEq, $P < 0.05$). Plasma potassium concentration decreased significantly during DOC administration and was accompanied by a significant cumulative increment in renal potassium excretion (+58 mEq, $P < 0.05$). A significant positive sodium balance (cumulative Δ balance, day 12 of DOC, +134 mEq, $P < 0.01$) was observed but no significant change in chloride balance occurred. The corresponding changes in potassium and nitrogen-corrected potassium balances were -77 mEq ($P < 0.05$) and -96 mEq ($P < 0.01$), respectively.

Group II

HCl feeding resulted in a level of hyperchloremic acidosis that was not significantly different from that observed in the

Group I animals fed a normal sodium diet (Fig. 2). In contrast to the Group I animals, however, DOC administration caused no significant changes in plasma bicarbonate, potassium or hydrogen ion concentration and did not alter urinary acid-base or electrolyte composition (Fig. 2, Tables 1 and 2).

Discussion

Several lines of evidence might lead one to predict that the stimulatory effect of mineralocorticoids on renal acid excretion in the intact animal would occur even in the absence of dietary sodium. First, chronic administration of DOC and a normal

Table 2. Effect of dietary Na⁺ intake on the steady-state renal response to DOC administration during chronic HCl acidosis (means ± SEM)

Period	pH	NH ₄ ⁺ mEq/24 hr	T.A. mEq/24 hr	HCO ₃ ⁻ mEq/24 hr	Net Acid mEq/24 hr	Pi mmole/24 hr	Na ⁺ mEq/24 hr	K ⁺ mEq/24 hr	Cl ⁻ mEq/24 hr	Volume ml/24 hr
Group I, normal Na ⁺ diet (N = 6)										
Control	5.98 ±0.06	43 ±1.9	29 ±1.2	1.8 ±0.4	70 ±2.8	44 ±1.4	37 ±2.5	47 ±1.4	44 ±2.0	936 ±25
HCl										
Final 7 days	5.35 ^ψ ±0.02	150 ^ψ ±3.2	32* ±0.6	0.1 ^ψ ±0.0	182 ^ψ ±3.6	42 ±0.8	44 ±1.8	46 ±1.1	166 ^ψ ±3.6	955 ±24
(Day 12, ΣΔ)		(+960 ^ψ) ±42	(+46) ±20	(-16) ±8	(+1023 ^ψ) ±67	(-14) ±13	(+90*) ±26	(+44*) ±16	(+1219 ^ψ) ±39	
HCl and DOC										
Days 8-12	5.50 ^ψ ±0.03	166 [†] ±5.3	33 ±1.2	0.2 [†] ±0.0	199* ±6.4	43 ±1.6	34 ^ψ ±2.6	50 ±2.0	170 ±6.0	961 ±38
(Day 12, ΣΔ)		(+168*) ±45	(+12) ±11	(+1 [†]) ±0	(+180*) ±49	(+9) ±13	(-116*) ±31	(+58*) ±21	(+62) ±44	
Group II, Na ⁺ restricted diet (N = 7)										
Control	5.90 ±0.03	45 ±2.6	31 ±1.5	0.9 ±0.1	75 ±3.9	45 ±2.0	2 [¶] ±0.6	44 ±2.1	1 [¶] ±0.4	925 ±47
HCl										
Final 7 days	5.44 ^{ψ§} ±0.01	163 ^{ψ§} ±3.7	30 ^{ψ§} ±0.8	0.2 ^{ψπ} ±0.0	193 ^{ψ§} ±4.3	40* ±1.0	3 [¶] ±0.4	43 ±1.8	128 ^{ψ¶} ±3.1	924 ±25
(Day 12, ΣΔ)		(+1041*) ±40	(-5) ±20	(-7*) ±2	(+1043 ^ψ) ±52	(-52) ±20	(+9 [§]) ±13	(+11) ±49	(+1149 ^ψ) ±19	
HCl and DOC										
Days 8-12	5.45 ±0.01	159 [§] ±4.4	31 ±0.9	0.3 ±0.0	190 ^π ±5.2	41 ±1.2	1* [¶] ±0.4	46 ±2.3	125 [¶] ±4.1	936 ±31
(Day 12, ΣΔ)		(-38 ^π) ±40	(+6) ±10	(+1 [†]) ±0	(-33 ^π) ±38	(+12) ±13	(-14 ^π) ±8	(+11) ±44	(-33) ±26	

Symbols are the same as Table 1. Symbol ΣΔ signifies the cumulative change in excretion values (within parentheses, mEq) over the respective 12-day period and reflects the accumulated daily differences from the mean excretion value for the previous steady-state period. Values for urinary excretion and balance data are normalized to 20 kg body wt.

sodium diet to dogs with chronic hyperchloremic metabolic acidosis pretreated with amiloride to block sodium-dependent distal acidification results in significant increases in both net acid excretion and plasma bicarbonate concentration [2]. This finding suggests that the augmentation in renal acidification induced by mineralocorticoid hormone in vivo is largely sodium-independent. Second, mineralocorticoid hormones cause augmented renal ammoniogenesis in vitro [16]; this raises the possibility that a direct stimulation of ammoniogenesis by mineralocorticoid in vivo might be sufficient to increase net acid excretion and plasma bicarbonate concentration. Third, sodium-independent hydrogen ion secretion is known to occur both in cortical collecting tubule [5], and in outer medullary collecting duct [8]; hydrogen ion secretion at both sites can be stimulated by mineralocorticoids.

The results of the present study stand in sharp contrast to the prediction derived from these lines of evidence. Mineralocorticoids administered in large doses to acidotic, sodium-restricted dogs (Group II) caused no detectable change in net acid excretion or plasma bicarbonate concentration. These observations imply that the sodium-independent mineralocorticoid-responsive hydrogen ion transport systems clearly identified in vitro are not able to make a sufficiently large contribution to overall tubular acidification in vivo to influence importantly extracellular or even urinary composition under these circum-

stances.¹ Moreover, these data underscore the hazards of predicting the integrated response of functioning nephron units or of the entire kidney from quantitative assessments of transport phenomena in certain tubular segments. Thus, finding that segments of cortical and medullary collecting ducts manifest a large capacity for sodium-independent hydrogen ion transport in response to mineralocorticoids does not necessarily imply that such transport systems contribute in a major way to the acidification response to these hormones in the intact animal. Alternatively, of course, one must consider the possibility that the sodium-independent, mineralocorticoid-responsive acidification mechanism demonstrated in the rabbit collecting tubule simply does not exist in the dog. Such a striking species difference in mammalian nephron transport characteristics seems to us highly improbable.

As already noted, previous studies have shown that amiloride-treated dogs with hyperchloremic acidosis given DOC and a normal sodium diet exhibited a significant increase

¹ Since Stone et al did not assess the chloride dependence of the outer medullary collecting duct acidification in response to mineralocorticoids [8], it remains to be determined whether the high luminal chloride concentration presumably achieved in the distal segment in the present studies might mitigate in vivo the potent acidification mechanism demonstrated in vitro.

in net acid excretion and plasma bicarbonate concentration [2]. Amiloride is known to obliterate the sodium-dependent, lumen-negative potential difference in cortical collecting tubules in vitro [17, 18]. Why does placing a limitation on distal sodium reabsorption by dietary sodium restriction block mineralocorticoid-induced urinary acidification when pharmacologic inhibition of distal sodium-dependent hydrogen ion transport did not? One possibility is that insufficient amiloride has been administered to block completely the sodium-dependent process; in view of the large dose administered, this seems unlikely. A second possibility is that mineralocorticoids stimulate sodium-dependent hydrogen ion secretion in tubular segments impervious to the effect of amiloride. Further studies will be required to clarify this issue.

The present results provide no support for the thesis that the stimulating effect of mineralocorticoid hormone on renal acid excretion is due to a direct augmentation of renal ammoniagenesis. Administration of aldosterone to rats ingesting a normal sodium diet resulted in significant increases in urinary ammonium excretion and metabolic alkalosis within 12 hr of hormone administration; renal ammoniagenesis was increased in the absence of a change in plasma potassium concentration or potassium balance [16]. However, the present study provides no evidence of DOC-induced augmentation of renal ammoniagenesis in the sodium-restricted Group II animals (Fig. 2, Table 2). If DOC administration did result in an increase in renal ammoniagenesis in the sodium-restricted Group II dogs, the increase was of insufficient magnitude to result in detectable changes in either net acid excretion or systemic acid-base equilibrium.

Could the failure of DOC administration to increase net acid excretion in Group II, sodium-restricted dogs be due to lesser degrees of basal hypokalemia and potassium depletion than those prevailing in animals receiving a normal sodium intake (Tables 1 and 2)? We believe not. Previous studies have demonstrated that chronic DOC administration does elicit a renal acidification response and causes plasma bicarbonate to rise significantly in sodium-replete animals given sufficient potassium to prevent appreciable potassium depletion [2]. However, since the DOC-induced increment in net acid excretion observed in the sodium-replete group of the present study occurred in the company of mineralocorticoid-stimulated kaliuresis and hypokalemia, our results cannot exclude a permissive role for potassium depletion in modulating the renal acid excretory response to the hormone.

The present studies also demonstrate that the ability of mineralocorticoids to stimulate potassium excretion in vivo, no less than their ability to stimulate acid excretion, is dependent on adequate sodium intake; in the absence of dietary sodium, the administration of mineralocorticoids caused neither hypokalemia nor augmented renal potassium excretion. The recent observation that potassium secretion in the rat superficial distal convoluted tubule is highly dependent on the magnitude of the lumen-negative potential difference, and hence, on the rate of sodium reabsorption is consistent with our findings [19]. That potassium secretion in isolated perfused rabbit cortical collecting tubules is also sodium-dependent has been demonstrated recently; potassium secretion ceased when mean luminal sodium concentration was reduced to values below 8 to 10 mEq/liter [20]. A major role for sodium-dependent transepithe-

lial voltage in regulating distal tubular potassium secretion, however, has not been a universal finding [21].

In conclusion, despite the existence of sodium-independent, mineralocorticoid-responsive hydrogen ion secretory processes within the mammalian distal nephron, the administration of supraphysiologic amounts of mineralocorticoids to the intact organism appears to enhance the bulk transfer of hydrogen ion into the urine only when a surplus of luminal sodium is available for augmented reabsorption. This finding strongly suggests that sodium-dependent hydrogen ion secretion, just as sodium-dependent potassium secretion, is the dominant mechanism accounting for the renal effects of mineralocorticoid excess on acid-base balance in the living organism.

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