Chronic Exposure to Vasopressin Upregulates ENaC and Sodium Transport in the Rat Renal Collecting Duct and Lung

Carole Nicco, Monika Wittner, Antonio DiStefano, Sylvie Jounier, Lise Bankir, Nadine Bouby

Abstract—Vasopressin is known to acutely stimulate sodium transport in the renal collecting duct. We investigated the long-term regulation by vasopressin of the epithelial sodium channel (ENaC) in the rat kidney. Five-day infusion of dDAVP (α V2 receptor agonist) to Brattleboro rats lacking vasopressin induced a marked increase in β- and γ-subunit ENaC mRNA levels in the renal cortex (β, 85%; γ, 100%), with no change in α-ENaC mRNA. Expression of β- and γ-ENaC mRNAs was also enhanced in lung (β, 49%; γ, 33%) but not in distal colon (an organ devoid of V2 receptors). Similar results were obtained in Sprague Dawley rats after either partial water restriction or dDAVP infusion for 5 days. Transepithelial voltage and transepithelial sodium and water net fluxes were measured in isolated perfused cortical collecting ducts of Brattleboro rats. Acute addition of 2×10^-10 mol/L dDAVP to the bath increased sodium and water fluxes in the same proportion, and to a far greater extent in dDAVP-infused than in control Brattleboro rats (change in Na^+ net flux, 337±30 versus 49±11 pmol · min^-1 · mm^-1, respectively; P<0.001). These effects were abolished by amiloride. Extrarenal water losses, partly originating from the lung, were reduced by high plasma vasopressin level. This study shows that vasopressin increases sodium transport in the renal collecting duct and probably in the lung, through a differential transcriptional regulation of ENaC subunits. This effect is followed by isosmotic water reabsorption and likely contributes to the process of water conservation. It could lead to less efficient sodium excretion, however, and thus participate in some forms of salt-sensitive hypertension. (Hypertension. 2001;38:1143-1149.)

Key Words: kidney ■ lung ■ colon ■ water transport ■ sodium

Excessive sodium reabsorption by the kidney has been known for a long time to participate in the pathogenesis of some forms of hypertension. The major role of the kidney in the control of blood pressure is now amply confirmed by the phenotypic consequences of recently discovered mutations in genes coding for membrane proteins involved in sodium reabsorption in the distal nephron, such as the epithelial sodium channel (ENaC).1,2 If a permanent activation of ENaC, as seen in Liddle’s syndrome, is sufficient to induce severe hypertension, more subtle dysfunction in the regulatory mechanism influencing ENaC activity might represent significant risk factors in essential hypertension. It is thus important to identify more precisely the factors involved in the control of ENaC activity in the kidney.

Aldosterone is considered to be the main hormone regulating sodium reabsorption in the distal nephron, distal colon, ducts of several exocrine glands, and airways; it increases the permeability of the luminal membrane of their epithelial cells to sodium. ENaC represents the limiting step for sodium reabsorption in these cells. In the kidney, the 3 homologous subunits that constitute the channel (α, β, and γ)3 are detected in the late distal convoluted tubule, connecting tubule, cortical and outer medullary collecting duct, and, to a lesser extent, the inner medullary collecting duct.4,5 The exact function of each subunit is not well elucidated. The α-subunit is functional when expressed alone in Xenopus oocytes, but the channel activity is highly increased by association with β- and γ-subunits.6 In transfected rat thyroid cells, sodium reabsorption requires the coexpression of all 3 subunits.7 The regulation of ENaC by mineralocorticoids is not homogenous with regard to both subunits and organs. In the distal colon, a large increase in β- and γ-subunit transcripts is observed in response to steroid treatment, whereas expression of α-subunit mRNA shows little variation.8–10 In contrast, in the kidney, aldosterone affects only the expression of α-subunit transcript or protein,11,12 although some investigators found no effect of this hormone on subunit abundance.4,8,13 Moreover, transgenic mice lacking the mineralocorticoid receptor exhibit a normal abundance of mRNAs encoding ENaC subunits in the kidney.14 These observations suggest that factors other than aldosterone are involved in the regulation of ENaC in the kidney. Now, vasopressin is known to stimulate sodium reabsorption in renal collecting duct15,16 and has been shown to exert synergistic effects with aldosterone.17,18
The present experiments were designed to investigate both the chronic influence of vasopressin on ENaC transcription in the rat kidney and the resulting consequences in terms of sodium transport in the collecting duct. Because vasopressin actions on sodium transport and water permeability in the collecting duct are mediated by V₁ receptors, we studied the influence of the selective V₁-receptor agonist dDAVP in Brattleboro rats with hereditary hypothalamic diabetes insipidus (DI), caused by a single base deletion in the vasopressin gene. To extend the results obtained in Brattleboro DI rats, an additional study of ENaC transcription was performed in Sprague Dawley rats in which the plasma vasopressin level was increased chronically by either dDAVP infusion or partial restriction in water intake.

Methods

Animals

All animal procedures were conducted in agreement with our institutional guidelines for the care and use of laboratory animals. Homozygous male Brattleboro rats were used in 2 series of experiments: series 1, body weight ~250 g (bred in our laboratory); and series 2, body weight ~150 g (Harlan, Indianapolis, Ind). Rats were continuously infused with the antiidiuretic analog of vasopressin, dDAVP (Ferring), for 18 hours or 5 days (treated-DI rats = T-DI). Control rats (C-DI) were infused with vehicle (NaCl 0.9%). All rats were fed a standard laboratory diet and had free access to tap water.

In series 3, male Sprague Dawley rats (Iffa Credo, France), BW ~290 g, with urinary concentrating activity increased either by intraperitoneal infusion of dDAVP for 5 days (1 μg · kg⁻¹ · day⁻¹; T-SD) or by reducing water intake to 15 mL/day (restricted water intake, R-WI), were used. Urinary concentrating activity was increased chronically by either dDAVP infusion or partial restriction in water intake.

Quantitation of ENaC mRNA Expression (Series 1 and 3)

Total RNA was isolated from distal colon (15 mm), lung (100 mg), superficial renal cortex, and deep outer stripe of renal outer medulla (50 to 100 mg of each) with RNA PlusKit (Quantum). Ten micrograms were run on 1.2% agarose/2.5% formaldehyde gel and blotted to nitrocellulose membranes (Hybond-C extra, Amersham). Membranes were hybridized with randomly primed ³²P-labeled probes for α1- (nt 279 to nt 2164), β- (nt 385 to nt 1440), and γ- (nt 511 to nt 2304) subunits. Human probe for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1000 nt length) was used as a reference. Radioactivity bound to transcripts (α, 3.7 kb; β, 2.2 kb; γ, 3.2 kb; and GAPDH, 1 kb) was measured with an electronic autoradiography system (InstantImager, Packard Instruments). Results are expressed as mean±SEM. Data obtained in the 2 groups of DI rats compared by Student’s t test. Those obtained in the 3 groups of Sprague Dawley rats were compared by 1-way ANOVA followed by protected least significant difference Fisher post-hoc test. P < 0.05 was considered statistically significant.

Physiological Study (Series 1 and 3)

Body weight, daily water and food intake, urine flow rate, and osmolality were measured during the last 2 days of the different treatments. Extrarenal water losses were calculated as the difference between daily water intake and urine flow rate. Under sodium pentobarbital anesthesia (6 mg/100 g body weight IP), a blood sample was collected, and kidneys, the distal part of the colon, and lungs were removed for immediate RNA extraction. Aldosterone concentration in urine and plasma was measured by radioimmunoassay (Dade Behring).

Physiological Data for C-DI and T-DI Rats

<table>
<thead>
<tr>
<th>Data</th>
<th>C-DI (n=5)</th>
<th>T-DI (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>254±2</td>
<td>257±5</td>
</tr>
<tr>
<td>Water intake, mL/day</td>
<td>170±6</td>
<td>24.9±0.9*</td>
</tr>
<tr>
<td>Urine flow rate, mL/day</td>
<td>133.1±5.1</td>
<td>10.7±0.2*</td>
</tr>
<tr>
<td>Extrarenal water loss, mL/day</td>
<td>37.2±2.2</td>
<td>14.2±0.8*</td>
</tr>
<tr>
<td>Urine osmolality, mOsm/kg H₂O</td>
<td>217±17</td>
<td>2106±46*</td>
</tr>
<tr>
<td>Na⁺ excretion, mmol/day</td>
<td>2.65±0.10</td>
<td>2.58±0.05</td>
</tr>
<tr>
<td>Plasma Na⁺, mmol/L</td>
<td>152±2</td>
<td>138±2*</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>47.3±0.7</td>
<td>47.6±0.4</td>
</tr>
<tr>
<td>Plasma aldosterone, ng/dL</td>
<td>18.3±4.5</td>
<td>20.1±3.9</td>
</tr>
<tr>
<td>Aldosterone excretion, ng/day</td>
<td>40.6±5.3</td>
<td>39.9±5.5</td>
</tr>
</tbody>
</table>

*P<0.001, Student’s t test.

Results

Physiological Data

As expected, dDAVP infusion in Brattleboro DI rats restored a urine concentrating activity close to that of normal rats, decreasing urine flow rate and increasing urinary osmolality ~10-fold (Table 1). Interestingly, the dDAVP treatment also reduced extrarenal water losses (to 38% of control, P<0.001). Daily sodium intake and excretion were similar in both groups, and the hypernatremia generally seen in DI rats was corrected by dDAVP infusion. Plasma concentration and excretion of aldosterone were not modified by dDAVP treatment (Table 1).

Measurement of Water and Sodium Transport in Isolated Perfused CCD (Series 2)

CCD were microdissected from the medullary rays and perfused in vitro as previously described. Briefly, the dissected tubules, 0.3 to 0.5 mm in length, were mounted on a set of concentric micropipettes and perfused at 37°C at rates of 2 to 3 nL/min. Asymmetrical conditions of osmolality and sodium concentration for bathing and perfused solutions were chosen to maximize water flux and to study precisely both the sodium and water movements. Fluid movements were monitored by addition of [methoxy-³H]-inulin to the perfusate. Sodium concentration in the perfused solution and the collected tubular fluid were measured with a flame microspectrophotometer. Transepithelial voltage (Vₑ) was measured continuously between calomel electrodes connected to 0.9% NaCl/4% agarose bridges inserted in the perfusion pipette and bathing solutions. Each experiment included a 20- to 30-minute equilibration period followed by collection of distal tubular fluid during 3 successive conditions (three 10-minute collections per condition): (1) basal, (2) 2 × 10⁻⁶ mol/L dDAVP added to the bathing solution, and (3) 10⁻⁶ mol/L amiloride added to the luminal perfusate, with dDAVP still present in the bathing solution. Transepithelial sodium (JₑNa) and water (JₑV) net fluxes per unit length of tubule were calculated with positive net flux values indicating net reabsorption. The three 10-minute collections for each experimental condition were averaged for each rat. Results were expressed as mean±SEM. Comparison of results obtained for the different conditions in C-DI and T-DI rats was performed by 2-way ANOVA (conditions and groups), followed by protected least significant difference Fisher post-hoc test. Comparison of the differences between dDAVP and basal conditions for each tubule in C-DI and T-DI rats was performed by paired t test. P < 0.05 was considered statistically significant.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.
TABLE 2. Physiological Data for C-SD, T-SD and RW-SD

<table>
<thead>
<tr>
<th>Data</th>
<th>C-SD</th>
<th>T-SD</th>
<th>RW-SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>297±8</td>
<td>288±5</td>
<td>293±4</td>
</tr>
<tr>
<td>Water intake, mL/day</td>
<td>23.8±1.7</td>
<td>17.2±1.7*</td>
<td>14.4±0.2*</td>
</tr>
<tr>
<td>Urine flow rate, mL/day</td>
<td>13.3±1.1</td>
<td>7.9±0.4*</td>
<td>6.8±0.3*</td>
</tr>
<tr>
<td>Extrarenal water losses, mL/day</td>
<td>10.5±1.2</td>
<td>9.4±1.9</td>
<td>7.5±0.4*</td>
</tr>
<tr>
<td>Urine osmolality, mosm/kg H2O</td>
<td>1734±179</td>
<td>2480±51*</td>
<td>2787±90*</td>
</tr>
<tr>
<td>Na+ excretion, mmol/day</td>
<td>2.07±0.05</td>
<td>2.04±0.04</td>
<td>1.91±0.04</td>
</tr>
<tr>
<td>Plasma Na+, mmol/L</td>
<td>142±2</td>
<td>143±1</td>
<td>147±2</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>46.8±0.5</td>
<td>47.4±1.0</td>
<td>45.5±1.1</td>
</tr>
<tr>
<td>Plasma aldosterone, ng/dl</td>
<td>38.7±8.9</td>
<td>46.5±9.8</td>
<td>37.9±8.0</td>
</tr>
<tr>
<td>Aldosterone excretion, ng/day</td>
<td>10.0±1.2</td>
<td>27.0±3.6*</td>
<td>12.7±1.9</td>
</tr>
</tbody>
</table>

*P<0.001, 1-way ANOVA, RW-SD or T-SD vs C-SD.
†P<0.001, RW-SD vs T-SD.

The 2 protocols used to stimulate the concentrating activity of the kidney in SD rats reduced urine flow rate by 45% and increased urine osmolality accordingly (Table 2). The amount of water lost by nonrenal tissues was also significantly reduced (by 30%) in the RW-SD group. Comparison between C-DI and C-SD rats (Tables 1 and 2) reveals that the lack of antidiuretic hormone results in markedly enhanced extrarenal water losses (37±2 versus 10±1 mL/d, P<0.001), although diarrhea was not seen. In a separate experiment, we observed that chronic treatment with either a V2 antagonist (SR121463A, Sanofi) or a V2 agonist (dDAVP) in SD rats (body weight, 300 g; n=4 per group)—leading to large differences in urine flow rate (58±7 and 9±1 mL/d, respectively; P<0.001) and extrarenal water losses (26±2 and 14±2 mL/day, respectively; P<0.01)—did not influence the amount of droppings (0.37±0.04 and 0.44±0.05 g/d per gram of food intake, P=NS) and their water content (57±1 and 53±2%, P=NS). No difference was observed among the 3 groups of SD rats for hematocrit, plasma sodium concentration, and urinary sodium excretion. Plasma aldosterone concentration was not significantly different among the 3 groups. For an unknown reason, T-SD group showed a higher aldosterone excretion compared with that of the other groups (Table 2).

ENaC mRNA Expression

After 5 days of dDAVP treatment, the expression of mRNA for β- and γ-subunits in Brattleboro DI rats was markedly enhanced in the cortex (85% and 100%, respectively; P<0.001), whereas expression for the α-subunit was barely modified (12%, P=NS) (Figures 1 and 2). The same pattern was observed in the outer strip, although with a lesser intensity (β: 39%, P<0.001; γ: 53%, P<0.001; α: −12%, P=NS). After 18 hours of dDAVP treatment, a moderate increase in mRNA abundance of β- and γ-subunits was already detectable in the cortex (β: 32%, P<0.05; γ: 36%, P<0.05; α: −14%, P=NS) but not in the outer stripe (data not shown). In the lung, β- and γ-subunit mRNA levels were significantly higher in the T-DI group than in the C-DI group (49%, P<0.01 and 33%, P<0.02, respectively), whereas the α-subunit mRNA level was similar in both groups (Figures 1 and 2). In the distal colon, dDAVP infusion did not induce any significant modification in the mRNA expression of any subunit (Figures 1 and 2).

In Sprague Dawley rats, enhancing the urinary concentrating activity by dDAVP infusion or by moderate dehydration induced a significant increase in the expression of β-subunit (33%, P<0.0001 and 34%, P<0.0001, respectively) and γ-subunit (49%, P<0.002 and 29%, P<0.05, respectively) mRNAs in the renal cortex, whereas the expression of mRNA for the α-subunit was almost unchanged (13% in each
ENaC Activity and Sodium and Water Transport in CCD

Under basal conditions, ie, in the absence of dDAVP in the bath solution, the transepithelial voltage was significantly more negative in T-DI rats than in C-DI rats (−6.3±1.4 mV versus −3.8±0.5 mV, P<0.0002) (Figures 4 and 5). The negative sodium net flux observed in C-DI (−151±19 pmol·min⁻¹·mm⁻¹) was significantly different from zero, P<0.01 and T-DI (−99±56 pmol·min⁻¹·mm⁻¹, P=NS) probably arose from passive paracellular sodium movements from bath to lumen, due to the difference in sodium concentration between perfusion and bath solutions (see Methods).

Addition of 2×10⁻³⁰ mol/L dDAVP to the bath induced in both groups a rapid fall in transepithelial voltage (maximal change achieved after 4 minutes) (Figures 4 and 5). This fall was significantly greater in T-DI rats than in C-DI rats (−6.0±0.5 versus −1.4±0.5 mV, P<0.0005). These voltage changes were accompanied by a reduction in transepithelial sodium net secretion in the C-DI group (from −151±19 to −102±20 pmol·min⁻¹·mm⁻¹, P=0.01) and a marked net reabsorption in the T-DI group (from −99±56 to 237±41 pmol·min⁻¹·mm⁻¹, P<0.002). The dDAVP-induced changes in sodium net flux (ΔJ Na⁻) were of much higher intensity in T-DI than in C-DI, amounting to 337±30 and 49±11 pmol·min⁻¹·mm⁻¹, respectively (P<0.001), as shown in Figure 6. The dDAVP-induced changes in both transepithelial voltage and sodium flux were rapidly reversed by the addition of 10⁻⁶ mol/L luminal amiloride (Figures 4 to 6), indicating that they resulted from an increased activity of ENaC.

Under basal conditions, no water net flux was observed in CCD of C-DI rats (0.00±0.08 nL·min⁻¹·mm⁻¹), and a small water net flux was found in that of T-DI rats (1.83±0.04 nL·min⁻¹·mm⁻¹). Addition of dDAVP to the bath induced a significant increase in water reabsorption in T-DI (P<0.01) and C-DI (P<0.05), with a lower intensity in the latter (ΔJw, P<0.01; T-DI versus C-DI; and #P<0.001, dDAVP or dDAVP+Amil versus Basal (ANOVA followed by Fisher post-hoc test).
dance of mRNA coding for the β- and γ-subunits of ENaC in the renal cortex and outer medulla and in the lung, but not in the colon. Aldosterone cannot be suspected to have mediated these effects because its plasma level was similar in all groups. Vasopressin V$_2$ receptor mRNA is expressed in the human lung, and this hormone influences sodium and fluid movements in that organ. In contrast, no vasopressin receptors and vasopressin-dependent sodium transport have been reported in the distal colon. The fact that dDAVP influenced ENaC mRNA expression only in organs possessing V$_2$ receptors suggests that this influence is exerted directly on vasopressin target cells and does not result from physicochemical changes in the internal milieu or from changes in other hormonal systems consecutive to the actions of this hormone on fluid handling in the body.

In both the kidney and lung, only β- and γ-subunit mRNAs were enhanced by dDAVP. This pattern is similar to that reported in a CD cell line exposed to vasopressin in vitro for several hours and to that observed in the colon after in vivo aldosterone or dexamethasone treatment for several days. This suggests that the long-term hormone-dependent regulation of ENaC expression is achieved mainly by changes in the abundance of these 2 subunits and that α-subunit abundance (mRNA or protein) is either not rate limiting for assembly of ENaC channels or regulated by other mechanisms.

**Vasopressin Influence on Sodium and Water Transport**

Under basal conditions, the transepithelial voltage, and the water and sodium fluxes in the isolated CCD were higher in dDAVP-treated rats than in C-DI rats. Moreover, a much greater change in both transepithelial voltage and net sodium flux was observed on acute dDAVP stimulation in chronically treated rats than in controls. The fact that these changes were reversed by amiloride proves that they result from an increased activity of ENaC. These observations indicate that the increase in β- and γ-ENaC gene transcription induced by chronic dDAVP treatment (present study) and that in β- and γ-ENaC protein abundance, recently demonstrated by Ecelbarger et al., result in a marked increase in vasopressin-sensitive sodium reabsorptive capacity in the CCD. However, the larger increase in the rate of net sodium transport than in subunit mRNA abundance suggests that additional mechanisms contributing to increased transport activity were also activated by vasopressin.

Acute dDAVP addition to the bath of isolated CCD also increased water flux to a far greater extent in dDAVP-treated than in C-DI rats, at the same proportion as the increase in sodium fluxes. This shows that water movement closely followed the sodium-induced osmotic driving force, as expected when water permeability of the CCD is not a limiting factor.

Our results show that vasopressin, through its V2 receptor–mediated actions, not only reduces urine flow rate but also reduces water losses in nonrenal sites. In the same line, Pouzet et al. reported that 1-week administration of a selective, nonpeptide V2 receptor antagonist in Sprague Dawley rats significantly enhanced extrarenal water losses. In rats, which do not possess sweat glands, the main site of
nonrenal water loss is the respiratory tract (in the absence of diarrhea). In the lung, water is reabsorbed through constitutively expressed aquaporin-1 and -3, driven by the osmotic force generated, for a large part, by ENaC-mediated sodium reabsorption. The differences in extrarenal water losses suggest that vasopressin increased sodium transport in the lung, as it does in the CD. Interestingly, vasopressin also increased the abundance of β- and γ-ENaC subunit message in the lung, as in the kidney. Thus, most likely, the transcriptional effects observed in the lung resulted in enhanced functional ENaC molecules and transport activity in this organ, as observed in the CD.

Possible Functional Consequences of Vasopressin Influence on ENaC

The secretion of vasopressin is not known to depend on sodium intake. It seems probable that vasopressin effects on ENaC and sodium reabsorption in kidney and lung serve the purpose of fluid conservation rather than of regulating sodium excretion. A stronger sodium reabsorption in the connecting tubule, the cortical, and the outer medullary CD (all vasopressin-responsive segments in which aquaporin-2 and ENaC are coexpressed) will drive more water out of the lumen and thus will increase the concentration of all solutes but sodium in the urine, as observed in vivo.34

The fact that vasopressin enhances sodium reabsorption in the last part of the nephron should likely result in some deficit in sodium excretion. Vasopressin has been reported to be natriuretic, but this effect is observed only when an intense water diuresis is interrupted by the systemic infusion of vasopressin (resulting in volume expansion).35 In contrast, distinct antinatriuretic effects of vasopressin have been observed in normovolemic rats and humans13,36–38 or in the isolated erythrocyte-perfused kidney.39 Thus, with high vasopressin levels, water conservation is probably achieved, at least temporarily, at the expense of a reduction in sodium excretion. No deficit in daily sodium excretion is observed, however, because compensatory mechanisms, such as pressure natriuresis, develop to maintain a stable sodium balance (as seen in our study). It is thus conceivable that individuals with a higher tendency to conserve water will be more prone to develop salt-sensitive hypertension. Actually, a limited capacity to excrete sodium and/or a tendency to develop salt-sensitive hypertension on one hand, and increased vasopressin levels or increased tendency to concentrate urine on the other hand, seem to be genetically associated in rodents and humans.40–44 Moreover, deoxycorticosterone acetate–salt hypertension can develop only in the presence of vasopressin or dDAVP.45,46

In summary, the present study shows that vasopressin not only exerts an acute influence on ENaC-dependent sodium transport, as was previously known, but also induces an increase in ENaC message and probably in ENaC protein, in both the kidney and lung. This enhances the capacity of the collecting duct and pulmonary epithelium to reabsorb sodium and water in response to short-term hormonal stimulation. The fact that vasopressin regulates ENaC abundance in the kidney and lung, together with the other known actions of vasopressin on the kidney, suggest that this influence partic-

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