A ROLE FOR SODIUM-RETAINING STEROIDS IN THE REGULATION OF PROXIMAL TUBULAR SODIUM REABSORPTION IN MAN

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SUMMARY

1. The response to an infusion of 4% (w/v) fructose in water was determined in fifteen women on a daily sodium intake of 100 mEq/day. The results were compared with those obtained during a similar infusion on another day after treatment with deoxycorticosterone (20 mg/day; seven subjects), or spironolactone (200 mg/day; eight subjects), for 1 day before the day of study.

2. Treatment with deoxycorticosterone significantly \( (P<0.01) \) decreased sodium excretion (from a mean value of 391 to 192 \( \mu \text{Eq/min} \)) and urine flow rate (from 14.3 to 12.4 ml min\(^{-1}\) 100 ml\(^{-1}\) of glomerular filtrate) without a change in urinary osmolality or the clearance of inulin. The steroid also increased the fractional reabsorption of sodium at the diluting segment of the nephron, but this increase in reabsorption was not sufficient to compensate for the decrease in delivery of sodium to the site, so that absolute free-water clearance decreased.

3. Treatment with spironolactone significantly \( (P<0.01) \) increased sodium excretion (from 349 to 437 \( \mu \text{Eq/min} \)) and urine flow rate (from 12.5 to 14.4 ml min\(^{-1}\) 100 ml\(^{-1}\) of glomerular filtrate) with essentially no change in urinary osmolality or in inulin clearance. Spironolactone also decreased the fractional reabsorption of sodium at the diluting segment of the nephron, but the degree of inhibition of reabsorption was not sufficient to prevent an increase in free-water clearance as a result of increased delivery of sodium to the site.

4. The findings support the concept that changes in circulating aldosterone can alter the renal excretion of sodium in man by affecting its reabsorption in the proximal tubule as well as in the distal tubule.

Key words: aldosterone, proximal renal tubule, deoxycorticosterone, spironolactone, salt-retaining hormones.

The administration of aldosterone intravenously to normal subjects during maximal water diuresis did not change or slightly decreased urine flow, decreased the excretion of sodium.

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chloride and increased the excretion of solute-free water ($C_{H_2O}$) (Sonnenblick, Cannon & Laragh, 1961). The authors postulated that aldosterone produced these changes by an increase in the resorption of sodium chloride without water in a distal portion of the nephron. In normal hydropenic man, aldosterone given intravenously decreased urine flow and sodium excretion, and increased total urinary solute concentration without a change in non-urea solute concentration; the steroid did not affect solute-free water reabsorption ($T^{*}_{H_2O}$) (Yunis, Bercovitch, Stein, Levitt & Goldstein, 1964). On the basis of these results, the authors presumed that aldosterone did not alter medullary sodium chloride concentration and therefore suggested that the steroid increased sodium reabsorption in a cortical segment of the distal nephron which was impermeable to urea, the distal convoluted tubule. This postulated locus of action of aldosterone may also explain the effects produced by aldosterone during maximal water diuresis (Sonnenblick et al., 1961). Whereas these studies indicate that aldosterone can affect the distal tubule, they do not answer the question whether aldosterone can affect sodium reabsorption in the proximal tubule. Indeed, indirect evidence suggests that the proximal tubular reabsorption of sodium may be increased in those disorders in which aldosterone production is also increased, such as hepatic cirrhosis (Schedl & Bartter, 1960), and raises the possibility that aldosterone may increase sodium reabsorption in the proximal portion of the nephron. The present experiments were performed to determine if sodium-retaining hormones affect sodium reabsorption in the proximal tubule, and were designed to take advantage of the observation that urine flow during maximal water diuresis approximates to the rate at which tubular fluid leaves the proximal nephron (Eknoyan, Suki, Rector & Seldin, 1967).

**METHODS**

The purpose of the investigation and the nature of all the procedures were written out and explained in detail and signed consent obtained from all patients before study. Fifteen women were given a sodium intake of 100 mEq/day for 3–5 days. On the day of study, breakfast was withheld and an infusion of 4% (w/v) fructose, containing inulin and p-aminohippurate, was given at 15 ml/min, starting at approx. 9 a.m. When urine flow stabilized, approx. 50 min after the start of infusion, urine was collected through an indwelling bladder catheter at 20 min intervals for four periods, and venous blood was drawn through an indwelling Cournand needle at mid-period. A similar study was performed 3 or more days later, after the subject had received either deoxycorticosterone acetate (DOCA) (20 mg intramuscularly at 7 a.m. the day before and the day of study, seven subjects), or spironolactone (50 mg orally at 9 a.m. the day before and every 6 h until the final dose at 9 a.m. on the day of study, eight subjects). In some cases, the order of the studies was reversed with a longer interval between them. The values for each study in the tables are the means for the four periods obtained after stable urine flow had been established.

Blood pressure was measured with a sphygmomanometer. The clearances of inulin ($C_{IN}$) and of p-aminohippurate ($C_{PAH}$) were determined as previously described (Gill, Carr, Fleischmann, Casper & Bartter, 1967). Serum and urinary sodium were determined by internal-standard flame photometer, and serum and urinary osmolality by a Precision Systems osmometer. $C_{H_2O}$ was calculated by the conventional formula, and the clearance of sodium by the formula $C_{Na} = U_{Na}V/S_{Na}$ where $U_{Na}V = sodium excretion and $S_{Na} = serum sodium concentration. $V, C_{H_2O}$ and $C_{Na}$ were expressed per 100 ml of glomerular filtrate for comparison of
the results from all the experiments. Significance of the results was determined by paired analysis.

RESULTS

The results of all the studies with DOCA are shown in Table 1. Mean urine flow and $U_{Na}V$ (14.3 ml min$^{-1}$ 100 ml$^{-1}$ of glomerular filtrate and 391 μEq/min, respectively) in the control studies, were significantly ($P<0.01$) lower after treatment with DOCA (12.4 ml min$^{-1}$ 100 ml$^{-1}$ of glomerular filtrate and 192 μEq/min, respectively). $C_{H_2O}$ was also significantly ($P<0.01$) lower in the studies with DOCA (8.3 ml min$^{-1}$ 100 ml$^{-1}$ of glomerular filtrate) than in the control studies (10 ml min$^{-1}$ 100 ml$^{-1}$), and urinary osmolality, $C_{IN}$ and $C_{PAH}$ were unchanged. Whereas the overall effect of DOCA was to decrease total $C_{H_2O}$, the steroid increased the fractional reabsorption of sodium at the diluting site (Fig. 1). This is based upon the assumption that $C_{H_2O} + C_{Na}$ is a minimal approximation of the amount of sodium delivered to the diluting segment of the tubule (Laragh, Cannon, Bentzel, Sicinski & Meltzer, 1963).

The results of the studies with spironolactone are shown in Table 2. Mean urine flow and $U_{Na}V$ (12.5 ml min$^{-1}$ 100 ml$^{-1}$ and 349 μEq/min, respectively) in the control studies, were significantly ($P<0.01$) higher after treatment with spironolactone (14.4 ml min$^{-1}$ 100 ml$^{-1}$ and 437 μEq/min, respectively). $C_{H_2O}$ was also significantly ($P<0.02$) higher in the studies with spironolactone (9.6 ml min$^{-1}$ 100 ml$^{-1}$ of glomerular filtrate) than in the control studies (8.4 ml min$^{-1}$ 100 ml$^{-1}$), and urinary osmolality, $C_{IN}$ and $C_{PAH}$ were essentially unchanged. Whereas the overall effect of spironolactone was to increase total $C_{H_2O}$, the steroid antagonist actually decreased the fractional reabsorption of sodium at the diluting site in five of the eight patients (Fig. 2).

DISCUSSION

Treatment with DOCA decreased urine flow during maximal water diuresis without a change in GFR or in urinary osmolality, and therefore presumably increased the fraction of glomerular filtrate reabsorbed by the proximal tubule (Eknoyan et al., 1967; Stein, Abramson, Kahn & Levitt, 1967). Spironolactone increased urine flow with essentially no change in GFR or in urinary osmolality, and therefore presumably decreased the fraction of glomerular filtrate reabsorbed by the proximal tubule by antagonism of the tubular effects of endogenous aldosterone (Bartter, 1960). The effects of the two agents, DOCA and spironolactone, on urine flow are plotted for comparison in Fig. 3. The mean increase in proximal tubular reabsorption with DOCA was 13%, the mean decrease with spironolactone 15%, as calculated from the changes in $V$.

A large body of information indicates that the effects of DOCA on the renal tubule are qualitatively similar to those of aldosterone. Thus, it is likely that aldosterone shares with DOCA the ability to increase proximal tubular reabsorption of sodium and water. The increase in $V$ with spironolactone also supports the concept that endogenous aldosterone induces proximal tubular reabsorption of sodium, and that it is present in concentrations sufficient to do so when sodium intake is 100 mEq/day. Indeed, aldosterone secretion does not appear to be entirely suppressed in normal subjects by a sodium intake as great as 250 mEq/day (Gill, Waldmann, Delea & Bartter, 1969).

A consequence of the DOCA-induced increase in proximal tubular reabsorption of sodium
<table>
<thead>
<tr>
<th>Patient</th>
<th>Regimen</th>
<th>V (ml/min)</th>
<th>U\textsubscript{Na}V (μEq/ml)</th>
<th>U\textsubscript{Na} (μEq/ml)</th>
<th>UN.V (mg/min)</th>
<th>CH\textsubscript{O} V (ml/min)</th>
<th>CH\textsubscript{O} (ml/min)</th>
<th>C\textsubscript{Na} (mEq/min)</th>
<th>C\textsubscript{Na} (mEq/min)</th>
<th>C\textsubscript{IN} (mEq/min)</th>
<th>C\textsubscript{IN} (mEq/min)</th>
<th>C\textsubscript{AH} (ml/min)</th>
<th>FF</th>
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<td>10.4 3.8 14.2</td>
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<td>9.3 409 116</td>
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<td>12.1 12.1 0.1</td>
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<td>9.7 102 0.21</td>
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C = Control study; DOC = study done after treatment with deoxycorticosterone; V = urine flow; UN.V = sodium excretion; UN.C = clearance of solute-free water; C\textsubscript{Na} = clearance of sodium; C\textsubscript{IN} = clearance of inulin; C\textsubscript{AH} = clearance of p-aminohippurate; FF = filtration fraction.
TABLE 2. Effect of spironolactone on the renal response to water diuresis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Regimen</th>
<th>$V$ (ml/min)</th>
<th>$U_{\text{Na}}$ (mg/min)</th>
<th>$U_{\text{HCO}_3}$ (mg/min)</th>
<th>$V$ (ml/min)</th>
<th>$C_{\text{IN},0}$ (mg/min)</th>
<th>$C_{\text{IN},0}^{+}$ (mg/min)</th>
<th>$C_{\text{IN},100}$ (mg/min)</th>
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<td>0.04</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>G.H.</td>
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<td>512.2</td>
<td>0.16</td>
<td>0.01</td>
<td>107.8</td>
<td>0.04</td>
<td>0.04</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>A.T.</td>
<td>C</td>
<td>510.3</td>
<td>0.16</td>
<td>0.01</td>
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<td>0.01</td>
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<tr>
<td>F.J.</td>
<td>S</td>
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<td>0.16</td>
<td>0.01</td>
<td>109.8</td>
<td>0.04</td>
<td>0.04</td>
<td>0.02</td>
<td>0.01</td>
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<td>A.P.</td>
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<td>0.16</td>
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<tr>
<td>S.S.</td>
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<tr>
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<td>0.04</td>
<td>0.04</td>
<td>0.02</td>
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</tr>
</tbody>
</table>

$C =$ Control study; $S =$ study done after treatment with spironolactone; for other abbreviations, see Table 1.
FIG. 1. Effect of deoxycorticosterone on the fractional reabsorption of the 'sodium load' at the diluting site $C_{\text{H}_2\text{O}}/(C_{\text{H}_2\text{O}}+C_{\text{Na}})$ and on the rate of urine flow ($V$). Deoxycorticosterone increased sodium reabsorption proximally (indicated by a decrease in $V$) and distally (indicated by an increase in fractional reabsorption at the diluting segment). ○, Control; ●, deoxycorticosterone.

FIG. 2. Effect of spironolactone on the fractional reabsorption of the 'sodium load' at the diluting site $C_{\text{H}_2\text{O}}/(C_{\text{H}_2\text{O}}+C_{\text{Na}})$ and on the rate of urine flow ($V$). Spironolactone decreased sodium reabsorption proximally (indicated by an increase in $V$) and distally in five of eight studies (indicated by a decrease in fractional reabsorption at the diluting segment). ○, Control; ●, spironolactone.
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and water, with decreased delivery distal to the diluting segment, was a corresponding decrease in $C_{\text{H}_2\text{O}}$. In the case of spironolactone, the decreased proximal tubular reabsorption of sodium and water, with increased delivery distally, was associated with an increase in $C_{\text{H}_2\text{O}}$. Whereas the net changes in $C_{\text{H}_2\text{O}}$ tended to reflect changes in delivery of sodium, there is evidence that DOCA and spironolactone also affected sodium reabsorption at the diluting site as previously reported for aldosterone (Sonnenblick et al., 1961): DOCA increased the fractional reabsorption of the estimated load of sodium delivered to the diluting site and spironolactone decreased it (Figs. 1 and 2). The increase with DOCA was $9\%$ and the decrease with spironolactone was $3\%$.

In previous studies in hydropenic man, aldosterone did not change non-urea solute concentration (though it did increase maximal urinary osmolality) nor did it alter $T_{\text{H}_2\text{O}}^v$ (Yunis et al., 1964). The authors presented these observations as indirect evidence that aldosterone did not increase medullary interstitial sodium concentration and proposed that aldosterone increased sodium reabsorption in the distal convoluted tubule, but not in the ascending limb of the loop of Henle. Such a locus of action could explain increases in $C_{\text{H}_2\text{O}}$ of the magnitude previously observed with aldosterone in normal man (Sonnenblick et al., 1961). Results of micropuncture studies in the dog during antidiuresis and during water diuresis (Clapp & Robinson, 1966) and in the rhesus monkey during antidiuresis (Bennett, Brenner & Berliner, 1968) indicate that the distal convoluted tubule is relatively impermeable to water. If one assumes that the distal convoluted tubule of normal man has permeability characteristics similar to those of dog and monkey, then a steroid-induced increase in sodium reabsorption in this portion of the tubule
J. R. Gill, Jr, C. S. Delea and F. C. Bartter could generate solute-free water. A similar distal site of action for DOCA would also explain the increase in fractional reabsorption of sodium without water observed in the present studies.

The decrease in $C_{\text{H}_{2}\text{O}}$ with DOCA suggests that total sodium reabsorption in the ascending limb of the loop of Henle decreased, probably as a result of decreased delivery of sodium chloride and water from the proximal tubule (Table 1). It is therefore possible that a decrease in sodium reabsorption in the loop was associated with a decrease in medullary sodium concentration. If such a decrease in medullary solute concentration did occur and were sufficient to decrease the rate of back diffusion of water from tubule lumen to interstitium, then the decrease in urine flow with DOCA would not fully reflect the extent of the increase in proximal tubular sodium reabsorption.

Conversely, antagonism of aldosterone-dependent sodium reabsorption in the distal convoluted tubule could account for the decrease in distal fractional sodium reabsorption with spironolactone. Spironolactone did not, however, prevent an increase in sodium reabsorption in the ascending limb of the loop of Henle with an increase in $C_{\text{H}_{2}\text{O}}$, presumably because it increased delivery of sodium chloride and water from the proximal tubule (Table 1). If an increase in medullary osmolality occurred as a result of an increase in sodium reabsorption in the loop, it did not increase the back diffusion of water in sufficient quantity to prevent an increase in urine flow (Table 2). However, the magnitude of the depression of proximal tubular sodium reabsorption with spironolactone may have been underestimated because of back diffusion. The failure of a twofold increase in papillary osmolality to prevent an increase in urine flow during infusion of hypo-osmotic saline and, presumably, depression of proximal tubular sodium reabsorption has been reported by others (Eknoyan et al., 1967). The results are therefore consistent with the interpretation that DOCA and spironolactone produced changes in urine flow as a result of changes in proximal sodium reabsorption and not as a result of changes in medullary solute concentration and in back diffusion of water out of the distal nephron.

Considerable evidence indicates that expansion of extracellular fluid volume produces changes in Starling forces in peritubular capillaries which in some way decrease net proximal tubular sodium and water reabsorption (Martino & Earley, 1967b, 1968; Brenner, Falchuk, Keimowitz & Berliner, 1969). Sodium and water retention produced by DOCA before the clearance was small and did not alter filtration fraction or, presumably, peritubular capillary oncotic pressure (Table 1); it also did not alter blood pressure (not shown) or the degree of renal vasodilatation as reflected by $C_{PAH}$ and therefore, presumably, did not affect peritubular capillary hydrostatic pressure. Spironolactone did not produce a significant change in filtration fraction, in $C_{PAH}$ (Table 2) or in blood pressure (not shown). These results suggest that DOCA and spironolactone altered proximal tubular sodium reabsorption through direct tubular effects and that the effects of each agent on the tubule were modified minimally or not at all by changes in peritubular capillary Starling forces.

The findings of a previous experiment in which aldosterone was given intravenously to normal subjects during maximal water diuresis differ from those of the present study in that urine flow showed no consistent change and $C_{\text{H}_{2}\text{O}}$ increased in all subjects (Sonnenblick et al., 1961). In those studies 1500 ml of water was followed by infusion of dextrose and water at a rate which exceeded urinary flow from the control periods which preceded the administration of aldosterone until a peak effect of the steroid on sodium excretion occurred 2-4 h later.
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A natriuresis occurred in some studies during the 20–60 min latent period between the administration of aldosterone and its onset of action and suggests that expansion of extracellular fluid volume with water may have depressed proximal tubular reabsorption of sodium so as to obscure a proximal effect of the steroid (Martino & Earley, 1967a). In the present studies the renal effects of DOCA were well developed at the start of the study and only that quantity of water required to produce a stable diuresis was infused before measurements of the effect of DOCA were begun. Aldosterone and DOCA increased the fractional resorption of sodium in the diluting segment but the increase in total C\textsubscript{H2O} reported for aldosterone did not occur with DOCA because DOCA decreased the distal delivery of sodium.

When treatment with sodium-retaining steroids is continued for many days, expansion of intravascular volume decreases sodium reabsorption by the proximal tubule (Wright, Knox, Howards & Berliner, 1969) and by the ascending limb of the loop of Henle (Eknoyan et al., 1967; Stein et al., 1967) and increases sodium excretion. In certain oedematous states, however, a decrease in effective circulating blood volume with an increase in circulating aldosterone produces a sodium retention probably by steroid-induced increase in sodium reabsorption by the proximal as well as by the distal tubule.

REFERENCES


