Increased blood pressure, aldosterone activity, and regional differences in renal ENaC protein during vasopressin escape

Jian Song, Xinquan Hu, Osman Khan, Ying Tian, Joseph G. Verbalis, and Carolyn A. Ecelbarger
Division of Endocrinology and Metabolism, Department of Medicine, Georgetown University, Washington, District of Columbia 20057

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Song, Jian, Xinquan Hu, Osman Khan, Ying Tian, Joseph G. Verbalis, and Carolyn A. Ecelbarger. Increased blood pressure, aldosterone activity, and regional differences in renal ENaC protein during vasopressin escape. Am J Physiol Renal Physiol 287: F1076–F1083, 2004.—The syndrome of inappropriate antidiuretic hormone (SIADH) is associated with water retention and hyponatremia. The kidney adapts via a transient natriuresis and persistent diuresis, i.e., vasopressin escape. Previously, we showed an increase in the whole kidney abundance of aldosterone-sensitive proteins, the α- and γ-(70-kDa band)-subunits of the epithelial Na⁺ channel (ENaC), and the thiazide-sensitive Na⁺-Cl cotransporter (NCC) in our rat model of SIADH. Here we examine mean arterial pressure via radiotelemetry, aldosterone activity, and cortical vs. medullary ENaC subunit and 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2) protein abundances in escape. Eighteen male Sprague-Dawley rats (300 g) were sham operated (n = 6) or infused with desmopressin (dDAVP; n = 12, a V₂ receptor-selective analog of AVP). After 4 days, one-half of the rats receiving dDAVP were switched to a liquid diet, i.e., water loaded (WL) for 5–7 additional days. The WL rats had a sustained increase in urine volume and blood pressure (122 vs. 104 mmHg, P < 0.03, at 7 days). Urine and plasma aldosterone levels were increased in the WL group to 844 and 1,658% of the dDAVP group, respectively. NCC and α- and γ-ENaC (70-kDa band) were increased significantly in the WL group (relative to dDAVP), only in the cortex. β- and γ-ENaC (85-kDa band) were increased significantly by dDAVP in cortex and medulla relative to control. 11β-HSD-2 was increased by dDAVP in the cortex and not significantly affected by water loading. These changes may serve to attenuate Na⁺ losses and ameliorate hyponatremia in vasopressin escape.

hyponatremia; syndrome of inappropriate antidiuretic hormone; pressure-natriuresis; renin; 11β-hydroxysteroid dehydrogenase; epithelial sodium channel; mean arterial pressure

HYPONATREMIA IS ONE OF THE MOST COMMON, AND POTENTIALLY SERIOUS, CLINICAL DISORDERS OBSERVED IN HOSPITALIZED PATIENTS (34). ONE CAUSE OF HYPONATREMIA IS THE SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE SECRETION (SIADH). WITH THIS DISORDER, PATIENTS SECRETE ABnormally HIGH LEVELS OF VASOPRESSIN (ANTIDIURETIC HORMONE; ADH) RELATIVE TO PLASMA OSMOLALITY, RESULTING IN INAPPROPRIATE FREE-WATER RETENTION AND A FALL IN SERUM NA⁺ CONCENTRATION ([Na⁺]). THIS WATER-RETENTIVE PHASE IS GENERALLY RELIEVED BY A PHYSIOLOGICAL PROCESS KNOWN AS “VASOPRESSIN ESCAPE,” IN WHICH BOTH HUMANS AND ANIMAL MODELS UNDERGO A NATURENURESIS FOLLOWED CLOSELY BY A DIURESIS OF INCREASINGLY MORE DILUTE URINE. THE MECHANISMS AND TRIGGERS OF BOTH THE DIURESIS AND THE NATURENURESIS ARE NOT FULLY UNDERSTOOD, ALTHOUGH INCREASED RENAL ARTERIAL PRESSURE APPEARS ESSENTIAL FOR THE PROCESS (16).

IT IS LOGICAL TO ASSUME THAT THIS NATURENURESIS MIGHT CONTRIBUTE TO A FURTHER REDUCTION IN SERUM NA⁺ LEVELS UNLESS ADAPTIVE COMPENSATORY MECHANISMS ARE INVOKED. SEVERAL REPORTS (1, 6, 7, 22, 23, 30), GOING BACK DECADES, HAVE REPORTED EVIDENCE OF INCREASED ALDOSTERONE ACTIVITY, I.E., PLASMA LEVELS, URINE EXCRETION, AND URINE K⁺–Na⁺ RATIOS IN HYPONATREMIA. THESE RESULTS WERE SURPRISING, BECAUSE SIADH IS OFTEN ASSOCIATED WITH VOLUME EXPANSION, WHICH IS KNOWN TO DECREASE ACTIVATION OF THE RENIN-ANGIOTENSIN SYSTEM, THEREBY REDUCING ANG II STIMULATION OF ADRENAL ALDOSTERONE SECRETION. ALSO, SOME REPORTS (5, 12, 14, 15) DID SUGGEST DECREASED RATHER THAN INCREASED ALDOSTERONE SECRETION IN SIADH IN HUMANS AND ANIMAL MODELS. FURTHERMORE, THE MECHANISMS PROPOSED TO UNDERLIE THESE INCREASES IN ALDOSTERONE ACTIVITY IN SIADH WERE NOT CLEAR, AND HYPOTHESES RANGED FROM A DIRECT EFFECT OF LOW NA⁺ ON THE ADRENAL TO INCREASED SENSITIVITY OF THE ADRENAL TO ANG II, ACTH, OR K⁺.

MUCH HAS BEEN DECREASED IN THE LAST DECADE WITH REGARD TO THE REGULATION OF ALDOSTERONE-RESPONSIVE NA⁺ TRANSPORT PROTEINS IN THE KIDNEY. ALDOSTERONE-SENSITIVE NaCl TRANSPORT HAS BEEN DEMONSTRATED IN THE DISTAL CONVOLUTED TUBULE VIA THE THIAZIDE-SENSITIVE Na⁺-Cl cotransporter (NCC; see Ref. 33) AND IN THE COLLECTING DUCT VIA THE AMILORIDE-SENSITIVE Na⁺ CHANNEL (ENaC; see Ref. 17). UTILIZING IMMUNOBLOTTING AND IMMUNOHISTOCHEMISTRY, KIM ET AL. (19) FOUND A MARKED INCREASE IN THE PROTEIN ABUNDANCE OF THE NCC WITH EITHER ALDOSTERONE INFUSION OR A LOW-NaCl DIET. NCC IS EXPRESSED EXCLUSIVELY IN THE DISTAL CONVOLUTED TUBULE IN THE CORTEX OF THE KIDNEY. SIMILARLY, MASILAMANI ET AL. (24) SHOWED AN INCREASE IN α-ENaC AND THE 70-kDA BAND ASSOCIATED WITH γ-ENaC IN THE COLLECTING DUCT IN THESE SAME ANIMAL MODELS. RECENTLY (12), WE SHOWED THE SAME PATTERN OF PROTEIN CHANGES IN WHOLE KIDNEY HOMOGENATES IN OUR RAT MODEL OF VASOPRESSIN ESCAPE. IN THOSE ANIMALS, WE WERE UNABLE TO DETECT A SIGNIFICANT INCREASE IN PLASMA ALDOSTERONE LEVELS COLLECTED AT THE TIME OF DEATH. HOWEVER, BECAUSE OF THE STRIKING NATURE OF THIS PATTERN OF CHANGES IN ENaC EXPRESSION, AND THE FACT THAT ALDOSTERONE REGULATION IN SIADH HAS REMAINED POORLY UNDERSTOOD, WE ELECTED TO REEVALUATE THIS PARAMETER. IN THIS STUDY, WE EVALUATED THE REGULATION OF ALDOSTERONE BY MEASURING IT BOTH IN THE PLASMA AND URINE. WE ALSO EVALUATED THE ABUNDANCES OF ENaC SUBUNITS, NCC, AND THE ENZYME, 11β-HYDROXysteroid dehydrogenase type 2 (11β-HSD-2) IN THE CORTEX, INNER STRIPE OF THE OUTER MEDULLA, AND INNER MEDULLA OF THE KIDNEY THROUGHOUT ESCAPE FROM VASOPRESSIN-INDUCED ANTIDIURETIC HORMONE SECRETION.
urinary antidiuresis. A decrease in the abundance and activity of 11β-
HSD-2 could explain an aldosterone-like pattern of protein changes in the
kidney. This enzyme is responsible for the conversion of corticosterone to cortisol in cells that express it. Thus, under normal conditions, it “shields” the mineralocorticoid receptor, which can bind both mineralocorticoids, such as aldosterone, and glucocorticoids, such as corticosterone, from cortico-
sterone, which circulates in plasma at 100-fold higher concentra-
tions.

We also utilized state-of-the-art radiotelemetry to continu-
ously monitor blood pressure over the course of vasopressin escape. Previously (35), we found a slight increase in mean arterial pressure (MAP) in our rat model of SIADH measured at the end of the study period. In this study, we determined the time course of blood pressure changes and correlated them with changes in aldosterone activity and the natriuresis and diuresis of vasopressin escape.

MATERIALS AND METHODS

Animals and study design. Three studies were performed. For all studies, male Sprague-Dawley rats, ~275 g, were obtained from Taconic Farms (Germantown, MD). In the first study, 18 rats were divided into the following three treatment groups (n = 6/group: 1) control, 2) desmopressin (1-deamino-[8-arginine]-vasopressin; dDAVP) infused, and 3) dDAVP infused plus feeding of a liquid diet. All rats were anesthetized with isoflurane (IsoFlo; Abbot Laboratories, North Chicago, IL) before subcutaneous osmotic minipump implantation (model 2002; Alzet, Cupertino, CA). dDAVP (Aventis Pharmaceuticals, Bridgewater, NJ), a selective vasopressin V$_2$ receptor agonist, was infused in rats in groups 2 and 3 at a rate of 5 ng/h, as previously described (9, 12, 13). At this time, all rats were fed a dry, pelleted AIN-76 formulation diet (BioServe, Frenchtown, NJ). After 4 days, rats in group 3 were switched to a liquid AIN-76 diet containing a high amount of water (80% by weight) or continued on the dry, pelleted diet and water ad libitum for 5 additional days. Rats were housed continuously in Nalgene metabolic cages (Harvard Apparatus, Holliston, MA) to facilitate urine collection. Finally, all rats were killed by decapita-
tion, and both heparinized and K$_2$EDTA blood was collected for plasma hormone analyses. The right kidney was removed rapidly at the end of the study, rats were anesthetized with isoflurane, and blood was collected for plasma hormone analyses. The left kidney was removed rapidly and frozen at -80°C for later dissection and immunoblotting analyses (see below).

Study 2 had the same design as study 1, except n = 4/treatment. At the end of the study, rats were anesthetized with isoflurane, and the left kidney was perfusion fixed with 2% paraformaldehyde after PBS to remove blood.

In study 3, 12 male Sprague-Dawley rats were implanted with radiotelemetric blood pressure transmitters (Data Sciences Interna-
tional, St. Paul, MN). Briefly, under isoflurane anesthesia, the pres-
sure-sensitive tip of the fluid-filled catheter of the radiotransmitter was advanced in the aorta via an incision in the femoral artery. The body of the radiotransmitter was secured in a pouch under the skin near the left hindlimb. Blood pressure was measured for 10 s at 10-min intervals for the entire study. After a 5- to 7-day recovery period, these rats were randomly assigned to one of the following two treatment groups (n = 6/group: 1) dDAVP infusion or 2) dDAVP infusion plus liquid diet (as above). Surgeries to implant minipumps were done as described above, and, when blood pressures were monitored for 7 additional days. Rats were killed by decapitation. All animals were maintained at all times under conditions and protocols approved by the Georgetown University Animal Care and Use Committee, an American Association for Accreditation of Laboratory Animal Care-approved facility.

Urine and plasma analyses. Urine was analyzed for Na$^+$, K$^+$ (ion-selective electrode system, EL-ISE electrolyte system; Beckman Instruments, Brea, CA), osmolality (freezing-point depression, The Advanced Osmometer model 3900; Advanced Instruments, Norwood, MA), and aldosterone (Coat-a-Count RIA kit; Diagnostic Products, Los Angeles, CA). Whole blood was centrifuged at 3,000 rpm (Sorvall RT 6000 D; Sorvall, Newton, CT) at 4°C for 20 min to separate plasma. Plasma was analyzed for Na$^+$, K$^+$, osmolality, aldosterone, renin activity (RIA; Diagnostic Products), and ACTH (RIA; DiaSorin, Stillwater, MN).

Immunohistochemistry for 11β-HSD-2. The left kidney from study 2 was processed to paraffin, and 5-μm sections were cut. Heat-
treated antigen retrieval was performed by boiling in citrate buffer, pH 6 (Zymed Laboratories), to unmask antigenic sites. Endogenous pero-
xisidase activity was removed by incubation with 2% H$_2$O$_2$ (DakoCy-
tomation) for 20 min. Tissues were incubated with 11β-HSD-2 antibody (1:1,000) overnight at 4°C. Sections were incubated with secondary antibody, rabbit anti-sheep IgG conjugated to horseradish peroxygenase (KPL, Gaithersburg, MD) for 1 h, and then with avidin-
biotin complex solution of the Vectastain Elite Peroxidase Kit (Vector Laboratories) for 1 h at room temperature. 3,3′-Diaminobenzidine tetrachloride dihydrate (DAB) was applied for 10 min, and the tissue dehydrated in ascending ethanol and cleared in xylene. Sections were mounted with DPX (Sigma-Aldrich). The left kidney from study 3 was processed to paraffin, and 5-μm sections were cut. Heat-
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Statistics. Data were evaluated by Sigma Stat (Chicago, IL). One-
way ANOVA followed by Tukey’s multiple-comparison test (when significant differences were determined between groups) was used to analyze data from study 1. For study 3, an unpaired t-test was used to evaluate differences between the two treatments in the daily MAPs. To determine a daily average MAP for each rat, each day, all blood pressure readings for that day were averaged, i.e., 144 measurements/day. The Data Sciences software program Dataquest was set to “clip” data that fell out of the range 50 < reading < 200 mmHg as being spurious data.

RESULTS

Verifying escape. Urine volume is plotted in Fig. 1A. Similar to previous studies (9, 12, 13), rats began to “escape” from the antidiuresis of dDAVP by day 2 or 3 after initiation of water loading. Similarly, urine osmolality (Fig. 1B) was reduced significantly in the rats undergoing escape, relative to dDAVP-treated rats, in this study by day 2 of water loading. The marked natriuresis of vasopressin escape began on day 1 (Fig. 2) and peaked by day 2, as observed previously (12).

Aldosterone activity. In Fig. 3A, we have plotted the urine K$^+$:to-Na$^+$ ratio as an index of aldosterone activity in the kidney. This ratio should increase, independently of feed in-
take, when aldosterone activity is high in the kidney. In the first 2 days of water loading, this ratio plunged in the water-loaded
group (during the period of natriuresis). However, starting on day 3, this ratio reversed and showed a sharp, upward slope of increase over the next 3 days, indicating increasing relative aldosterone activity. Figure 3B shows urine aldosterone excretion in these rats. Urine aldosterone was low and not different among all treatments until day 3, on which the water-loaded rats had increased excretion of aldosterone that continued to increase remarkably over the rest of the experiment. Tukey’s multiple-comparison test was applied only when 1-way ANOVA detected a significant (P < 0.05) difference among means. Letters were assigned based on the outcome of Tukey’s multiple-comparison test. Means with letters in common are not statistically different from each other.

Fig. 1. Urine volume and osmolality during vasopressin escape. A: urine volume over the course of the experiment with water loading (WL) commencing on day 0. Urine volume was increased significantly in the desmopressin (dDAVP) + water load group relative to the dDAVP/dry diet group by day 3. B: urine osmolality over the course of the experiment. Osmolality was increased significantly by water loading in the dDAVP-treated rats relative to dDAVP treatment alone by day 2. Tukey’s multiple-comparison test was applied only when 1-way ANOVA detected a significant (P < 0.05) difference among means. Letters were assigned based on the outcome of Tukey’s multiple-comparison test. Means with letters in common are not statistically different from each other.

Fig. 2. Urine Na⁺ excretion during vasopressin escape. dDAVP + water loading led to a marked natriuresis that peaked on day 2.

Fig. 3. Urine K⁺/Na⁺ ratio and aldosterone. A: the ratio of K⁺ to Na⁺ was measured as an index of renal aldosterone activity. This ratio was suppressed significantly in the early part of water loading (through day 2). However, after that, there was a remarkable recovery, indicating increasing Na⁺ relative to K⁺ retention in the escaping rats as days progressed. B: aldosterone excretion over the course of vasopressin escape. Urine aldosterone was low, and not different, among groups until day 3, on which the water-loaded rats had increased excretion of aldosterone that continued to increase remarkably over the rest of the experiment. Tukey’s multiple-comparison test was applied only when 1-way ANOVA detected a significant (P < 0.05) difference among means. Letters were assigned based on the outcome of Tukey’s multiple-comparison test. Means with letters in common are not statistically different from each other.

MAP, ALDOSTERONE, AND ENA:C IN VASOPRESSIN ESCAPE

Material and methods. Materials. All rats were housed and handled as previously described (1). The dDAVP used in this study was a gift from Sugen, Inc. (Sunnyvale, CA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

Plasma biochemistry. As expected, rats receiving the water load were hyposmotic and hyponatremic (Table 1). Plasma aldosterone levels, by day 5, were increased significantly by the water load to 1658%, whereas renin activity was suppressed markedly to 32% of the dDAVP-treated level by day 5. No significant differences were observed for plasma ACTH levels among the groups.

Increased MAP in rats undergoing escape. In Fig. 4, the daily treatment means of the MAP are plotted for the rats in study 2. MAP rose slightly in both groups of rats after they began the dDAVP infusion. However, divergence between
treatment means for MAP began at 2 days of water loading, where MAP for the water-loaded group continued to rise for three additional days (to day 5). At that point, it plateaued at an ~20-mmHg elevation relative to the dDAVP treatment alone.

**NCC and differential regional regulation of ENaC subunit abundances.** In Fig. 5, example immunoblots derived from cortex and outer or inner medullary homogenates of the rats in study 1 probed with antibodies against NCC (cortex only) and α-, β-, and γ-ENaC are shown. NCC was not examined in the inner and outer medulla because it is expressed exclusively in the distal convoluted tubule found only in cortical homogenates. Table 2 provides a densitometric summary of these data and statistics. Similar to our previous findings using whole kidney homogenates (12), we found a mineralocorticoid-like pattern of protein changes in the cortex. That is, there was an increase in abundance of NCC, α-ENaC, and the 70-kDa band of γ-ENaC (broad band region) with water loading. In contrast, this pattern of changes was different in the outer or inner medulla. α-ENaC was actually decreased in abundance by dDAVP infusion in the inner and outer medulla and was not affected at all by the water loading. The 70-KDa band of γ-ENaC was increased by dDAVP in the medulla, irrespective of whether the animals were water loaded. On the other hand, β-ENaC was increased by dDAVP infusion in all three regions. However, in the cortex, it was increased more in the dDAVP + WL rats relative to the dDAVP alone-treated group. Similarly, the upper (85-kDa) band of γ-ENaC was strongly upregulated by dDAVP infusion in all three kidney regions. Finally, in the cortex, our anti-γ-ENaC antibody also recognized a band ~60 kDa, the nature of which is not known. This band did not appear to be regulated in this study.

**Regulation of 11β-HSD-2 protein.** A decrease in the activity of the enzyme 11β-HSD-2 would predict an increase in both

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**Table 1. Plasma physiology in vasopressin escape**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>dDAVP</th>
<th>dDAVP + WL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmolality, mosmol/kg H2O</td>
<td>288 ± 4Δ</td>
<td>290 ± 4Δ</td>
<td>206 ± 3²</td>
</tr>
<tr>
<td>Na⁺, mM</td>
<td>143 ± 1Δ</td>
<td>143 ± 2Δ</td>
<td>102 ± 2²</td>
</tr>
<tr>
<td>Aldosterone, nM</td>
<td>0.61 ± 0.10 AB</td>
<td>0.24 ± 0.06 AB</td>
<td>3.98 ± 1.03³</td>
</tr>
<tr>
<td>Renin activity, ng·ml⁻¹·h⁻¹</td>
<td>7.3 ± 0.6Δ</td>
<td>10.1 ± 1.8Δ</td>
<td>0.4 ± 0.1²</td>
</tr>
<tr>
<td>ACTH, pM</td>
<td>11.3 ± 2.7</td>
<td>6.4 ± 1.1</td>
<td>5.3 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. dDAVP, desmopressin; WL, water load. Plasma was collected at death. Renin activity, generated ANG I. ³ Within a row, the mean is significantly (P < 0.05) increased relative to means labeled with “B.” ² Mean is significantly decreased relative to means labeled with “A.” ²Β Mean is not significantly different from means labeled with either “A” or “B,” as assessed by 1-way ANOVA followed by Tukey’s multiple-comparison test.
MAP, ALDOSTERONE, AND ENaC IN VASOPRESSIN ESCAPE

Table 2. Densitometry summary of immunoblots

<table>
<thead>
<tr>
<th>Protein Region</th>
<th>Treatment</th>
<th>Control</th>
<th>dDAVP</th>
<th>dDAVP + WL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCC CTX</td>
<td>100±11B</td>
<td>94±14B</td>
<td>299±14A</td>
<td></td>
</tr>
<tr>
<td>α-ENaC OM</td>
<td>100±5B</td>
<td>104±11B</td>
<td>196±11A</td>
<td></td>
</tr>
<tr>
<td>α-ENaC IM</td>
<td>100±5A</td>
<td>72±3B</td>
<td>72±5B</td>
<td></td>
</tr>
<tr>
<td>β-ENaC CTX</td>
<td>100±21C</td>
<td>314±35B</td>
<td>545±56A</td>
<td></td>
</tr>
<tr>
<td>β-ENaC OM</td>
<td>100±15B</td>
<td>212±11A</td>
<td>203±26A</td>
<td></td>
</tr>
<tr>
<td>β-ENaC IM</td>
<td>100±10B</td>
<td>143±15A</td>
<td>208±26A</td>
<td></td>
</tr>
<tr>
<td>γ-ENaC (85 kDa) CTX</td>
<td>100±12B</td>
<td>185±10A</td>
<td>194±20A</td>
<td></td>
</tr>
<tr>
<td>γ-ENaC (85 kDa) OM</td>
<td>100±10B</td>
<td>256±10A</td>
<td>217±17A</td>
<td></td>
</tr>
<tr>
<td>γ-ENaC (85 kDa) IM</td>
<td>100±16B</td>
<td>227±53AB</td>
<td>313±41A</td>
<td></td>
</tr>
<tr>
<td>γ-ENaC (70 kDa) CTX</td>
<td>100±9B</td>
<td>116±8B</td>
<td>251±20A</td>
<td></td>
</tr>
<tr>
<td>γ-ENaC (70 kDa) OM</td>
<td>100±6</td>
<td>111±8</td>
<td>122±19</td>
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<td>γ-ENaC (70 kDa) IM</td>
<td>100±16B</td>
<td>145±35AB</td>
<td>254±4B</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE, expressed as %control. NCC, Na-Cl cotransporter; ENaC, epithelial Na⁺ channel; CTX, cortex; OM, outer medulla; IM, inner medulla. Mean is significantly (P < 0.05) increased relative to means labeled with “B.” Mean is significantly decreased relative to means labeled with “A.” AB Mean is not significantly different from means labeled with either “A” or “B,” as assessed by 1-way ANOVA followed by Tukey’s multiple-comparison test.

The interplay between Na⁺ and water balance in the body is quite intricate. Differential regulation of transport along the renal tubule by vasopressin and aldosterone, respectively, allows for selective reabsorption of Na⁺ or water, as needed. SIADH, characterized by hyponatremia, is primarily the result of dysregulated water balance. Na⁺ in the plasma and the cells, is diluted as water is retained. However, concurrent with water retention, a marked transient natriuresis occurs. This is likely an adaptive response of the body to reduce plasma volume as increased arterial pressure is detected by baroreceptors. To prevent excessive Na⁺ losses resulting from natriuresis, it is not unreasonable to predict that adaptive Na⁺-retentive mechanisms may come into play to reestablish Na⁺ balance. One possibility to accomplish this is upregulation of aldosterone production and excretion and its regulated proteins.

What is the stimulus for hyperaldosteronism in vasopressin escape? Although not considered a classic stimulus, as high extracellular K⁺ concentration, ANG II, and ACTH are recognized to be, several studies (1, 6, 7, 23, 28, 30, 37) suggest that hyponatremia is associated with direct or indirect stimulation of adrenal aldosterone synthesis. Balla et al. (1) used peritoneal dialysis with a 5% glucose solution in dexamethasone-pretreated rats to produce hyponatremia. They found an increase in plasma aldosterone levels when Na⁺ levels dropped <132 mM. Furthermore, Decaux and associates (7) have

Fig. 6. Immunoblotting of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2) protein in kidney regions during vasopressin escape. Left: each lane of each blot is loaded with a different rat’s sample (n = 6/treatment) of whole homogenate of either cortex or outer or inner medulla (as indicated). Within each blot, equal amounts of total protein were loaded in each lane, and a Coomassie-stained gel confirmed equality of loading for each sample set. Blots were probed with the polyclonal antibody against 11β-HSD-2. Right: summary of densitometry for cortex (top), outer medulla (middle), and inner medulla (bottom). One-way ANOVA followed by Tukey’s test revealed a significant increase in band density for dDAVP + WL vs. control rats in the cortex. Means with letters in common are not statistically different from each other.

AJP-Renal Physiol • VOL 287 • NOVEMBER 2004 • www.ajprenal.org
demonstrated a corticosteroid-dependent nature of the hyperaldosteronism. They showed that panhypopituitaric rats treated with water and dDAVP do not develop hyperaldosteronism, as do the same rats treated with corticosteroids. We found no significant differences in the level of corticosterone (previous study and Ref. 12) or ACTH (Table 1) between treatment groups; however, whether their presence at any level was required to produce hyperaldosteronism was not tested.

A few groups (23, 28, 30, 37) have examined potential cellular signaling mechanisms for the hyperaldosteronism. Makara et al. (23) demonstrated increased sensitivity of rat adrenal glomerulosa cells to K⁺ when the cells were incubated...
in hyposmotic conditions (250 mosmol/l), as demonstrated by increased Ca\(^{2+}\) currents and aldosterone secretion. Schneider and associates (28, 30, 37) have reported that osmolality, independently of Na\(^{+}\) concentration, affects basal and K\(^{+}\)- and ANG-stimulated aldosterone secretion in bovine or canine glomerulosa cells. They suggest that aldosterone secretion is likely mediated via hyposmotic activation of voltage-dependent or stretch-activated Ca\(^{2+}\) channels (37).

There are several possible reasons why the effect of hyponatremia on aldosterone secretion is not well recognized. First, there have been conflicting reports as to whether it really occurs (5, 12, 14, 15). Cogan et al. (5) and Fichman et al. (14, 15) have shown depressed or normal aldosterone levels, respectively, in patients with SIADH. Second, hyponatremia is considered a pathophysiological state, and the classic stimuli are generally considered to be the major contributors to aldosterone stimulation under normal physiological conditions. Third, there appears to be a delay in the rise in aldosterone relative to the development of hyponatremia, making it unclear what the stimulus for secretion truly is (22). As early as 1965, Knochel and associates (22) reported a delayed rise in aldosterone excretion in a patient with SIADH administered a water load. We speculate, as do others (22), that the “delay” in the appearance of hyperaldosteronism relative to the rapid development of hyponatremia may be the result of the competition between factors that decrease aldosterone, i.e., volume expansion, and factors that increase it, i.e., hyponatremia. This might also explain conflicting findings of many groups, including ourselves (12).

In these studies, we show a gradual, but sustained, increase in MAP (10–20 mmHg) in rats infused with dDAVP after they were switched to a “liquid diet.” Blood pressure begins to increase 1–2 days after the start of the liquid diet and plateaus by day 4. The mechanism for the increase in blood pressure cannot be determined here. SIADH is not commonly associated with hypertension in humans (16, 27). However, because these patients are generally quite ill to begin with, mild elevations in blood pressure might be overlooked. Case reports of elevated blood pressure in association with SIADH do exist (18, 26, 31); however, the cause and effect are unclear in these complex disorders. Several years ago (35), we showed an elevation in blood pressure of a similar magnitude in our rat vasopressin escape model. However, in that study, blood pressure was monitored for only 120 min at the completion of the study in conscious animals via an indwelling aortic catheter, as opposed to continuously via telemetry from the outset of the study. In dogs, using a servo-controlled renal arterial pressure-clamped model, Hall and associates (16) demonstrated that a rise in renal arterial pressure was necessary to elicit the diuresis and natriuresis of vasopressin escape.

It seems plausible that high aldosterone activity was the cause of the increase in the protein abundances of α-ENaC, NCC, and the 70-kDa band of γ-ENaC, because this is the pattern that is produced by aldosterone infusion or feeding of a low-NaCl diet to rats (19, 24), although we did not directly test that assumption here. In this study, we also found an increase in β-ENaC in the cortex of the water-loaded rats relative to both other groups. In our previous studies (12), we did not observe this difference; however, in those studies, we examined whole kidney rather than cortex homogenates and were using a different polyclonal antibody. Curiously, we find that regulation of ENaC subunit abundance is different in the outer and inner medulla relative to the cortex. Although regulation of the ENaC subunit abundances by aldosterone, ANG II, and vasopressin in the whole kidney or the cortex has been well described, little has been done examining regulation in the outer and inner medullary portion of the collecting duct. This may partly relate to the fact that the presence of ENaC subunit expression in the inner medullary collecting duct is controversial (4, 8, 32, 36), with some reporting little or no ENaC mRNA in these cells (8, 32). Nevertheless, recently, Nielsen and associates (25) have examined the regulation of ENaC subunit protein abundances in all three regions of the collecting duct. They have reported differential regional regulation in rat models of lithium toxicity (25) and puromycin aminonucleoside nephrotic syndrome (20). With lithium toxicity, β- and γ-ENaC were downregulated in protein abundance in cortex and outer medulla, but not in inner medulla or connecting tubule. They suggested, as one possible explanation, a cell-specific resistance to aldosterone and vasopressin in the cortical and outer medullary collecting duct. Differences in sensitivity may relate to differences in expression patterns of both the mineralocorticoid receptors and 11β-HSD-2. We have confirmed others’ (2, 29) findings of decreased inner medullary relative to outer medullary and cortical abundance of 11β-HSD-2, which could explain increased sensitivity to changes in circulating aldosterone levels in the cortex relative to the medulla. However, Wolf et al. (38) have reported an increase in α-ENaC mRNA in the cortex and outer and inner medulla of rats fed a low-NaCl diet, suggesting aldosterone sensitivity of at least this subunit in the inner medulla. We did see a trend toward an increase in abundance of 11β-HSD-2 with dDAVP infusion, as Brooks et al. (3) have reported, but we did not observe any relative downregulation of this abundance with water-loading, which might explain the mineralocorticoid-like pattern of protein changes present only in the cortex. However, it is possible that activity of this enzyme, however, is diminished without a change in abundance. Additional studies will be needed to understand the mechanisms underlying differential regional or cellular regulation of ENaC subunits during physiological and pathophysiological states.

Overall, these studies clearly show an increase in plasma and urine aldosterone and MAP in rats undergoing vasopressin escape. Renal distal tubule proteins known to be regulated by aldosterone are also strongly upregulated in this model. These adaptive changes may facilitate the escape process, while attenuating the severity of Na\(^{+}\) losses.

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