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# Regulation of nephron acidification by corticosteroids

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## Abstract

The present paper reviews work from our laboratories evaluating the importance of adrenal cortical hormones in acidification by proximal and cortical distal tubules. Proximal acidification was determined by stationary microperfusion, and measurement of bicarbonate reabsorption using luminal pH determination was performed with $H^+$-ion-sensitive microelectrodes. Rats were adrenalectomized (ADX) 48 h before the experiments, and corticosteroids (aldosterone (A), corticosterone (B), and 18-OH corticosterone (18-OH-B)) were injected intramuscularly 100 and 40 min before the experiments. In ADX rats stationary pH increased significantly to 7.03 as compared to sham-operated rats (6.78). Bicarbonate reabsorption decreased from 2.65 ± 0.18 in sham-operated rats to 0.50 ± 0.07 nmol $cm^{-2}$ $s^{-1}$ after ADX. The administration of the three hormones stimulated proximal tubule acidification, reaching, however, only 47.2% of the sham values in aldosterone-treated rats. Distal nephron acidification was studied by measuring urine minus blood $pCO_2$ differences (U-B $pCO_2$) in bicarbonate-loaded rats treated as above. This $pCO_2$ difference is used as a measure of the distal nephron ability to secrete $H^+$ ions into an alkaline urine. U-B $pCO_2$ decreased significantly from 39.9 ± 1.26 to 11.9 ± 1.99 mmHg in ADX rats. When corticosteroids were given to ADX rats before the experiment, U-B $pCO_2$ increased significantly, but reached control levels only when aldosterone (two 3-µg doses per rat) plus corticosterone (220 µg) were given together. In order to control for the effect of aldosterone on distal transepithelial potential difference one group of rats was treated with amiloride, which blocks distal sodium channels. Amiloride-treated rats still showed a significant reduction in U-B $pCO_2$ after ADX. Only corticosterone and 18-OH-B but not aldosterone increased U-B $pCO_2$ back to the levels of sham-operated rats. These results show that corticosteroids stimulate renal tubule acidification both in proximal and distal nephrons and provide some clues about the mechanism of action of these steroids.

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- Corticosterone
- Bicarbonate reabsorption
- Amiloride
- Urine
- $pCO_2$

## Introduction

It is known that the main action of mineralocorticoids on urinary acidification occurs in the distal nephron via stimulation of apical vacuolar $H^+$-ATPase (1,2). Both whole animal studies on the adrenalectomized (ADX) rat and microperfusion studies of adrenalectomized rabbit collecting duct have demonstrated the importance of adrenocortical steroids for urinary acid excretion and bicarbonate reabsorption (3,4). The mechanism of action of these hormones involves the regulation of gene transcription and incorporation of new transporters ($Na^+$-$K^+$-ATPase) and $Na^+$ channels into cell membranes (5,6). In addition, rapid, non-genomic stimulation of electrolyte transport has been observed in a number of extrarenal cells, including lymphocytes and vascular smooth muscle cells. This effect is mediated by specific membrane receptors for aldosterone, which lead to the incorporation or activation of pre-existing sodium channels, elevation of cell sodium and secondary activation of the basolateral $Na^+$-$K^+$-ATPase, responsible for an increase in electrolyte transport within a few minutes after hormone addition (7).

Corticosteroids have been shown to affect other acid/base transporters besides $H^+$-ATPase. Aldosterone stimulates $Na^+/H^+$ exchange in renal early distal amphibian tubules (1,2), and in cultured MDCK cells (8), specifically on their basolateral surface (9). These cells are derived from canine kidney, and have several properties in common with distal nephron $\alpha$-intercalated cells. Aldosterone has also been shown to stimulate $Cl^-/HCO_3^-$ exchange, both in MDCK cells (8) and in cardiac cells (10), as well as the $H^+$-$K^+$-ATPase of the apical membrane of MDCK cells (11). In addition, glucocorticoids have been shown to stimulate $Na^+/H^+$ exchange in OKP cells in culture and in rat distal colon cells (12,13). Glucocorticoids act via stimulation of the expression of different forms of these exchangers, in particular NHE1 and NHE3 (14,15).

The observation that corticosteroid hormones act on the $Na^+/H^+$ exchanger and $H^+$-$K^+$-ATPase, in addition to the vacuolar $H^+$-ATPase led us to study the effect of these hormones on $H^+$-ion secretion in renal proximal tubules by microperfusion techniques. In addition, we studied the role of corticosteroid hormones in distal nephron acidification by the determination of urine minus blood $pCO_2$ (U-B $pCO_2$) differences. Urine $pCO_2$ may have a number of origins, including delayed dehydration of carbonic acid, trapping of $CO_2$ in the countercurrent system, mixture of urines at different pH values from different nephrons, and ampholyte properties of bicarbonate buffer, among others (16). However, U-B $pCO_2$ has also been considered to represent the magnitude of distal nephron (mostly collecting duct) $H^+$-ion secretion at high urinary bicarbonate concentrations (17,18). In these experiments, corticosterone (B), aldosterone (A) or 18-OH corticosterone (18-OH-B) was given to ADX rats in order to investigate the role of each of them in supporting the normal rate of renal tubule $H^+$ secretion. 18-OH-B is a natural steroid of the biosynthetic pathway of aldosterone, and has been shown to stimulate titratable acid excretion and to reduce urine pH in ADX rats (19,20). In the present paper we review some of the work performed in our laboratories in this area.

## Material and Methods

### Microperfusion studies

Wistar rats were sham operated and adrenalectomized (ADX) under ether anesthesia 48 h before the experiments. Rats received a standard pellet diet and saline substituted for drinking water. Hormone supplementation was administered as follows (21): 220 µg corticosterone was given intramuscularly 100 min before the experiment,

and 3 µg aldosterone or 6 µg 18-OH-B was given 100 and 40 min before the experiment. These doses lead to blood levels of these hormones that are within the upper limits of the physiological range (22,23). Rats were prepared for micropuncture as previously described (24). Stationary microperfusion was performed with double-barrelled pipettes blocking droplets of 25 mM bicarbonate Ringer solution with castor oil in the tubule lumen. These fluid droplets were punctured with pH-sensitive microelectrodes and the pH was followed from the initial alkaline value (approximately pH 8) to the stationary level. Bicarbonate concentrations were calculated from these curves and from arterial blood $pCO_2$, and bicarbonate fluxes were obtained from their rate of disappearance and tubular geometry (24). Urine was collected from the bladder during experiments, and GFR was determined by inulin clearance. Sodium and potassium in urine were determined by flame photometry.

### Urine-blood $pCO_2$ studies

Adrenalectomy and hormone supplementation were performed as described above. The rats received an infusion of 0.6 M $NaHCO_3$ plus 5% mannitol during the experiments which raised urine pH to about 7.8. Urine pH and $pCO_2$ were determined with a radiometer model BMS3 MK2 blood micro system (Radiometer, Copenhagen, Denmark). Urine $pCO_2$ was plotted against urine bicarbonate concentration, and the $pCO_2$ at 150 mM (or 120 mM in experiments in which amiloride was given) urine bicarbonate was obtained from the respective regression lines. Comparisons between groups were made on the basis of these values. In one group of rats, a priming dose of amiloride of 0.4 mg/100 g body weight was given, followed by an infusion of 1 mg 100 $g^{-1}$ $h^{-1}$, and similar procedures as described above were performed.

## Results and Discussion

Figure 1 shows the general conditions of the rats in this study. The mean control arterial blood pressure of 129.4 ± 5.75 mmHg decreased to 100.9 ± 8.67 mmHg ($P<0.05$) in ADX, but did not recover in supplemented rats. Urine sodium/potassium ratios increased significantly after ADX, confirming corticoid hormone depletion. Hormone supplementation caused complete recovery only when aldosterone was given. GFR decreased

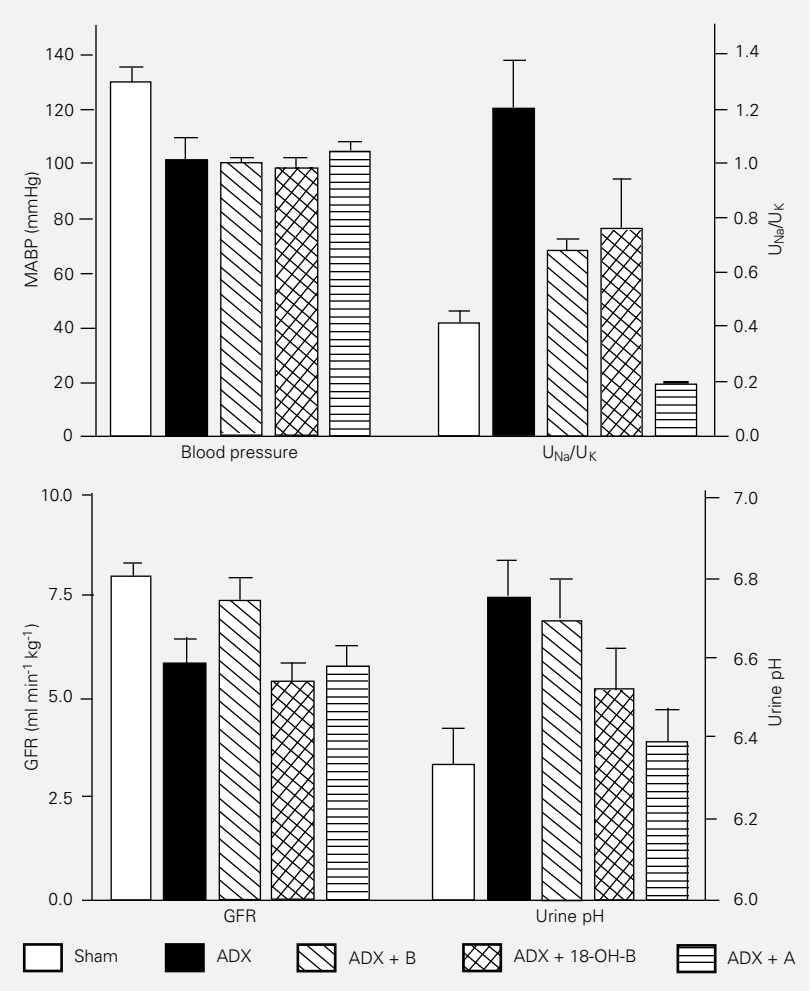


Figure 1 - General conditions of sham-operated and adrenalectomized (ADX) rats, and the effect of corticosterone (B), aldosterone (A) and 18-hydroxycorticosterone (18-OH-B) on ADX rats. $U_{Na}/U_K$, Urine $Na^+/K^+$ concentration ratio; GFR, glomerular filtration rate per kg rat; MABP, mean arterial blood pressure. Data are reported as means ± SEM. Data from Ref. 21, with permission.

from a control value of 7.98 ± 0.36 ml $min^{-1}$ kg body $weight^{-1}$ to 5.77 ± 0.66 ml $min^{-1}$ $kg^{-1}$ in ADX, and recovered only in corticosterone-supplemented rats, as previously shown (25). Urine pH increased markedly from 6.76 ± 0.020 in sham-operated rats to 7.03 ± 0.028 in ADX rats, and recovered entirely only in aldosterone-treated animals. These whole-animal data show that the rats in our experiments conform to the findings generally encountered in corticosteroid-depleted animals, indicating the role of these hormones in urinary acidification.

Figure 2 shows results obtained in the experiments of *in vivo* microperfusion of convolute cortical proximal tubules (S2 segments). Stationary bicarbonate concentrations increased markedly from 5.97 ± 0.23 mM in controls to 14.1 ± 0.88 mM in ADX, corresponding to a rise in tubule lumen pH from 6.76 ± 0.020 in controls to 7.03 ± 0.028 in ADX. Stationary bicarbonate returned to normal with aldosterone and almost to normal after supplementation with the other steroids. Bicarbonate reabsorption ($J_{HCO_3^-}$) fell markedly from 2.65 ± 0.18 nmol $cm^{-2}$ $s^{-1}$ in sham-operated rats to 0.50 ± 0.07 nmol $cm^{-2}$ $s^{-1}$ in ADX rats. During hormone supplementation recovery was only partial with all steroids, reaching only a value in the range of 1.2 to 1.3 nmol $cm^{-2}$ $s^{-1}$. This reduction in reabsorption rates was mostly due to a delay in the rate of fall in luminal bicarbonate concentrations. Half-times of luminal disappearance of bicarbonate increased from 3.73 ± 0.17 s in sham-operated rats to 11.43 ± 0.72 s in ADX rats. Proximal acidification was reduced in these experiments by 73% in ADX, which cannot be explained only by inhibition of proximal $H^+$-ATPase, since this transporter is responsible for at most 30% of proximal bicarbonate reabsorption (26). This finding suggests a role of these hormones in the stimulation of luminal insertion or turnover of $Na^+/H^+$ exchanger molecules.

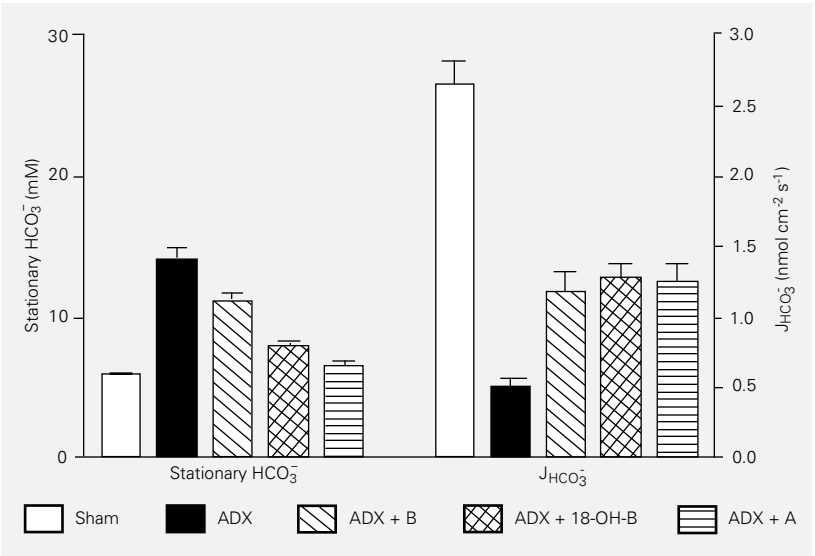


Figure 2 - Stationary bicarbonate concentrations ($HCO_3^-$s) and net bicarbonate reabsorption ($J_{HCO_3^-}$) in proximal tubules from sham-operated and ADX rats. Experimental groups as in Figure 1. Data from Ref. 21, with permission.

A recent series of experiments using brush-border membrane vesicles and fluorometric pH determination with acridine orange have detected a significant reduction in the rate of $Na^+/H^+$ exchange in vesicles from ADX rats, therefore localizing the hormonal effect to the apical (brush-border) membrane of proximal tubule cells. The origin of this reduction was shown to involve a decrease in $V_{max}$ of the exchanger, without alteration of $K_m$ for $Na^+$ (Igarreta MP, Calvo JC, Paladini A and Damasco MC, unpublished data). Also, in these experiments the administration of corticosterone and 18-OH-B to ADX rats reversed the inhibition of the exchanger.

The following series of experiments was performed to analyze the role of several corticosteroid hormones in acid secretion by the distal nephron, using the urine minus blood $pCO_2$ difference (27). Figure 3 shows a plot relating U-B $pCO_2$ to urine bicarbonate concentrations in control and ADX rats. $CO_2$ is generated when $H^+$ is secreted into a bicarbonate-containing fluid. This $pCO_2$ difference increases with bicarbonate concentration, which is a property of the $CO_2/HCO_3^-$ buffer system. Therefore, it is important that these differences are measured at well-de-

fined urine bicarbonate levels (28). Differences between $H^+$ secretion in experimental groups are not related to the slope of these lines, but to the level of the line. It is clear that in ADX rats, U-B $pCO_2$ values were significantly decreased with respect to controls. Figure 4 shows the mean $pCO_2$ differences plotted against mean urine bicarbonate concentration in the different experimental groups. Again, the marked reduction in U-B $pCO_2$ in ADX rats is apparent. Supplementation with hormones given within 100 min before the experiments raised these values significantly; however, only when aldosterone and corticosterone were given together did the U-B $pCO_2$ values return to the control level, indicating that both hormones contribute to normal urine $pCO_2$. The role of corticosterone in maintaining urine $pCO_2$ may be related to its known effect of increasing GFR, as discussed above, which leads to an enlarged urinary buffer load, represented mostly by phosphate salts. In the rats studied in these experiments, phosphate excretion fell from 2.30 ± 0.17 µEq/min in sham-operated rats to 1.17 ± 0.20 µEq/min in ADX rats, and recovered to 1.90 ± 0.19 µEq/min when corticosterone was given. This rise in urine phosphate excretion may be due to the increased GFR after corticosterone administration. It is also known that the phosphate level in urine is an important factor regulating urine $pCO_2$ (29), which may explain the role of corticosterone in increasing U-B $pCO_2$.

In another group of experiments, we studied the role of amiloride, a blocker of sodium channels which affects the transepithelial potential difference (PD) in hormonal regulation of distal $H^+$ secretion as evaluated by U-B $pCO_2$ (30). Sham-operated and ADX rats treated as above received amiloride during the experiments. The administration of amiloride reduced U-B $pCO_2$ from 49.3 ± 2.7 mmHg in controls to 29.8 ± 3.2 mmHg ($P<0.01$). This finding is commonly attributed to the reduction of collecting duct trans-

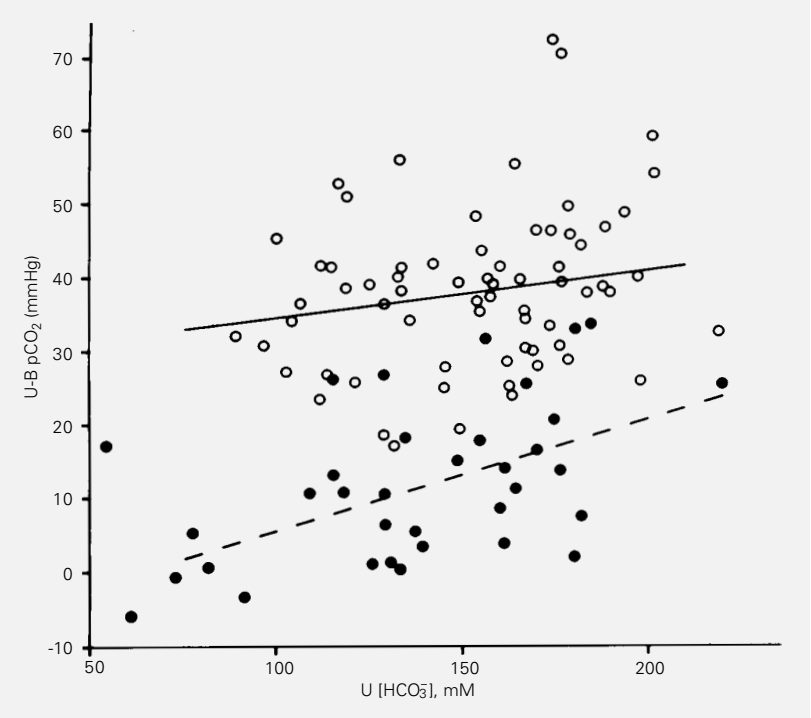


Figure 3 - Urine minus blood $pCO_2$ (U-B $pCO_2$) differences plotted against urine bicarbonate concentration (U) in control (open circles) and adrenalectomized (filled circles) rats. From Ref. 27, with permission.

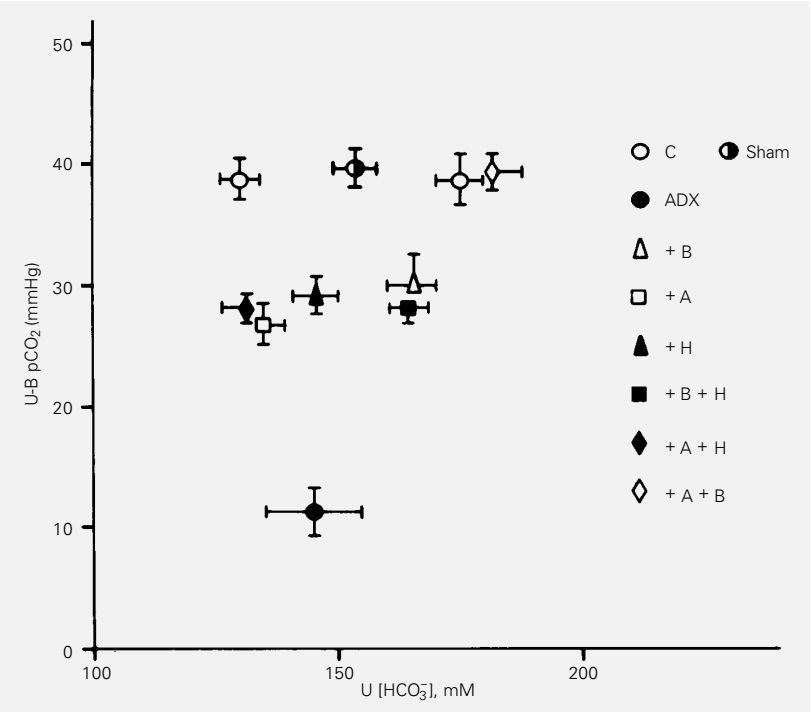


Figure 4 - U-B $pCO_2$ plotted against urine bicarbonate concentrations under different experimental conditions. Data are reported as means ± SEM. Symbols as in Figure 1, except H = 18-OH corticosterone. From Ref. 27, with permission.

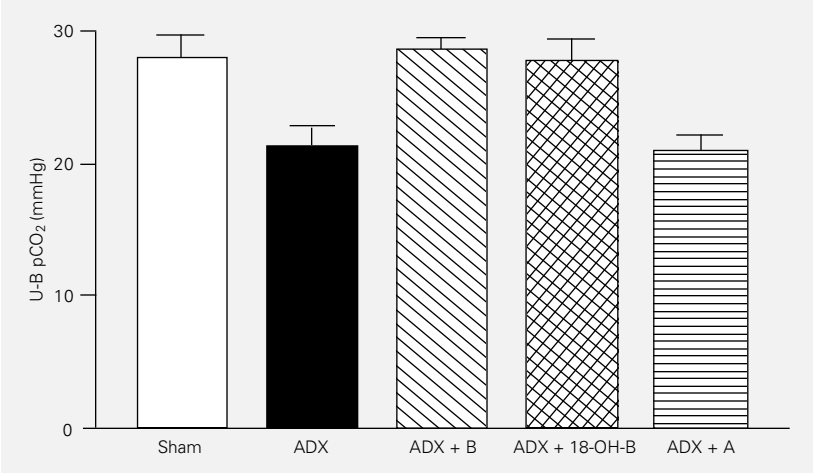


Figure 5 - U-B $pCO_2$ in amiloride-treated rats. Experimental groups as in Figure 1. Data from Ref. 30, with permission.

epithelial PD and, possibly, also to the effect of amiloride on $Na^+/H^+$ exchange. U-B $pCO_2$ was further reduced to 21.3 ± 1.6 mmHg ($P<0.05$) in ADX rats, as shown in Figure 5. Supplementation with corticosterone and 18-OH-B led to recovery toward the $pCO_2$ difference of sham-operated rats. However, in aldosterone-treated rats no change of U-B $pCO_2$ was observed. This finding supports the view that aldosterone stimulates $H^+$-ion secretion by a process related to the presence of activated sodium channels, possibly transepithelial PD. It is well known that amiloride reduces this PD in late distal tubule and in cortical collecting ducts from 30-50 mV, lumen negative, to near zero, impairing a factor that stimulates the transfer of the positive $H^+$ ion into the lumen. On the other hand, corticosterone, as discussed above, may act mainly by its effect on GFR and buffer filtration, leading to greater urinary phosphate excretion, an effect which is not affected by amiloride.

What is the mechanism by which corticosteroid hormones act on acid excretion? A classical explanation proposed by AlAwqati and colleagues (31) based on experiments in turtle bladder assumes that these hormones stimulate $H^+$-ATPase expression or turnover. In the mammalian nephron, several transporters involved in urine acidification have been described. It has been shown that aldosterone stimulates $Na^+/H^+$ exchange in a number of both amphibian (32) and mammalian (8,9,33) tissues and cells in culture. Glucocorticoids have also been shown to stimulate $Na^+/H^+$ exchange in cells in culture (13). This stimulation is thought to occur by membrane insertion or activation of transporters, which is compatible with our findings in the proximal tubule. In these experiments there was only a relatively small effect on the transepithelial pH gradient, which depends on the sodium gradient across the apical cell membrane, and a large modification of the acidification half-time, which, according to a model of proximal tubule cells, depends on the number or turnover of transport sites within the membrane (34). In proximal tubules, not all $H^+$-ion secretion is due to $Na^+/H^+$ exchange, but some 20-30% have been shown to depend on vacuolar $H^+$-ATPase, which of course could also undergo the action of these hormones (26). For the distal nephron, the vacuolar $H^+$-ATPase is the most prevalent $H^+$-ion transporter, although more recently the importance of the gastric type $H^+$-$K^+$-ATPase has been stressed (35). The stimulation mechanisms of these ATPases are probably similar to those of $Na^+$-$K^+$-ATPase and sodium channels of the collecting duct principal cells, although the stimulation of $H^+$-ion transporters by these hormones has not been investigated in comparable detail. Additional factors involved in corticosteroid stimulation of distal $H^+$-ion secretion, as supported by our data, are the transepithelial PD which is known to increase along the collecting duct in hormone-stimulated animals due to the higher density of $Na^+$ channels, depolarizing the apical membrane of collecting duct principal cells (36), and the increase in buffer (phosphate) excretion caused by the higher GFR induced by steroids such as corticosterone.

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