Clinical Studies

Converting-Enzyme Inhibitor Administration Lowers Urinary Free 19-Nor-deoxycorticosterone Levels

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SUMMARY 19-Nor-deoxycorticosterone (19-nor-DOC) is a human mineralocorticoid. The regulation of its secretion is poorly understood, as renin angiotensin II (ANG II) stimulation has minimal effects on 19-nor-DOC. This study sought to determine if ANG II inhibition would decrease 19-nor-DOC production. Six normal subjects on fixed electrolyte intake were admitted to a metabolic unit. After a 5-day control period to establish electrolyte balance, enalapril, p.o., 10 mg/day, was administered for 28 days. This treatment resulted in ANG II inhibition, which was reflected by a rise in plasma renin activity, a blunting of the postural plasma aldosterone increment, and a decrease in aldosterone secretion rate (ASR). Levels of urinary free (UF) 19-nor-DOC progressively decreased from 294 ± 108 ng/day on Day 0 to 164 ± 70 on Day 3, 101 ± 38 on Day 7, 68 ± 18 on Day 21, and 106 ± 31 on Day 28. The decrease in 19-nor-DOC levels was synchronous, with the fall in ASR (R = 0.94, n = 5, p < 0.005), but it was of greater magnitude (71% decrease in 19-nor-DOC levels versus 41% decrease in ASR). In addition, the decrease in 19-nor-DOC levels correlated with a fall in urinary potassium and an increase in both urinary sodium and chloride (R = 0.68, –0.79, –0.87 respectively; n = 6, p < 0.05). The fall in ASR, on the other hand, was not significantly correlated with the changes in these urinary electrolyte levels (R = 0.65, 0.64, 0.57 respectively; n = 5). Converting-enzyme inhibitor administration with enalapril appears to lower UF 19-nor-DOC levels, which suggests either that ANG II regulates basal production or that other non-ANG II-related enalapril actions alter production or metabolism of 19-nor-DOC. Furthermore, decreases in 19-nor-DOC levels could partially account for some of the ability of converting-enzyme inhibitors to reduce mineralocorticoid activity. (Hypertension 7: 178-181, 1985)

The mineralocorticoid 19-nor-deoxycorticosterone (19-nor-DOC) has been isolated from the urine of rats with regenerating adrenal glands and, more recently, from humans. The levels of urinary free (UF) 19-nor-DOC are consistent with biological activity in both rats and humans, but the factors regulating 19-nor-DOC production remain unknown. Previous studies have demonstrated that urinary 19-nor-DOC levels are markedly stimulated by adrenocorticotropic hormone administration and are suppressed by dexamethasone administration. The influence of the renin-angiotensin system on 19-nor-DOC production, however, is more controversial. In human studies this laboratory found that neither dietary sodium restriction (10 mEq/day), sodium supplementation (250 mEq/day), or long-term diuretic-induced renin stimulation (hydrochlorothiazide, 50 mg/day) substantially influenced 19-nor-DOC secretion. Gomez-Sanchez and colleagues, however, described a threefold increase in UF 19-nor-DOC levels following 3 days of sodium restriction in rats, and they concluded that 19-nor-DOC production may be influenced by angiotensin II (ANG II).

This study was undertaken to determine if ANG II inhibition by the converting-enzyme inhibitor (CEI) enalapril would decrease UF 19-nor-DOC levels. The results of the study indicate that enalapril lowers 19-nor-DOC levels in parallel with, but to a greater degree than, aldosterone secretion. Although this finding suggests that ANG II may be important for basal 19-nor-DOC production, it is possible that enalapril's action on 19-nor-DOC production or metabolism may be independent of ANG II. In addition, enalapril's ability...
to alter normal urinary electrolyte ratios could be partly responsible for the observed decrease in UF 19-nor-DOC levels.

Methods
Written informed consent, approved by the Institutional Review Board, was obtained from six normal subjects. There were four men and two women with a mean weight of 164 ± 20 lbs (SD) and a mean age of 25 years (range, 23–28 years). Subjects stopped taking concomitant medications (including estrogens, contraceptives, diuretics, or corticosteroids) at least 4 weeks before the study began.

Subjects were admitted to a metabolic unit and fed an isocaloric, constant diet (sodium 128 mEq/day and potassium 80 mEq/day). After a 5-day control period (Days -4 to 0) to establish electrolyte balance, volunteers received a CEI (enalapril, p.o., 10 mg/day) for 7 days (days 1–7). The volunteers left the metabolic unit on Day 8 and continued the enalapril regimen on an ad libitum diet on Days 8 through 13, 15 through 20, and 22 through 27. They returned to the metabolic unit and resumed the metabolic diet on Days 14, 21, and 28.

Blood and urine specimens were collected daily while the subjects were in the metabolic unit. Blood was drawn at 0800 hours (before drug administration and after 8 hours of overnight recumbency) and again at 1200 hours (4 hours after drug administration and upright posture). Blood measurements included electrolyte levels, plasma renin activity (PRA), and plasma aldosterone levels obtained by standard assay methods. In addition, 24-hour urine collections were monitored for electrolyte levels, creatinine excretion, and urinary tetrahydroaldosterone. The aldosterone secretion rate (ASR) was measured on Days 0 (Day 5 of the control period), 7, 14, 21, and 28 of the study. The ASR was calculated by methods previously described. Briefly, $^{3}H$-aldosterone of known specific activity was injected, and the specific activity of urinary $^{3}H$-tetrahydroaldosterone was measured by radioimmunoassay following derivatization and chromatography. The interassay variation is 10.3% and the intraassay variation is 9.1% for this method.

The ASR (Figure 1) appeared to fall slightly on Day 7, but this decrease did not reach statistical significance. The CEI action was manifested by an increase in upright PRA. Upright PRA was maximal on Day 4 (20.5 ± 5.8 ng ANG II/ml/hr versus 3.3 ± 0.8 ng ANG II/ml/hr on Day 0; $p < 0.05$), and it remained slightly elevated up to Day 28 (9.0 ± 2.9 ng ANG II/ml/hr; $0.1 > p > 0.05$). Supine PRA increased to a lesser extent with administration of enalapril (3.9 ± 0.6 ng ANG II/ml/hr on Day 4 versus 1.8 ± 0.6 ng ANG II/ml/hr on Day 0).

The CEI action also produced a blunting or reversal of the postural plasma aldosterone increment (upright plasma aldosterone minus supine plasma aldosterone increments). This finding was evident as early as Day 1 (postural aldosterone increment $-3$ ng/dl versus 4 ng/dl on Day 0) and persisted throughout the study. The ASR (Figure 1) appeared to fall slightly on Day 7, but this decrease did not reach statistical significance.
until Day 14 (p < 0.05). The decrease in ASR plateaued on Day 14 and remained significantly suppressed for the remainder of the study (p < 0.05). Urinary tetrahydroaldosterone levels decreased in conjunction with, but to a lesser extent than, the ASR.

The levels of UF 19-nor-DOC decreased following the administration of CEI (Figure 2). This decrease began on Day 3 of drug administration and continued to fall until Day 21. The decline in 19-nor-DOC was synchronous with the fall in the ASR (R = 0.94, n = 5, p < 0.0005), and both hormones remained low throughout the 28 days of enalapril therapy. The decline in 19-nor-DOC levels, however, was much more marked than that of aldosterone (average decrease 71% and 44% respectively).

During the initial 7-day metabolic unit treatment period (after a 5-day period to establish electrolyte balance) changes in urinary electrolyte balance were noticed. Urinary potassium levels decreased from 80 to 73 mEq/day (49 mEq, 7-day net gain), urinary sodium levels increased from 117 to 122 mEq/day (35 mEq, 7-day net loss), and urinary chloride levels increased from 135 to 141 mEq/day (42 mEq, 7-day net loss). Later metabolic unit studies on Day 14, 21, and 28 reflected similar changes, but the overall electrolyte balance could not be assessed because of the interim ad libitum diet. Nevertheless, when the metabolic unit urinary electrolyte studies are considered in aggregate, the decline in urinary 19-nor-DOC levels was correlated with a decrease in urinary potassium levels (R = 0.68, n = 6, p < 0.05) and an increase in urinary sodium levels (R = 0.79, n = 6, p < 0.05) and urinary chloride levels (R = 0.87, n = 6, p < 0.01). The ASR, on the other hand, was less correlated (p < 0.05) with the changes in these urinary electrolytes (potassium: R = 0.65, n = 5; sodium: R = 0.64, n = 5; chloride: R = 0.57, n = 5).

Discussion

The results of this study indicate that inhibition of ANG II with the CEI enalapril is associated with a decrease in urinary free 19-nor-DOC levels. This decrease in 19-nor-DOC levels also appears to be correlated with the ability of enalapril to reduce the mineralocorticoid’s effects on urinary electrolyte levels. On the other hand, although the decrease in ASR paralleled that of 19-nor-DOC, it was of a smaller magnitude and did not correlate as well with changes in urinary electrolyte levels. These findings suggest that either basal UF 19-nor-DOC production is dependent on ANG II to a greater degree than is aldosterone secretion, or, alternatively, a non-ANG II-related enalapril action alters the production or metabolism of 19-nor-DOC.

Because 19-nor-DOC has a unique and complex pattern of biosynthesis, its regulation may involve several factors. The mineralocorticoid 19-nor-DOC is derived from an adrenocortical precursor, 19-oxygenated deoxycorticosterone (probably 19-oic-DOC). This precursor is then converted by extraadrenal, nonendocrine tissues to 19-nor-DOC. Adrenocorticotropic hormone is an important stimulator of this pathway and probably acts by increasing adrenocortical precursor production. The effects of the renin-angiotensin system on 19-nor-DOC production are more controversial. In human studies, dietary sodium depletion, sodium supplementation, or diuretic-induced renin stimulation had only minimal effects on 19-nor-DOC production. In the rat, however, sodium depletion produced a threefold increase in levels of urinary free 19-nor-DOC. These conflicting results are not reconcilable, as there may be species differences in the regulation of 19-nor-DOC biosynthesis. Furthermore, the degree of sodium depletion and renin stimulation may have been greater in the animal studies. The present study suggests that ANG II may have a role in maintaining basal 19-nor-DOC production. This possibility also implies that the precursor of circulating 19-nor-DOC may originate in the adrenal zona glomerulosa, as ANG II selectively activates this portion of the adrenal gland.

The urinary electrolyte studies indicate that 19-nor-DOC could have an important influence on the electrolyte balance. The fall in urinary potassium and rise in urinary sodium and chloride during enalapril administration was closely correlated with the decrease in UF 19-nor-DOC. This relationship was stronger than the association between the urinary electrolyte levels and aldosterone secretion. Furthermore, the levels of UF 19-nor-DOC measured in this study are consistent with substantial biological activity. Compared with aldosterone, 19-nor-DOC has 140% of the mineralocorticoid receptor affinity in rat renal cytosol, and it is not different in terms of sodium transport across toad bladder epithelia. Although 19-nor-DOC is probably more tightly bound to plasma proteins than is aldosterone, comparison of the UF levels of both of these compounds should provide a reliable index of comparative biological activity as it is the free, unbound
fraction that is available for binding to mineralocorticoid receptors. In previous studies the level of UF aldosterone in normotensive subjects was 350 ± 150 ng/day (SD).14 Therefore, as the extrapolated biological potencies of aldosterone and 19-nor-DOC are comparable, the greater decrease in 19-nor-DOC (71%) versus aldosterone (44%) in the present study is consistent with a greater urinary electrolyte effect of 19-nor-DOC compared with aldosterone in this situation.

Conclusion

The findings of this study indicate that ANG II inhibition with enalapril is associated with a decrease in UF 19-nor-DOC levels. This fall in 19-nor-DOC levels paralleled that of aldosterone but was greater in magnitude than the fall in aldosterone secretion. This difference suggests that either (1) basal UF 19-nor-DOC is dependent on ANG II to a greater degree than is basal aldosterone secretion or (2) that a non-ANG II-related enalapril action affects 19-nor-DOC production or metabolism, independent of aldosterone. In addition, the greater fall and higher correlation of 19-nor-DOC with urinary electrolyte changes (compared with those of aldosterone) argue strongly for the putative biological importance of 19-nor-DOC. The results of this study also suggest that at least part of the effects of CEI on urinary electrolytes could be mediated by the inhibition of 19-nor-DOC.

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