Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 630–634, January 1984
Physiological Sciences

Na^+–H^+ exchange activity in renal brush border membrane vesicles in response to metabolic acidosis: The role of glucocorticoids

(hormonal regulation of kidney transport/dexamethasone/adrenal glucocorticoids/excretion of phosphate and ammonium/acid excretion)

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Communicated by Robert W. Berliner, September 26, 1983

ABSTRACT **Amiloride-sensitive Na^+–H^+ exchange activity in brush border membrane vesicles isolated from rat proximal tubule was increased in metabolic acidosis. The enhancement of exchange activity required an intact adrenal gland or glucocorticoid supplements. Ammonium and phosphate excretions were increased during acidosis and these were also largely dependent on an intact adrenal gland or glucocorticoid supplements. Amiloride-insensitive Na^+ uptake and passive H^+ permeability were not altered by acidosis or the glucocorticoid status of the animal. These findings are consistent with glucocorticoids having an important regulatory role in the kidney by orchestrating the proximal tubular adaptation to metabolic acidosis.**

The kidney responds to metabolic acidosis by increasing secretion of acid, phosphate, and ammonium and by enhancing reabsorption of bicarbonate (1). Acid secretory processes provide a mechanism for bicarbonate reabsorption, ≈80% of which occurs in the proximal tubule (2). This nephron segment is the locus of the Na^+–H^+ exchanger (3). The carrier, found in the brush border membrane, mediates the electroneutral antiport of Na^+ for H^+ (4–6). Amiloride, at relatively high concentrations (K_i = 0.05 mM), competitively inhibits exchange activity (7).

Metabolic acidosis is also associated with increased levels of adrenal corticosteroids (8–10). Adrenalectomy decreases renal net excretion of titratable acids and ammonium (8, 9) and hyperglucocorticoid states are concomitant with metabolic alkalosis (11). Glucocorticoids increase endogenous acid production, stimulate acid secretion, enhance ammonium production, and induce phosphaturia (12–14). The site of action of glucocorticoids in decreasing phosphate reabsorption is largely confined to the proximal tubule (15), where glucocorticoid receptors have been found (16). In addition, we have recently reported that the glucocorticoid dexamethasone, but not the mineralocorticoid aldosterone, increases amiloride-sensitive Na^+–H^+ exchange activity and selectivity decreases Na^+ gradient-dependent phosphate uptake in proximal tubule brush border membrane vesicles (17).

These findings raise the possibilities that metabolic acidosis induces changes in renal brush border Na^+–H^+ exchange activity and phosphate and ammonium excretion and, further, that glucocorticoids may have a role in mediating these effects. The present communication addresses these questions.

METHODS AND MATERIALS

Animals: Dexamethasone Administration and Acid–Base Status. Male Sprague–Dawley rats weighing 200–320 g were fed Purina rat chow pellets ad libitum and had 0.9% saline in their drinking water for 6–8 days. Acidotic rats were given, in addition, 1% NH_4Cl in the water. The adrenals of adrenalectomized animals were removed under light ether anesthesia on day 1. Dexamethasone-treated animals were given two injections (30 μg/100 g of body weight) of dexamethasone in 0.9% saline, 24 and 16 hr prior to sacrifice. The glucocorticoid dose approximated that reported to lead to a large increase in total acid elimination in the rat (18).

Urines were collected for the 24-hr period prior to sacrifice and aortic blood was obtained at sacrifice. Blood gases and pH were determined with a IL213 blood gas analyzer. Plasma and urine Na^+ and K^+ were measured by flame photometry. Urinary pH was measured with a pH glass electrode. Titratable acids were estimated by titrating 2 ml of urine to pH 7.2 with 0.1 M NaOH. Total urinary NH_4^+ was measured by titrating 1 ml of urine to pH 7.2 with 0.1 M NaOH after the addition of 0.5 ml of 34% formaldehyde (18).

Plasma electrolytes, blood pH and pCO_2, and urinary pH for the rats on the different treatments are reported in Table 1. Acidotic animals, whether not treated further, adrenalectomized or adrenalectomized and then given dexamethasone, had comparable blood pH and pCO_2 and urinary pH values, 7.15–7.22, 22.4–27.2, and 5.77–5.84, respectively. These values were significantly lower than those of the nonacidotic groups, 7.39–7.41, 35.4–40.5, and 6.67–6.81, for the corresponding parameters. Plasma [Na^+] values were nearly the same in all groups, except that the concentrations were slightly lower in the two groups that were made both acidotic and adrenalectomized. Plasma [K^+] values were higher in the adrenalectomized animals because these rats were not fed a K^+-restricted diet.

Brush Border Membrane Vesicles. Rat renal cortex brush border vesicles were prepared as described (17, 19). In experiments in which Na^+ uptake was measured, the intravesicular medium was 150 mM KCl/25 mM 2-(*N*-morpholino)ethanesulfonic acid/4 mM KOH, pH 5.5. In experiments in which H^+ flux was determined, the intravesicular medium was 150 mM sodium gluconate/10 mM Tris/16 mM Hepes, pH 7.5. Previous studies showed that the quality of the membrane preparations evaluated by specific activities and enrichment factors of maltase, γ-glutamyltransferase, and alkaline phosphatase in different treatment groups was essentially the same (17).

Transport Measurements. Uptake of $^{22}Na^+$ was measured at 20°C by a Millipore filtration technique with 0.65-μm filters (17). The membrane vesicle suspension (20 μl containing 170–350 μg of protein) was preincubated for 1 min at 20°C and incubation was initiated by addition of 30 μl of uptake medium to give (final concentration) 142 mM KCl/14.7 mM KOH/10 mM 2-(*N*-morpholino)ethanesulfonic acid/9 mM Hepes/1 mM NaCl (containing 0.1–0.2 μCi of $^{22}Na^+$; 1 Ci = 37 GBq), adjusted to pH 7.5. Uptakes were terminated by addition of 3 ml of ice-cold solution containing 0.1 mM amiloride/150 mM KCl/15 mM Hepes/7.8 mM KOH, pH 7.5.

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Table 1. Blood pH, pCO_2, Na^+ and K^+ concentrations, and urinary pH in different treatment groups

Parameter	Normal	Acidotic	ADX	Acidotic ADX	Acidotic ADX, DEX	ADX, DEX
Blood H^+, 10^{-8} M	3.88 ± 0.12 (pH = 7.41)	6.40 ± 0.36 (pH = 7.19)	4.08 ± 0.15 (pH = 7.39)	5.99 ± 0.35 (pH = 7.22)	7.05 ± 0.67 (pH = 7.15)	3.89 ± 0.14 (pH = 7.41)
Blood pCO_2, mm of Hg	38.5 ± 0.8	25.4 ± 1.1	35.4 ± 1.1	22.4 ± 1.1	27.2 ± 1.2	40.5 ± 2.9
Plasma Na^+, mM	147 ± 2	142 ± 2	143 ± 2	138 ± 2	137 ± 2	145 ± 3
Plasma K^+, mM	3.7 ± 0.2	3.8 ± 0.2	4.5 ± 0.2	4.7 ± 0.3	4.2 ± 0.1	4.7 ± 0.5
Urine H^+, 10^{-7} M	1.56 ± 0.25 (pH = 6.81)	17.13 ± 1.64 (pH = 5.77)	2.12 ± 0.70 (pH = 6.67)	14.29 ± 1.74 (pH = 5.84)	15.59 ± 0.71 (pH = 5.81)	1.60 ± 0.23 (pH = 6.80)

ADX, adrenalectomized; DEX, dexamethasone treated. Each datum represents the mean ± SEM; $n = 12$ for each group.

All incubations were carried out in triplicate with fresh membranes. Each experiment was repeated six times with different membrane preparations (two rats for each preparation).

The rate of pH gradient dissipation was measured by monitoring changes in fluorescence of 9-aminoacridine with time (17). Membrane vesicles suspended with pH 5.5 buffer containing 0.5 mM 9-aminoacridine were diluted 1:100 with 150 mM KCl/15 mM Hepes/7.8 mM KOH, pH 7.5. The internal buffering capacity of membrane vesicles from different treatment groups was assumed to be the same. As the pH gradient dissipated, 9-aminoacridine diffused from the intravesicular to the extravesicular space, resulting in an increase in fluorescence. Fluorescence was determined by using 420 nm and 480 nm as the exciting and emission wavelengths, respectively. Fluorescence of 100% was set by the transmission after complete dissipation of the pH gradient with time, a value equal to that found when the pH gradient was dissipated with nigericin.

The uptake of H^+ in the membrane vesicles was measured by changes in absorbance of acridine orange (20). An Aminco DW-2 spectrophotometer was used in the dual-beam mode with 492 nm as the absorbing wavelength and 600 nm as the reference wavelength. The membrane vesicles loaded with the sodium gluconate (pH 7.5) medium were diluted 1:100 with 150 mM tetramethylammonium gluconate/10 mM Tris/16 mM Hepes, pH 7.5, buffer. Because initially there was no pH gradient across the membrane, the outwardly directed Na^+ gradient provided the driving force for intravesicular H^+ accumulation via amiloride (1 mM)-sensitive Na^+–H^+ exchange. This uptake was detected by monitoring the accumulation of the weak base acridine orange into the more acidic intravesicular space, where it was self-quenched. Extravesicular acridine orange concentration, thus its absorbance, was decreased.

RESULTS

Effect of Metabolic Acidosis on Na^+–H^+ Exchange. Previous studies of Na^+–H^+ exchange in renal brush border membrane vesicles demonstrated that a H^+ gradient induced a Na^+ gradient across the membrane; a Na^+ gradient induced a H^+ gradient across the membrane; and amiloride inhibited both of these modes of Na^+–H^+ exchange by competing with Na^+ (5, 7). Fig. 1 shows that Na^+–H^+ exchange activity increased in metabolic acidosis. With membrane vesicles from acidotic rats, the initial (5 s) rate of uptake of 1 mM Na^+ in the presence of a pH gradient ($pH_i = 5.5$, $pH_o = 7.5$) was 3.24 ± 0.17 nmol/mg of protein, a value 43% greater than the uptake found with vesicles from control animals, 2.26 ± 0.19 ($P < 0.005$). Accumulation of Na^+ was greater at 1 min, but at 1 hr of incubation, at which time both H^+ and Na^+ gradients presumably were dissipated (21), uptakes were the same. The finding that Na^+ uptakes at 1 hr were identical indicated that the average intravesicular volumes of vesicles from control and acidotic rats were comparable and suggested that the difference in uptake of Na^+ between vesicles from acidotic and control animals was not due to an alteration in vesicle size. When amiloride was present in the incubation, Na^+ uptake rates by vesicles from acidotic and control animals were very low (about 10% of the uptakes in the absence of the inhibitor) and were essentially identical. These observations were consistent with the hypothesis that metabolic acidosis altered Na^+ uptake mediated by an amiloride-sensitive process.

Because it was feasible that the increased Na^+ uptake in membrane vesicles from acidotic rats resulted from a decrease in the rate of H^+ gradient dissipation rather than from increases in Na^+–H^+ exchange activity, passive changes in intravesicular pH in vesicles of acidotic or control animals were measured. A typical trace of 9-aminoacridine fluorescence with time and a replot of the data are shown in Fig. 2 *A* and *B*, respectively. The rate constant k of H^+ efflux from vesicles from control and acidotic rats was not significantly different, 0.55 ± 0.15 and 0.65 ± 0.11 min^{-1} ($n = 4$), respectively (data not illustrated). This finding demonstrated that the metabolic acidosis-dependent stimulation in rate of Na^+ uptake in vesicles (Fig. 1) was not the consequence of a change in the rate of dissipation of the pH gradient.

Because amiloride-sensitive pH gradient ($[H^+]_i > [H^+]_o$)-dependent Na^+ uptake was enhanced in vesicles from acidotic rats and this increase was not due to a decreased rate of passive H^+ efflux, one would predict that Na^+-dependent H^+ flux would be greater in membrane vesicles from acidotic rats compared to those from control rats. That this was indeed the case is shown in Fig. 3. Initially in this experiment there was no pH gradient across the membrane ($pH_i = pH_o$

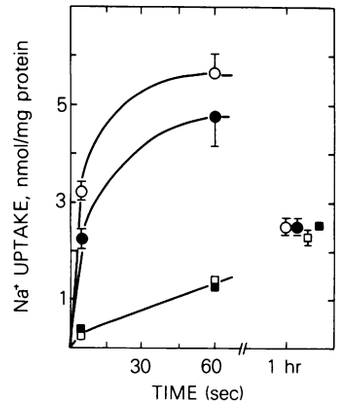

FIG. 1. Effect of metabolic acidosis on brush border membrane Na^+–H^+ exchange activity. Na^+ (1 mM) uptake was measured in membrane vesicles from acidotic (○, □) and normal (●, ■) rats, in the absence (○, ●) and presence (□, ■) of 1 mM amiloride. The initial pH gradient was $pH_i = 5.5$, $pH_o = 7.5$. Data represent the mean ± SEM ($n = 6$).

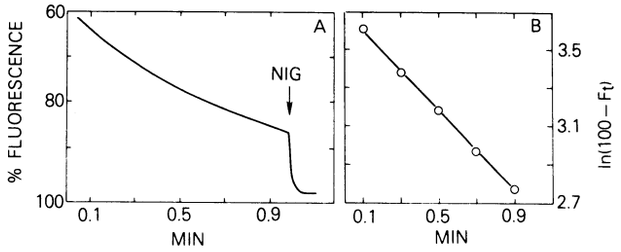

FIG. 2. Rate of the passive dissipation of the pH gradient for a representative experiment. (*A*) Change in fluorescence of 9-aminoacridine was measured. Brush border membrane vesicles initially contained 150 mM KCl and 25 mM 2-(*N*-morpholino)ethanesulfonic acid/KOH, pH 5.5, and were diluted into 150 mM KCl and 15 mM Hepes/KOH, pH 7.5. Nigericin (NIG) (5 μg) was added at 1 min to collapse the pH gradient. (*B*) Replot of the data in *A* showing that the dissipation of the gradient follows first-order reaction kinetics.

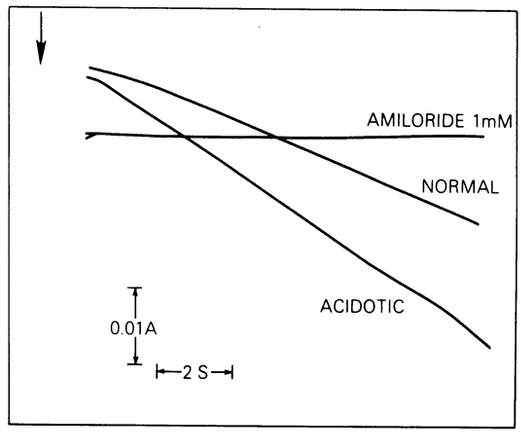

FIG. 3. Generation of a pH gradient in Na^+-loaded brush border membrane vesicles from acidotic and normal rats. Membrane vesicles were loaded with 150 mM sodium gluconate/15 mM Hepes/KOH, pH 7.5, and diluted 1:100 with 150 mM tetramethylammonium gluconate/15 mM Hepes/KOH, pH 7.5, and 20 μM acridine orange. Representative experiments showing traces in the absence and presence of 1 mM amiloride are illustrated. The absorbance difference ($A_{492} - A_{600}$) was measured with time. Membrane protein concentrations were the same for all conditions.

= 7.5), but as intravesicular Na^+ exchanged for extravesicular H^+, a pH gradient ($pH_i < pH_o$) developed. This was detected by monitoring the absorbance of acridine orange. As shown, the initial rate of H^+ accumulation was more rapid in vesicles from the acidotic animals. The maximal pH gradient generated in vesicles from acidotic and control rats was approximately the same and this level was attained between 5 and 10 min (data not illustrated). When the experiments were repeated in the presence of 1 mM amiloride, there was no detectable change in acridine orange distribution (Fig. 3). This finding indicated that the H^+ flux was mediated by amiloride-sensitive Na^+–H^+ exchange. Thus, the results showed that both the rate of H^+ flux and the rate of Na^+ uptake were greater in vesicles from acidotic animals. In control experiments, to test whether amiloride interfered with the absorbance of acridine orange, we found that the Na^+–H^+ exchange ionophore monensin decreased acridine orange absorbance despite the presence of amiloride, although the rate of change and the maximal spectroscopic absorbance were reduced by 33% compared to that found in the absence of amiloride (not illustrated).

Role of Glucocorticoids in the Renal Adaptation to Metabolic Acidosis: Na^+–H^+ Exchange. Previously, we showed that treating animals with glucocorticoids but not with mineralocorticoids enhanced renal Na^+–H^+ exchange activity (17). Additionally, it was reported that the synthesis and circulating levels of glucocorticoids were increased during chronic metabolic acidosis (8–10). Therefore, we tested whether glucocorticoids were involved in the stimulation of Na^+–H^+ exchange activity found here with metabolic acidosis (Figs. 1 and 3). Initial (5 s) rates of amiloride-sensitive Na^+ uptake in brush border membrane vesicles from differently treated rats are illustrated in Fig. 4. In this series of experiments, the Na^+ uptake in membrane vesicles from normal and acidotic animals was 1.95 ± 0.21 and 2.97 ± 0.17 nmol/mg of protein, respectively. The uptake in vesicles from adrenalectomized rats, 2.11 ± 0.13, was essentially the same as in vesicles from the control group. However, if acidotic rats were also adrenalectomized, Na^+ uptake was not enhanced, 2.15 ± 0.27 compared to 2.97 ± 0.27 in membrane vesicles from acidotic rats with intact adrenals. These findings suggested that an intact adrenal gland was necessary for an increase in Na^+–H^+ exchange activity with metabolic acidosis. Na^+ uptake in vesicles from adrenalectomized, acidotic animals given dexamethasone was increased to 3.56 ± 0.26 nmol/mg of protein. The glucocorticoid, as we reported before (17), stimulated amiloride-inhibitable Na^+ uptake even in the absence of acidosis, 3.78 ± 0.06 nmol/mg of protein. Amiloride-insensitive Na^+ uptake (5 s) did not significantly differ between the groups and averaged 0.27 nmol/mg of protein (data not shown). Fig. 4 also shows that after 1 hr of uptake, the amount of Na^+ that accumulated per milligram of protein was not significantly altered by the different treatments, indicating equivalent intravesicular spaces.

Membrane vesicles from rats treated as described above (Fig. 4) were also tested for their passive rates of pH gradient dissipation by monitoring the distribution of 9-aminoacridine (Fig. 2). The rate constant k ($t_{1/2}$) for pH gradient dissipation between any group was not significantly different ($P > 0.05$, $n = 4$) by analysis of variance and Duncan's multiple-range tests. For example, the k values for vesicles from acidotic, acidotic and adrenalectomized, and acidotic and adrenalectomized with dexamethasone were 0.65 ± 0.11, 0.69 ± 0.04, and 0.69 ± 0.11 min^{-1}, respectively. Thus, the differences in amiloride-sensitive, H^+ gradient-dependent Na^+ uptakes between the groups were not due to alterations in passive H^+ gradient dissipation.

Role of Glucocorticoids in the Renal Adaptation to Metabolic Acidosis: Phosphate and Ammonium Excretion. Phosphate

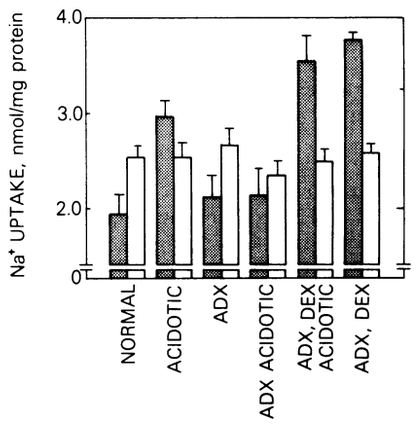

FIG. 4. Role of the adrenals and glucocorticoids in the stimulation of Na^+–H^+ exchange activity in brush border membranes from acidotic animals. Rats were treated for 6–8 days. Initial (5 s) Na^+ uptake was calculated as the difference between 1 mM Na^+ uptake in the absence and presence of 1 mM amiloride (crosshatched bars). The uptake of Na^+ after 1 hr of incubation is shown as open bars. Results represent the mean ± SEM ($n = 6$) for each treatment. ADX, adrenalectomized; DEX, dexamethasone.

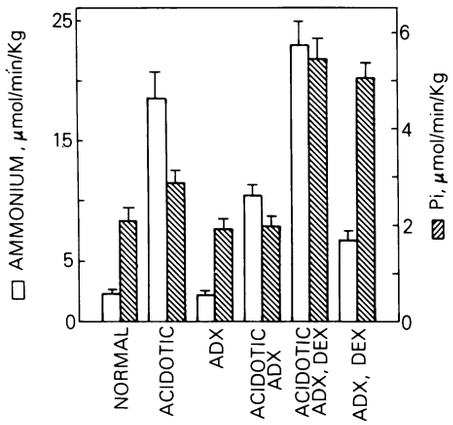

FIG. 5. Effect of acidosis, adrenalectomy (ADX), and the glucocorticoid dexamethasone (DEX) on ammonium and phosphate excretion. Results represent the mean ± SEM (n = 12) for each treatment.

and ammonium excretion was known to increase in metabolic acidosis (1). In addition, we previously reported that glucocorticoids but not mineralocorticoids decreased Na^+-dependent phosphate uptake in brush border membrane vesicles (17). Therefore, we next examined the role of glucocorticoids in the excretion of phosphate and ammonium by the kidney in response to metabolic acidosis. Fig. 5 shows excretion rates of phosphate and ammonium, determined from urinary collections for 24 hr prior to sacrifice of the rats. Acidotic animals had an increased output of phosphate relative to the normal animals, 2.88 ± 0.27 vs. 2.08 ± 0.27 μmol·min^{-1}·kg^{-1}. Phosphate excretion by adrenalectomized rats was the same as that for normal animals. The increment of phosphate excretion due to acidosis was completely blocked by adrenalectomy of the acidotic rats, only 1.98 ± 0.22 μmol·min^{-1}·kg^{-1} was produced. Substantial and equivalent increases in phosphate excretion with glucocorticoid treatments were found in the presence or absence of acidosis, 5.48 ± 0.37 and 5.07 ± 0.31 μmol·min^{-1}·kg^{-1}, respectively.

The action of glucocorticoids in regulating ammonium excretion in response to metabolic acidosis followed a similar pattern. Acidosis resulted in a large increase in ammonium excretion, 18.54 ± 2.20 vs. 2.26 ± 0.28 μmol·min^{-1}·kg^{-1}. Adrenalectomy in the absence of acidosis had little affect, 2.22 ± 0.33 μmol·min^{-1}·kg^{-1}. There was a partial response to acidosis, in the adrenalectomized animal, 10.46 ± 0.83 μmol·min^{-1}·kg^{-1}. A full response was elicited if the adrenalectomized acidotic rat was given dexamethasone 22.97 ± 2.01 μmol·min^{-1}·kg^{-1}. Dexamethasone in the absence of acidosis increased ammonium excretion 6.73 ± 0.70 compared to 2.26 ± 0.28 μmol·min^{-1}·kg^{-1} in the normal rat, but the effect was much less than that found in acidosis.

DISCUSSION

The present study demonstrated that Na^+–H^+ exchange activity—i.e., amiloride-sensitive Na^+ and H^+ flux—increased in renal brush border membrane vesicles from acidotic rats. Cohn *et al.* recently reported in a preliminary communication that exchange activity was increased in brush border membrane vesicles isolated from acidotic dogs (22). Further, we found that the increased activity was dependent on glucocorticoids, for activity was not stimulated if the acidotic animals were adrenalectomized, whereas if dexamethasone was given to the adrenalectomized, acidotic rats the uptake of Na^+ was enhanced to a level at least equivalent to that of the acidotic rat with adrenals intact. The amount of dexamethasone used here was relatively high and comparable to that used in another study to produce a large increase in total acid secretion (18). We previously showed that a lower dose of dexamethasone stimulated Na^+–H^+ exchange activity (17) and the fact that adrenalectomy abolished the acidosis-induced adaptation in Na^+–H^+ exchange might suggest that physiological levels of glucocorticoids were effective in this response. However, the effect of physiological levels of glucocorticoids as a mediator of the response in acidosis remains to be determined. Although the contribution of mineralocorticoids was not examined in this paper, recent studies showed that aldosterone was quantitatively considerably less important than glucocorticoids in renal acid excretion during acidosis (18) and, previously, we reported that aldosterone did not affect Na^+–H^+ exchange activity in renal brush border membrane vesicles (17).

The mechanism of the glucocorticoid-related acidosis-induced change in Na^+–H^+ exchange activity is not known. In other studies, we found a significant increase in amiloride-sensitive Na^+ uptake 4 hr after dexamethasone administration with a maximal stimulation of 24 hr (23). Additionally, dexamethasone was found to increase the V_{max} of amiloride-sensitive Na^+ uptake without altering the apparent affinity for Na^+ (23). The experiment described in Fig. 3 showing an increased amiloride-sensitive Na^+-dependent H^+ flux in acidosis would be consistent with a V_{max} effect. The Na^+ concentration (150 mM) used was probably saturating (K_{Na^+} = 10–15 mM) (7, 23, 24). With saturating substrate, only a change in V_{max} would have been detected, as was the case. Another possible mechanism may be that acidosis induced a change in the H^+ modifier site of the Na^+–H^+ exchanger (25). It is also recognized that the rate of proximal tubule acidification in the intact animal may be stimulated by hypokalemia, hypercapnia, volume contraction, hypoparathyroidism, hypercalcemia and vitamin D, in addition to glucocorticoids. These other effectors were not examined in this investigation.

It was found in the present study that phosphate excretion was increased in metabolic acidosis and the enhancement was dependent on an intact adrenal gland or glucocorticoid supplements. Increases in phosphate excretion in metabolic acidosis were previously reported (12, 18, 26, 27). Further, elevated levels of glucocorticoids were found in metabolic acidosis (8–10). In addition, we reported that glucocorticoids, but not mineralocorticoids, specifically decreased Na^+-dependent phosphate uptake in renal brush border membrane vesicles (17). These findings support the concept that glucocorticoids may be important regulators of phosphate excretion in metabolic acidosis. On the other hand, it should be recognized that the possible interaction between glucocorticoids and parathyroid hormone on phosphate excretion was not examined in the present study. However, others reported a decrease in brush border Na^+-dependent phosphate uptake in acidotic animals that was independent of parathyroid hormone (28, 29).

Ammonium excretion increased substantially in metabolic acidosis, as had been found previously (8, 18). The present paper shows that this increase was contingent, at least in part, on glucocorticoids. Adrenalectomized acidotic rats did excrete more ammonium than normal animals, but the increase was not as great as measured from nonadrenalectomized acidotic animals or adrenalectomized acidotic rats given dexamethasone. This may be related to the report of an ammoniagenic factor in the plasma of acidotic adrenalectomized rats that was blocked by the α-adrenergic antagonist phentolamine (30). It was noted (Fig. 5) that dexamethasone increased ammonium excretion even in the absence of acidosis. This finding is in accord with the recent report that glucocorticoids were necessary to promote the excretion of ammonium (18). Interestingly, it was found that the Na^+–H^+ carrier also mediated the antiport of Na^+ for NH_4^+ (24). A physiological role for Na^+–NH_4^+ exchange in the proximal

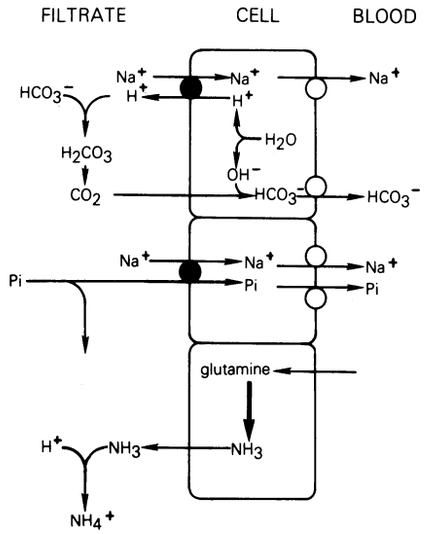

FIG. 6. Schematic model of the processes in the renal proximal tubule involved in the adaption to metabolic acidosis. The closed circles represent tubular functions that may be regulated by glucocorticoids.

tubule has not been ascertained because NH_3 readily diffuses across the renal plasma membranes (31).

We propose that glucocorticoids are important in orchestrating proximal tubular adaptation to metabolic acidosis. An outline for this model is presented schematically in Fig. 6. The major responses of the proximal tubule to acidosis are to enhance the retention of HCO_3^- and the excretion of H^+ (1). Glucocorticoids, by increasing the activity of Na^+–H^+ exchange (ref. 17 and Fig. 4), would contribute to the acidification of the tubular fluid. There, HCO_3^- would act as a H^+ acceptor giving rise to H_2CO_3 and, thereby, CO_2. Carbon dioxide could diffuse across the luminal membrane, where upon reassociation with OH^-, it would form intracellular HCO_3^-. The anion would then be transported across the basolateral membrane, effecting the transepithelial movement of HCO_3^-. If the rate-limiting step for the reabsorption of HCO_3^- in the proximal tubule in metabolic acidosis is the activity of Na^+–H^+ exchange, then the glucocorticoid-induced increase in exchange activity would enhance HCO_3^- reabsorption. Phosphate is reabsorbed by a Na^+-dependent mechanism in the proximal tubule (3). Others have shown that the V_{max} for phosphate uptake in brush border membrane vesicles is lowered in acidosis (28, 29) and we have demonstrated that dexamethasone decreases phosphate transport *in vivo* (Fig. 5) and in isolated brush border membrane vesicles (17). Because circulating levels of glucocorticoids are known to be elevated in acidosis (8–10), it seems reasonable to hypothesize that the increased excretion of phosphate in acidosis might be related to higher levels of plasma glucocorticoids. Analogously, ammonia production from its major precursor glutamine is enhanced in acidotic animals (32). Moreover, glucocorticoids are required for the maximal excretion of ammonium (Fig. 5). Thus, the possibility that the increased excretion of ammonium in acidosis is mediated by glucocorticoids merits consideration.

In summary, we suggest that regulation by glucocorticoids provides a mechanism for the concerted response of the proximal tubule to metabolic acidosis. The consequences of the hormonal actions would be the greater retention of HCO_3^- and the increased excretion of H^+ in the form of phosphate buffer and ammonium.

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