Mechanism of Increased Renin Release in the Adrenalectomized Rat
Adrenal Insufficiency and Renin

WILLIAM J. WELCH, M.S., COBERN E. OTT, PH.D., GORDON P. GUTHRIE, JR., M.D., AND THEODORE A. KOTCHEN, M.D.

SUMMARY We have previously suggested that inhibition of renin release by sodium chloride is related to absorptive chloride transport in the loop of Henle. Infusion of sodium chloride fails to inhibit renin release in the adrenalectomized (Adx) rat, and dexamethasone restores renin responsiveness to sodium chloride. The purpose of the present study was to evaluate the relationship between loop function (urinary diluting and concentration capacity) and plasma renin concentration (PRC) in the Adx rat. After hypotonic sodium chloride infusion, free water clearance ($C_{\text{H}_20}$) of Adx rats (0.56 ml/hr/100 g ± 0.17 se) was decreased ($p < 0.01$) compared to controls (2.86 ml/hr/100 g ± 0.29 se); PRC of Adx rats (61.9 units/ml ± 11.2 se) was increased ($p < 0.01$) above controls (6.0 units/ml ± 1.7 se). These differences persisted after administration of $d(CH_2)_5Tyr(Et)VAVP$, a potent ADH antagonist. In separate groups of animals, after water deprivation, urine concentration of Adx rat (1,401 mOsm/kg ± 45 SE) was less ($p < 0.01$) than that of controls (2,117 mOsm/kg ± 169 SE). Dexamethasone normalized both $C_{\text{H}_20}$ and urinary concentrating ability and also decreased PRC in Adx rats. Thus, in the glucocorticoid deficient rat, increased renin release is associated with impaired loop function. The loop defect may account for high PRC that is not suppressed by sodium chloride. (Hypertension 5 (supp I): 1-47-1-52, 1983)

METHODS

PRC, $C_{\text{H}_20}$, and maximum urinary concentrating capacity were determined in three groups of male Sprague Dawley rats: adrenalectomized rats (Adx); adrenalectomized rats treated with dexamethasone (Adx + Dex); and sham-operated controls. In each protocol, Adx or sham surgery was carried out 7 days before the acute study. Adx was performed under pentobarbital (50 mg/kg) anesthesia through bilateral retropitoneal incisions, and total adrenalectomy was subsequently confirmed by measurement of plasma corticosterone concentrations. Sham-operated animals were treated identically, except that the adrenal glands were located but not removed.

During the 7 days after Adx or sham surgery, animals were maintained in individual metabolic cages and fed normal rat chow (240 uEq Na\(^+\)/g, 430 uEq K\(^+\)/g, 270 uEq Cl\(^-\)/g) according to a paired feeding protocol. Adx + Dex and sham were fed 1 day behind the Adx group. Both Adx and Adx + Dex drank 0.9% sodium chloride, and sham-operated animals drank distilled water. Net electrolyte balance was computed as the difference between dietary intake and urinary excretion. Adx + Dex were injected intramuscularly with dexamethasone (5.0 µg/100 g body weight) on Days 4 through 7 following Adx.
Hypotonic Saline Infusion

For measurement of $C_{\text{H}_{2}O}$, all animals (n = 8/group) were anesthetized with inactin (100 mg/kg). Following tracheostomy, two jugular veins were cannulated (PE No. 50), one for infusion of inulin and the other for infusion of hypotonic saline. A femoral artery catheter (PE No. 50) was inserted to collect blood. Mean arterial pressure was monitored by connecting the femoral artery catheter to a Statham pressure transducer, and recorded on a Grass recorder. A bladder catheter was inserted to collect urine.

After completion of these preparative surgical procedures, animals received a bolus of inulin (0.2 ml/100g body weight) and then a maintenance infusion of 4% inulin at 0.5 ml/100g body weight per hour for the duration of the experiment. After a 30-minute recovery period, urine was collected during a 15-minute control period, and 600 µl of blood was taken at the end of the control period for measurement of inulin, osmolality, and hematocrit. Hypotonic sodium chloride (0.45% NaCl, volume equivalent to 10% body weight) was then infused for 30 minutes. A second timed urine was collected between 30 and 60 minutes after completion of hypotonic saline infusion, and 600 µl of blood was obtained at the end of this 30-minute period. Inulin and osmolality were measured in the urine and plasma, and plasma Na+, K+, and Cl- concentrations were also measured. At the end of the collection period, radioiodinated serum albumin (0.2 ml) was injected for measurement of plasma volume. Ten minutes following injection, blood was obtained for measurement of plasma volume, PRC, hematocrit, and plasma corticosterone.

Hypotonic Saline Infusion in Animals Treated with d(CH2)5Tyr(Et)VAVP

To eliminate the possibility that group differences in $C_{\text{H}_{2}O}$ reflect differences of endogenous ADH, even after hypotonic volume expansion, the above study was repeated in separate groups of Adx, Adx + Dex, and sham controls treated with the ADH antagonist, d(CH2)5Tyr(Et)VAVP. Animals were handled as in the experiment above, except that d(CH2)5Tyr(Et)VAVP was administered intravenously (30 µg bolus) midway through the infusion of hypotonic saline. In a study with a separate group of six control, hydropenic, inactivated rats, administration of d(CH2)5Tyr(Et)VAVP reduced urine osmolality from 1530 mOsm/kg ± 92 se to 129 mOsm/kg ± 9 se.

Water Deprivation

To evaluate urine concentrating ability, separate groups of Adx, Adx + Dex, and sham controls were prepared as in the previous protocols. Seven days following either Adx or sham operation, all animals were deprived of fluid, and urine osmolality was measured in a 12-hour urine sample collected between 36 and 48 hours of fluid deprivation. At 48 hours, animals were sacrificed by decapitation, and blood was collected for measurement of PRC and plasma corticosterone concentration.

Sodium and potassium concentrations were measured with a flame photometer (Instrumentation Laboratory, Morris Plains, New Jersey), and chloride concentrations were measured with a Buchler chloridometer. Inulin was measured by an anthrone method. Plasma volume was measured with radiodinated albumin, with appropriate correction for plasma trapping. Plasma corticosterone concentrations were measured by radioimmunoassay. Similar to other investigators, we observed that plasma renin substrate concentration, measured as previously described, was lower ($p < 0.01$) in Adx rats (36 ng/ml ± 11 se) and higher ($p < 0.01$) in Adx + Dex (555 ng/ml ± 70 se) vs sham controls (378 ng/ml ± 45 se). Because of the differences in endogenous substrate concentration, plasma renin concentration (PRC) rather than plasma renin activity was measured. To measure PRC, excess renin substrate prepared from plasma of anephric sheep by the method of Rosenthal, was added to each plasma sample (2000 ng substrate/0.2 ml plasma). In vitro incubations were carried out at pH 7.4 for 60 minutes, and the concentrations of angiotensin I generated was measured by radioimmunoassay by the method of Haber et al.

Glomerular filtration rate (GFR) was estimated from the clearance of inulin. Free water clearance was calculated from the following formula: $C_{\text{H}_{2}O} = V/C_{\text{In}}$, where $V$ is urine flow rate and $C_{\text{In}}$ is the osmolar clearance.

To test statistical significance, two group comparisons were made with the Student's $t$ test, and a paired or unpaired $t$ test was used, as appropriate. The Bonferroni modification of the $t$ test was used for three group comparisons.

Results

Hypotonic Saline Infusion

As shown in table 1, during the week after Adx and before hypotonic saline infusion, net sodium balance of saline drinking Adx animals was more positive than Adx + Dex or sham controls ($p < 0.01$). Potassium balance was less positive ($p < 0.01$) in the groups of animals drinking saline. There were no group differences of body weight on Day 1 or Day 7 of the balance period.

Arterial pressure decreased ($p < 0.01$) after hypotonic saline infusion in Adx and sham (table 2). Blood pressure of Adx was slightly but significantly lower, both before and after infusion, than in the other two groups ($p < 0.05$). There were no group differences in GFR before infusion, and GFR did not change during infusion. After infusion, GFR of sham animals was higher than that of the other two groups ($p < 0.05$). Urine flow rates increased ($p < 0.01$) in all groups. Plasma osmolality did not differ among groups and decreased ($p < 0.01$) comparably in all three groups. After infusion, the urine osmolality of the sham-operated animals was lower ($p < 0.01$) than that of the other two groups. As seen in table 3, compared to that...
TABLE 1. Net Electrolyte Balances during the 7 Days after Adx or Sham Surgery and Before Hypotonic NaCl Infusion

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight (g)</th>
<th>Na⁺ (mEq/7 da)</th>
<th>Cl⁻ (mEq/7 da)</th>
<th>K⁺ (mEq/7 da)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 1</td>
<td>day 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adx (n = 8)</td>
<td>249 ± 11SE</td>
<td>289 ± 9</td>
<td>18.3 ± 1.4*</td>
<td>15.4 ± 1.5</td>
</tr>
<tr>
<td>Adx + Dex (n = 8)</td>
<td>269 ± 5</td>
<td>262 ± 6</td>
<td>14.4 ± 1.9</td>
<td>14.7 ± 1.6</td>
</tr>
<tr>
<td>Sham (n = 8)</td>
<td>284 ± 11</td>
<td>304 ± 12</td>
<td>12.4 ± 0.6</td>
<td>13.6 ± 2.7</td>
</tr>
<tr>
<td>Adx (n = 8)</td>
<td>296 ± 12</td>
<td>295 ± 9</td>
<td>18.7 ± 2.8*</td>
<td>14.7 ± 2.5</td>
</tr>
<tr>
<td>Adx + Dex (n = 8)</td>
<td>286 ± 12</td>
<td>264 ± 13</td>
<td>12.8 ± 1.4</td>
<td>13.8 ± 1.9</td>
</tr>
<tr>
<td>Sham (n = 8)</td>
<td>286 ± 12</td>
<td>292 ± 12</td>
<td>13.9 ± 1.1</td>
<td>10.6 ± 2.0</td>
</tr>
</tbody>
</table>

* p < 0.01 compared to the other two groups.

Hypotonic Saline Infusion in Animals Treated with d(CH₂)₃Tyr(Et)VAVP

Net electrolyte balances during the week before acute infusion were similar to results in the above study (table 1). Arterial pressure decreased in all groups during the acute study, as shown in table 4; however, blood pressure of Adx animals and sham controls did not differ either before or after infusion although blood pressure of Adx + Dex were slightly elevated (p < 0.05). There were no group differences in GFR, although during infusion, GFR increased (p < 0.01) in Adx + Dex and sham. Urine osmolality of Adx + Dex was lower (p < 0.01) than that of Adx, and urine osmolality of sham controls was lower (p < 0.01) than Adx + Dex. The C_H₂O of Adx was lower (p < 0.001) than respective values in the other two groups (table 3); C_H₂O of Adx + Dex and sham controls did not differ. PRC of Adx was higher (p < 0.01) than the other two groups, and PRC of sham controls was lower (p < 0.01) than Adx + Dex. There were no group differences of plasma Na⁺, K⁺, or Cl⁻ concentrations, plasma osmolality, or plasma volume.

TABLE 2. Arterial Pressure, GFR, Hematocrit, and Plasma and Urine Osmalalities in the Study without the ADH Antagonist

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Art press (mm Hg)</th>
<th>GFR (ml/min)</th>
<th>Hct (%)</th>
<th>Plasma Osm (mOsm/kg)</th>
<th>Urine Osm (mOsm/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adx</td>
<td>pre 119±3*</td>
<td>post 98±6†</td>
<td>48±1</td>
<td>256±3</td>
<td>181±15</td>
</tr>
<tr>
<td>Adx + Dex</td>
<td>pre 188±0.18</td>
<td>post 1.78±0.18</td>
<td>42±1†</td>
<td>256±3</td>
<td>174±13</td>
</tr>
<tr>
<td>Sham</td>
<td>pre 132±4</td>
<td>post 122±5</td>
<td>47±1</td>
<td>259±3</td>
<td>116±47</td>
</tr>
</tbody>
</table>

*p < 0.05 compared to the other two groups.  †p < 0.01 compared to the other two groups.  ‡p < 0.01 compared to preinfusion value in same group.

TABLE 3. Plasma Electrolytes, Volume, Corticosterone, C_H₂O and PRC after Hypotonic NaCl Infusion

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Na⁺ (mEq/liter)</th>
<th>K⁺ (mEq/liter)</th>
<th>Cl⁻ (mEq/liter)</th>
<th>Plasma volume (ml/100 g)</th>
<th>Plasma corticosterone (µg/dl)</th>
<th>C_H₂O (ml/hr/100 g)</th>
<th>PRC (units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study without ADH antagonist</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adx</td>
<td>132±2</td>
<td>4.6±0.2</td>
<td>106±3</td>
<td>5.2±0.2</td>
<td>0.5±0.1</td>
<td>0.56±0.17</td>
<td>61.9±11.2*</td>
</tr>
<tr>
<td>Adx + Dex</td>
<td>134±3</td>
<td>4.0±0.3</td>
<td>109±4</td>
<td>4.8±0.2</td>
<td>0.9±0.1</td>
<td>1.32±0.31*</td>
<td>9.6±2.2</td>
</tr>
<tr>
<td>Sham</td>
<td>137±3</td>
<td>4.0±0.3</td>
<td>110±3</td>
<td>4.8±0.1</td>
<td>10.4±0.6*</td>
<td>2.86±0.29*</td>
<td>6.0±1.7</td>
</tr>
<tr>
<td>Study with d(CH₂)₃Tyr(Et)VAVP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adx</td>
<td>136±3</td>
<td>4.4±0.2</td>
<td>105±2</td>
<td>4.9±0.3</td>
<td>0.3±0.1</td>
<td>0.64±0.11*</td>
<td>75.9±8.8*</td>
</tr>
<tr>
<td>Adx + Dex</td>
<td>140±2</td>
<td>4.0±0.3</td>
<td>109±2</td>
<td>4.8±0.2</td>
<td>0.2±0.1</td>
<td>2.95±0.20</td>
<td>17.1±2.4*</td>
</tr>
<tr>
<td>Sham</td>
<td>139±3</td>
<td>3.8±0.2</td>
<td>109±2</td>
<td>4.4±0.2</td>
<td>34.2±2.1*</td>
<td>3.50±0.23</td>
<td>10.0±2.1</td>
</tr>
</tbody>
</table>

*p < 0.01 compared to the other two groups.
TABLE 4.  Arterial Pressure, GFR, Hematocrit, and Plasma and Urine Osmalalies in the Study with d(CH2)5Tyr(Et)VAVP

<table>
<thead>
<tr>
<th></th>
<th>Adx</th>
<th>Adx + Dex</th>
<th>Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Art press (mm Hg) pre</td>
<td>122 ± 3</td>
<td>134 ± 2*</td>
<td>126 ± 5</td>
</tr>
<tr>
<td>post</td>
<td>99 ± 6</td>
<td>118 ± 2*†</td>
<td>105 ± 5*</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>1.9 ± 0.24</td>
<td>1.84 ± 0.27</td>
<td>1.55 ± 0.21</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>48 ± 2</td>
<td>50 ± 2</td>
<td>49 ± 2</td>
</tr>
<tr>
<td>Plasma Osm (mOsm/kg) post</td>
<td>262 ± 4</td>
<td>271 ± 3</td>
<td>270 ± 3</td>
</tr>
<tr>
<td>Urine Osm (mOsm/kg) post</td>
<td>160 ± 9</td>
<td>120 ± 5†</td>
<td>88 ± 5†</td>
</tr>
</tbody>
</table>

* p < 0.05 compared to the other two groups.
† p < 0.01 compared to the other two groups.
‡ p < 0.01 compared to preinfusion value in same group.

Water Deprivation

After water deprivation (table 5), compared to respective values in the other two groups, urine osmolality of Adx was decreased (p < 0.01) and PRC was increased (p < 0.01). In comparing Adx + Dex with sham, urine osmolality did not differ; however, PRC of Adx + Dex was higher (p < 0.01).

Discussion

Both the ability to excrete free water and the capacity of the hydropenic animal to concentrate urine are dependent on absorptive chloride transport in the thick ascending limb of the loop of Henle. Consistent with previous observations of others, both C(H2O) after hypotonic saline infusion and maximum urine concentrating ability after water deprivation were decreased in Adx animals compared to respective values in sham controls. However, plasma and urine vasopressin levels are increased in adrenalectomized animals, and despite hypotonic volume expansion, differences in vasopressin rather than a loop defect might have accounted for the low C(H2O). To rule out this possibility, C(H2O) measurements were repeated after treatment with d(CH2)5Tyr(Et)VAVP, a potent antagonist of the in vivo responses to endogenous and exogenous ADH. Despite treatment with this agent, C(H2O) was again found to be decreased in Adx animals. Similarly, free water excretion is also impaired in adrenalectomized Brattleboro rats lacking ADH. Thus, even in these animals, low C(H2O) after hypotonic saline infusion appears to be related to altered loop function rather than to ADH. Renal papillary sodium concentration is decreased in adrenalectomized rats, also suggesting decreased active transport out of the ascending limb of the loop of Henle. If differences in net sodium or potassium balance, consistent differences in arterial pressure, plasma volume, or GFR, PRC was increased in Adx animals compared to sham controls. We have previously suggested that renin secretion is inversely related to the magnitude of absorptive chloride transport in the loop of Henle. Acute and chronic administration of sodium salts other than sodium chloride fail to inhibit renin release, and in a micropuncture study, failure of sodium bicarbonate to inhibit renin release was associated with decreased chloride uptake in the loop compared to animals infused with sodium chloride. Koletsky and coworkers have confirmed that dietary chloride intake modifies the renin response to sodium deprivation. Similar to our previous results in adrenalectomized animals, we have also demonstrated that both acute and chronic sodium chloride administration fail to inhibit renin release in the potassium depleted rat, whereas renin was inhibited by albumin induced vascular volume expansion. Similar to adrenal insufficiency, potassium depletion also results in impaired function of the loop of Henle. Taken together, these results are consistent with the hypothesis that failure of sodium chloride to inhibit renin release in the adrenalectomized rat is related to altered loop function and increased renin release in the adrenalectomized rat. Inhibition of chloride uptake in the thick ascending limb of the loop of Henle may also account for stimulation of renin release in response to furosemide and for increased renin release in patients with Bartter's Syndrome.

Although our studies would suggest decreased loop transport in adrenalectomized rats, Cortney, in a micropuncture study, concluded that loop transport was no different following adrenalectomy compared to normal since fractional sodium reabsorption in the loop was the same in both groups. These results, however, are very difficult to compare to ours since in the micropuncture study the GFR was only one-half normal. Thus, a similar fractional reabsorption would indicate a significant decrease in loop transport in adrenalectomized rats because of loop delivery. There is also the additional suggestion that loop transport was decreased since the tubular fluid-to-plasma concentration ratio for sodium and osmolality were significantly increased in early distal tubular fluid when compared to control animals. A very recent micropuncture study of chloride transport in the adrenalectomized rat also concluded that fractional loop reabsorption was unchanged by adrenalectomy. However, both single nephron filtration rate and blood pressure were significantly decreased by adrenalectomy. These results also suggest that loop transport would be decreased due to the decreased delivered load.

TABLE 5. Urine Osmolality and PRC after Water Deprivation

<table>
<thead>
<tr>
<th></th>
<th>Adx (n = 6)</th>
<th>Adx + Dex (n = 6)</th>
<th>Sham (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max U_osm (mOsm/kg)</td>
<td>140 ± 45*</td>
<td>2119 ± 53</td>
<td>2117 ± 169</td>
</tr>
<tr>
<td>PRC (units/ml)</td>
<td>83.0 ± 17.1*</td>
<td>13.6 ± 2.6*</td>
<td>4.0 ± 0.8</td>
</tr>
</tbody>
</table>

* p < 0.01 compared to the other two groups.
In the absence of ADH, free water clearance is generally regarded as a measure of loop transport. It is possible that adrenalectomy alters loop delivery or water permeability in segments distal to the loop. Although loop delivery was not measured in this study both absolute C H2O and C H2O/GFR were significantly decreased in Adx animals (p < 0.001), thus, whether C H2O is decreased by decreased delivery to the loop or diminished absolute loop transport, the present study is consistent with the idea that absolute loop transport is decreased in Adx animals. Additional support for this concept is provided by the data for minimum Uosm which was significantly increased in the Adx group. While the possibility exists that changes in water permeability distal to the loop may have also been altered, the consistent interpretation of the data is diminished loop function. An increased water permeability distal to the loop would have artificially decreased C H2O in the hypotonic saline loaded animals but could not have explained the diminished maximum Uosm in the water deprivation studies. In considering all the evidence, the most likely explanation is that the Adx animals showed decreased loop function.

In the present study, the dose of dexamethasone used corrected the loop defect and tended to normalize PRC in the adrenalectomized rat, without a consistent effect on arterial pressure, electrolyte balance, plasma volume, or GFR. However, dexamethasone may not have totally normalized loop function. After hypotonic sodium chloride infusion, both with and without administration of the ADH antagonist, C H2O tended to be lower and PRC tended to be higher in Adx + Dex than in sham controls, although these differences were not always statistically significant. However, dexamethasone totally restored the ability to concentrate urine. This finding, coupled with the striking increase of C H2O and the striking reduction of PRC compared to respective values in glucocorticoid deficient, adrenalectomized animals further supports an association between loop function and renin release. Other investigators have also reported that glucocorticoid replacement in inhibited renin release in the adrenalectomized rat (abstr). 1982 meeting of American Society of Nephrology. In press.

In conclusion, we have previously suggested that inhibition of renin release by sodium chloride is related to increased absorptive chloride transport in the thick ascending limb of the loop Henle. Renin release is increased in the adrenalectomized rat and is not inhibited by infusion of sodium chloride. Increased renin release is associated with impaired loop function as estimated by decreased C H2O and an inability to concentrate urine. Dexamethasone decreased PRC and corrected these indices of loop function. These results suggest that stimulation of renin release in the glucocorticoid deficient rat is related to impaired loop function and further supports the hypothesis that inhibition of renin release by sodium chloride is specifically related to increased chloride uptake in the loop.

Acknowledgments
The ADH antagonist, d(CH2)3Tyr(ET)VAVP, was generously provided by Dr. Maurice Manning, Medical College of Ohio, Toledo, Ohio. Nicole Braude provided assistance with manuscript preparation.

References


Mechanism of increased renin release in the adrenalectomized rat. Adrenal insufficiency and renin.

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