Effects of Corticosteroids on Urinary Ammonium Excretion in Humans

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(J. Am. Soc. Nephrol. 1994; 4:1531–1537)

ABSTRACT

This study was designed to examine the selective effects of glucocorticoid and mineralocorticoid classes of steroid hormones on urinary ammonium excretion in humans. In 22 10-day studies, normal male volunteers received either 9a-fludrohydrocortisone or hydrocortisone, alone or with the receptor antagonist spironolactone or mifepristone. The small but significant increase in ammonium excretion noted with the administration of 9a-fludrohydrocortisone was associated with a significant decrease in serum potassium. In contrast, a significantly larger increase in ammonium excretion was noted with hydrocortisone, without concomitant electrolyte changes. Spironolactone did not alter the effect on ammonium excretion by either corticosteroid, whereas mifepristone markedly blunted the hydrocortisone-induced increase in urinary ammonium excretion. It was concluded that glucocorticoids increase urinary ammonium excretion in humans and that this effect occurs through binding to the Type II (glucocorticoid) receptor rather than by cross-occupancy of the Type I (mineralocorticoid) receptor.

Key Words: Glucocorticoids, 9a-fludrohydrocortisone, steroid receptors, receptor antagonists, mifepristone

It is well recognized that adrenal hormones are critical for the maintenance of acid-base homeostasis, particularly during periods of acute and chronic metabolic acidosis. Despite a large number of studies in various animal models (1–8; see reference 8 for additional references), the specific roles of mineralocorticoid and glucocorticoid hormones in urinary acid excretion have remained controversial, and their mechanisms of action remain to be elucidated.

Many studies have focused on the critical role of aldosterone in distal acidification processes, predominantly through the enhancement of proton-ATPase activity (9–11), and sodium reabsorption, and subsequent luminal electronegativity (12). Through subsequent investigations, it has become increasingly clear that glucocorticoids are perhaps even more pivotal in maximizing renal acid elimination, through proximal tubule effects. In vitro data have demonstrated that glucocorticoids increase apical sodium-proton antiporter activity (13,14), increase antiluminal sodium-bicarbonate symporter activity (15), and decrease sodium-phosphate cotransport (16), with subsequent increased urinary phosphate excretion. Recently, a direct effect of glucocorticoids to increase acidification in rabbit proximal tubule has been shown (17). These findings are consistent with previous reports indicating that the glucocorticoid-mediated increase in urinary ammonium excretion occurred by augmented ammoniagenesis, a recognized proximal tubule event (1–6,8,18,19). The composite data have implied different mechanisms for mineralocorticoid- and glucocorticoid-induced increases in ammonium excretion: for mineralocorticoids, an effect to increase ammonium excretion, and for glucocorticoids, an effect primarily on ammonium production.

Efforts to elucidate the differential physiologic effects of mineralocorticoids and glucocorticoids and their individual sites of action have been complicated by the significant cross-occupancy of the mineralocorticoid receptor (Type I) and the glucocorticoid receptor (Type II) by these corticosteroids (20–22). Autoradiographic techniques have suggested that aldosterone-binding sites are located primarily in the distal tubule and the collecting duct (23), findings confirmed recently by immunolocalization studies (24). Despite some evidence of proximal glucocorticoid binding, results with these methods to determine glucocorticoid-binding sites have been decidedly less consistent (24–26) and without correlation with the observed physiologic glucocorticoid effects.

The purpose of this study was to attempt to estab-
lish a selective role for mineralocorticoids and glu-
corticoids in ammonium excretion in humans. We
administered 9α-fludrohydrocortisone (9αFF) or hy-
drocortisone (HC) with either spironolactone (Sp) or
mifepristone (RU486), receptor antagonists of the
Type I and Type II receptors, respectively, to normal
male volunteers. The results indicate that HC mark-
edly increases urinary ammonium excretion, an
effect that is almost eliminated by Type II, but not by
Type I, receptor blockade.

METHODS

Fifteen normal male volunteers (age range, 23 to
31 yr) participated in 22 10-day studies, portions of
which have been reported previously (27,28). Exclu-
sion criteria included renal disease, diabetes melli-
tus, hypertension, or cardiac or pulmonary disease.
None of the subjects was taking medications. The
study was approved by the Human Investigation
Committee of the Medical College of Virginia, and
informed consent was obtained from each man. The
subjects were admitted to the Clinical Research Cen-
ter and consumed a constant daily diet of their
choice, each calculated to contain approximately 125
mmol of sodium, 80 mmol of potassium, and 1.5 g/
kg of protein, with total calories to approximate their
usual daily consumption. Subjects were weighed
each morning before breakfast, and supine and up-
right blood pressure determinations were obtained at
7:00 a.m. and 7:00 p.m. The initial 5 days of the
study were required for the stabilization of urinary
electrolyte and acid excretion on the constant diet
before steroid administration.

The men were then divided into five groups: Group
I (N = 4) received 9αFF, 1.5 mg orally every 12 h;
Group II (N = 4) received 9αFF, 1.5 mg orally every
12 h, plus Sp, 100 mg orally every 6 h; Group III (N =
4) received HC, 60 mg orally every 6 h; Group IV (N =
6) received HC, 60 mg orally every 6 h, plus Sp, 100
mg orally every 6 h; Group V (N = 3) received HC, 60
mg orally every 6 h, plus RU486, 300 mg orally every
6 h on Days 6 and 7 only. RU486 is very slowly
metabolized, and at this dosage, the HC-induced in-
crease in serum insulin is still suppressed at the end
of the study by the Type II receptor antagonist (27).
These are stress-level HC dosages and 10-time re-
placement doses of 9αFF. Fasting blood samples were
obtained at the initiation of the study, on the morning
of Day 6 (before drug administration), and on Day 9
(after 3 days of drug). Freshly voided urine was col-
cected over a 24-h period under a 1/4-inch layer of
mineral oil on Days 3 through 10 of all studies. Multi-
ple aliquots of each sample were placed in 20-
ml scintillation vials, capped, and then frozen at
−70°C for the subsequent determination of urinary
electrolytes and net acid excretion.

Serum electrolytes were determined by the use of
a Technicon Automated Analyzer (Tarrytown, NY).
Urinary Na⁺ and K⁺ were assayed with an IL flame
photometer 943 (Instruments Laboratories, Lexing-
ton, MA). Titratable acid and ammonium concen-
trations were determined in the laboratory of Dr. J.C.M.
Chan by the titrimetric method (29). Briefly, samples
were thawed at room temperature for ~2 h. A 1-mL
aliquot was pipetted into thermostated vials contain-
ing 1 mL of 0.075 N HCl. The mixture was heated in
a boiling water bath for 10 min to drive out all CO₂
and then cooled for 10 min. The samples were ti-
trated with 0.05 N NaOH to pH 7.40 at 37°C on a
Radiometer Autotitrator TTT 85 (including combi-
nation pH probe, NaOH measuring probe, stirrer, and
burette) with an ABU 80 autoburette (Radiometer,
Westlake, OH). The blanks (deionized water) were
treated identically. The concentration of titratable
acidity was calculated as the difference in volumes of
NaOH required to titrate 1 mL of sample and blank,
multiplied by the normality of the NaOH, and the
results are expressed as micromoles per liter. One
milliliter of 8% formaldehyde was then added to acid-
ify the sample. After the titrator was reset, the titra-
tion to pH 7.4 was repeated to allow the calculation
of the ammonium (NH₄⁺) concentration (28,30). The
blank was treated in the same fashion. Titrations
were carried out in triplicate for each sample as well
as blank.

Statistical comparisons between electrolyte and
acid excretion within groups under baseline versus
experimental conditions, as well as between groups,
were determined by analysis of variance with AN-
COM software (Scilab, Inc., Guildersky, NY). Values
of P < 0.05 were considered significant, taking into
account the number of analyses performed.

RESULTS

Effects of 9αFF, HC, Sp, and RU486 on Serum
Electrolytes and Urinary Potassium Excretion

Control values for weight, urine volume, and uri-
nary electrolyte excretion for all of the subjects are
summarized in Table 1. Serum values obtained in
the early morning on Day 6 (after 3 days on control
diet but before the initiation of corticosteroids ± an-
tagonists) and on Day 9, as well as 24-h creatinine
clearances, are summarized in Table 2. Serum potas-
sium concentrations and urinary potassium excre-
tion were not different between groups at baseline.
There was a significant decrease in serum potassium
in Group I during the experimental period (3.9 versus
3.4 mmol/l P < 0.05). This corresponded to a cumulative
increase in K⁺ excretion, compared with control, of
78 ± 18 mmol over 5 days. The hypokalemic and
kaliuretic responses appeared to be blunted by Sp
(serum K⁺ 4.1 versus 4.0 mmol; not significant), with
TABLE 1. Control values for urinary electrolytes*

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight (kg)</th>
<th>Urine Vol</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Cl⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>9aFF</td>
<td>73.7 ± 1.5</td>
<td>1.45 ± 0.04</td>
<td>114.2 ± 13.2</td>
<td>59.8 ± 5.0</td>
<td>106.9 ± 11.7</td>
</tr>
<tr>
<td>(9aFF + Sp)</td>
<td>70.0 ± 1.2</td>
<td>1.56 ± 0.17</td>
<td>123.8 ± 17.1</td>
<td>56.1 ± 6.2</td>
<td>112.8 ± 14.9</td>
</tr>
<tr>
<td>(HC)</td>
<td>68.5 ± 0.8</td>
<td>1.45 ± 0.24</td>
<td>118.5 ± 15.6</td>
<td>77.4 ± 14.6</td>
<td>116.7 ± 19.0</td>
</tr>
<tr>
<td>(HC + Sp)</td>
<td>71.4 ± 1.5</td>
<td>1.87 ± 0.21</td>
<td>104.2 ± 5.9</td>
<td>49.4 ± 3.0</td>
<td>101.4 ± 4.9</td>
</tr>
<tr>
<td>(HC + RU486)</td>
<td>68.6 ± 1.3</td>
<td>0.97 ± 0.10</td>
<td>136.2 ± 19.8</td>
<td>52.4 ± 6.6</td>
<td>125.5 ± 15.1</td>
</tr>
</tbody>
</table>

*Urine volume is expressed in liters/24 h. Urinary electrolytes are expressed as millimoles/24 h.

TABLE 2. Serum electrolytes and creatinine clearances*

<table>
<thead>
<tr>
<th>Group</th>
<th>[Na⁺]</th>
<th>[K⁺]</th>
<th>[Cl⁻]</th>
<th>[CO₂]</th>
<th>Creatinine Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>9aFF</td>
<td>143 ± 1.0</td>
<td>3.9 ± 0.1</td>
<td>105 ± 0.6</td>
<td>25.8 ± 0.7</td>
<td>107 ± 15</td>
</tr>
<tr>
<td>(9aFF + Sp)</td>
<td>144 ± 0.3</td>
<td>3.4 ± 0.4*</td>
<td>107 ± 1.1</td>
<td>26.2 ± 0.6</td>
<td>139 ± 13</td>
</tr>
<tr>
<td>(HC)</td>
<td>141 ± 0.9</td>
<td>4.1 ± 0.2</td>
<td>104 ± 0.4</td>
<td>26.5 ± 1.0</td>
<td>116 ± 5</td>
</tr>
<tr>
<td>(HC + Sp)</td>
<td>144 ± 0.0</td>
<td>4.0 ± 0.1</td>
<td>104 ± 0.4</td>
<td>24.0 ± 0.6</td>
<td>106 ± 17</td>
</tr>
<tr>
<td>(HC + RU486)</td>
<td>142 ± 1</td>
<td>4.2 ± 0.2</td>
<td>104 ± 1.3</td>
<td>25.3 ± 1.4</td>
<td>123 ± 12</td>
</tr>
<tr>
<td>Day 6</td>
<td>143 ± 1</td>
<td>4.1 ± 0.1</td>
<td>105 ± 0.4</td>
<td>26.8 ± 0.9</td>
<td>115 ± 8</td>
</tr>
<tr>
<td>Day 9</td>
<td>142 ± 1</td>
<td>4.0 ± 0.1</td>
<td>104 ± 0.7</td>
<td>25.5 ± 0.9</td>
<td>131 ± 9</td>
</tr>
<tr>
<td>Day 6</td>
<td>142 ± 1</td>
<td>4.2 ± 0.1</td>
<td>104 ± 0.7</td>
<td>26.3 ± 1.0</td>
<td>133 ± 9</td>
</tr>
<tr>
<td>Day 9</td>
<td>144 ± 1</td>
<td>3.8 ± 0.1</td>
<td>105 ± 0.3</td>
<td>25.7 ± 0.5</td>
<td>81.4 ± 1.8</td>
</tr>
<tr>
<td>Day 6</td>
<td>145 ± 0.3</td>
<td>3.8 ± 0.1</td>
<td>111 ± 0.5</td>
<td>25.3 ± 0.5</td>
<td>90.4 ± 13</td>
</tr>
</tbody>
</table>

*Electrolyte concentrations are expressed in millimoles per liter, as mean ± SE. Creatinine clearance is expressed in milliliters per minute per 1.73 m², also as mean ± SE. Brackets indicate concentrations of substances.

*P < 0.05, Day 6 versus Day 9.

a cumulative increase in K⁺ excretion of 25 ± 58 mmol, a value not different than controls.

The administration of HC alone (Group III) and with either Sp (Group IV) or RU486 (Group V) effected no change in serum potassium values. Cumulative K⁺ excretion was slightly increased over baseline in all groups; however, this can be attributed predominantly to increased kaliuresis on Day 6 (data previously published [27,28]). Other serum electrolytes and creatinine clearances did not change significantly in any group during the experimental period.

Effects of 9aFF, HC, Sp, and RU486 on Urinary Ammonium Excretion

The effect of 9aFF and HC on urinary ammonium excretion is shown in Figure 1. Baseline ammonium excretion was approximately the same in all groups. Although there was a slight rise in ammonium excretion with the administration of 9aFF, the increase was significant only on Day 9 (Day 4 of 9aFF treatment). The increase seen with HC was considerably greater and was significant on Days 7 to 10 (Days 2 to 5 of HC treatment). The cumulative change in urinary ammonium excretion during the experimental period (Table 3) was 219 ± 17.4 mmol with HC (P < 0.05) compared with 84.9 ± 26.1 mmol with 9aFF; (P > 0.05; not significant).

The coadministration of Sp with either steroid did not significantly alter the individual responses. In contrast, RU486 completely attenuated the HC-induced increase in urinary ammonium excretion (Fig. 1; Table 3). This finding indicates that the glucocorticoid stimulus to enhanced renal ammoniagenesis is mediated specifically by the Type II receptor.

Mean daily urine pH values for Groups I and II are shown in Figure 2. Urine pH decreased significantly in Group I (9aFF) only on Day 6 (Day 1 of treatment)
TABLE 3. Control and cumulative change in ammonium excretion for each treatment group

<table>
<thead>
<tr>
<th>N</th>
<th>Control</th>
<th>Cumulative Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (9αFF)</td>
<td>4</td>
<td>34.3 ± 2.1</td>
</tr>
<tr>
<td>Group II (9αFF + Sp)</td>
<td>4</td>
<td>23.3 ± 2.8</td>
</tr>
<tr>
<td>Group III (HC)</td>
<td>4</td>
<td>38.3 ± 2.8</td>
</tr>
<tr>
<td>Group IV (HC + Sp)</td>
<td>6</td>
<td>36.4 ± 3.5</td>
</tr>
<tr>
<td>Group V (HC + RU486)</td>
<td>3</td>
<td>42.4 ± 2.5</td>
</tr>
</tbody>
</table>

*Control values are given in millimoles per 24 h; cumulative change values are in millimoles. All results are given as mean ± SE. Control ammonium excretion was determined from the average of 3 days' measurements for each subject. The cumulative change in ammonium excretion was calculated as the sum of the difference between excretion on each of five treatment days minus control. One treatment day's urine collection was not obtained in one subject in Group I; hence the N was 4 instead of 5.

* P < 0.05 versus 9αFF.
* P < 0.05 versus HC alone.

Compared with each of the three control days (P < 0.05). There was no change in urine pH from control values in any of the other groups (P > 0.05). When mean urine pH of the three control days was compared with that of the five treatment days, no difference was observed with either 9αFF or HC treatment. In addition, although any tendency for urine pH to decrease with 9αFF was obliterated by Sp, this effect was also not significant. 9αFF control and treatment urine pH values were 6.77 ± 0.26 and 6.23 ± 0.05, respectively (P > 0.05); 9αFF + Sp control and treatment urine pH values were 6.52 ± 0.16 and 6.42 ± 0.17, respectively (P > 0.05). Likewise, HC control and treatment urine pH values were 6.36 ± 0.09 and 6.47 ± 0.09, respectively (P > 0.05).

There was no significant increase in titratable acid excretion in any group (data not shown). When cumulative change in titratable acid excretion during the experimental period was calculated, there was also no significant change. For example, cumulative change in titratable acid excretion was 25.7 ± 6.1 and 20.7 ± 11.3 mmol, respectively, with 9αFF and 9αFF Sp (P > 0.05).
DISCUSSION

The critical role of adrenal hormones in the maintenance of acid-base homeostasis has long been appreciated (1,2). However, there have been significant difficulties in elucidating the differential physiologic effects of mineralocorticoids and glucocorticoids and their specific mechanisms of action, particularly in humans (31,32). This is largely related to the significant binding of glucocorticoids to Type I receptors and of mineralocorticoids to Type II receptors (20-22,33); therefore, despite the use of highly selective steroids, there has continued to be a question of cross-occupancy. With this question in mind, a principal goal of this study was the elucidation of the specific steroid receptor through which the glucocorticoids exert their effect on renal ammoniagenesis in humans.

Sp is a synthetic 17-spirolactone steroid with recognized selectivity and specificity as a competitive Type I receptor antagonist (34). As presented in a previous article, the dosage of Sp chosen was adequate to completely inhibit the sodium retention induced by the same amount of 9αFF (28). In this study, Sp inhibited the hypokalemia and the associated kaliuresis but did not inhibit the small increase in ammonium excretion caused by 9αFF; in contrast, it had no effect on the substantial glucocorticoid-mediated increase in ammonium excretion. Together, these findings suggest that the increase in ammonium excretion observed in Group I was due to a glucocorticoid effect of 9αFF, indicating cross-occupancy of the glucocorticoid receptor by high-dose 9αFF.

Although predominantly recognized as an antagonist of progesterone receptors, RU486, an 11-β substituted derivative of norethindrone, first synthesized in 1980, also binds with high affinity to the rat glucocorticoid receptor (35,36). It does not bind to mineralocorticoid receptors. The dosage used in this study was sufficient to inhibit the HC-mediated rise in fasting serum glucose concentrations, as well as the subsequent increase in insulin level, as we reported previously (27). The marked diminution in ammonium excretion demonstrated by Group V as compared with Group IV (corticosteroid + Type II inhibitor versus corticosteroid alone) provides strong evidence that the Type II receptor is the putative corticosteroid receptor involved in glucocorticoid-mediated renal ammonium excretion. To our knowledge, this is the first study in humans in which specific receptor antagonists have been used to delineate this relationship.

The data from earlier in vivo animal studies were strongly suggestive of a separate role for mineralocorticoids and glucocorticoids in renal acidification processes. In adrenalectomized dogs supplemented with glucocorticoid replacement only, Hulter et al. demonstrated that urinary ammonium excretion did not change in these normokalemic animals, whereas urinary pH was comparatively higher than that of acid-loaded, mineralocorticoid-replete animals (5). Adrenalectomized dogs supplemented with mineralocorticoid exhibited a marked increase in urinary ammonium and net acid excretion that exceeded the concomitant increase in exogenous acid production when high- compared with low-dose dexamethasone replacement was given (7,8). Wilcox et al. demonstrated in adrenalectomized rats that mineralocorticoids principally reduced urinary pH whereas glucocorticoids increased buffer excretion, both as phosphate and ammonia (6). These data suggest that the major effect of mineralocorticoids is to increase renal H+ secretory capacity, with a resultant increase in ammonium excretion; glucocorticoids, in contrast, increase ammonium production by an undefined mechanism in the proximal tubule. The tendency for urine pH to decrease in Group I of our study is consistent with this formulation.

The critical role of potassium homeostasis in renal ammoniagenesis was confirmed in previous studies (36). In vitro data suggested that potassium depletion resulted in an adaptive increase in renal ammonium production, whereas hyperkalemia had a suppressive effect (37,38). Indeed, the relatively intact maintenance of urinary ammonium excretion in glucocorticoid-replaced, normokalemic adrenalectomized dogs noted above was not seen in the hyperkalemic group (5). Wilcox et al. maintained all of their adrenalectomized animals on a low-potassium diet to eliminate this potential variable in the study of corticosteroid effects on renal acid elimination (6). In this study, although Sp reversed the sodium retention (28) and hypokalemia induced by 9αFF, it did not inhibit the modest increase in ammonium excretion resulting from the latter agent. This finding suggests that the decrease in serum potassium, although significant, may not have been sufficient to stimulate ammonium production and excretion substantially.

The importance of acid-base status on urinary ammonium excretion has also been recognized. Welbourne established in intact rats that the increase in ammonium excretion observed under acidic conditions, resulting from elevated renal ammonium production and attendant glutamine utilization (4), was significantly blunted in adrenalectomized animals, but was restored by the administration of low doses of triamcinolone (18). Even under normal acid-base conditions, high doses of administered glucocorticoid resulted in increased ammonium production (1,18). Welbourne has suggested that the stimulation of a pituitary-adrenal-renal axis occurs in acidosis, with corticosterone being the putative hormone involved in the activation of the renal mitochondrial pathway.
in rats (2). However, this hypothesis is not supported by data obtained in humans (32,39).

Previous studies in humans to date have addressed the differential effect of the corticosteroid classes on urinary acidification processes (40,41). In this study, through the use of specific Type I and Type II receptor antagonists, we have established in humans that the physiologic glucocorticoid HC causes a marked increase in urinary ammonium excretion that is significantly greater than that seen with 9aFF (Table 3). The inhibition of the HC-induced increase in ammonium excretion by RU486, but not by Sp (Figure 1 and Table 3), indicates that this effect is mediated specifically via the Type II, glucocorticoid, receptor. Glucocorticoids play a significant and selective role in renal acid elimination in humans. They likely exert their effect in the proximal tubule through increased ammonium production and excretion, which is mediated through binding to the Type II receptor. There has been marked expansion in the understanding of the glucocorticoid induction pathway, of the structure and function of steroid receptors (21,42,43), and of the biochemistry of renal ammoniagenesis (31,35,44). Extensive investigations have suggested integrative mechanisms through which glucocorticoids might influence the critical enzymatic pathways involved (18). Nonetheless, because of the complex mode of action of the steroid hormones, more studies are needed to delineate the individual steps and the regulation of the signal transduction pathway by which glucocorticoids stimulate renal ammoniagenesis.

ACKNOWLEDGMENTS

This work was supported in part by Grants DK36822, DK35341, and RR00065 from the NIH and by an A.D. Williams Grant, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA. Mifepristone was generously provided by Dr. Andre Ulmann, Institute/Roussel-UCLAF (Romainville, France). The authors thank Mrs. Martha D. Welions and Dr. James CM. Chan for technical advice.

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