EXAGGERATED NATRIURETIC RESPONSE OF BRATTLEBORO RATS TO EXTRACELLULAR VOLUME EXPANSION

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A state of chronic dehydration with reduced plasma volume, decreased blood pressure, and increased plasma renin activity (PRA) has been demonstrated in rats with hereditary hypothalamic diabetes insipidus (DI rats). In this situation decreased renal perfusion and glomerular filtration rate might result in sodium retention. On the other hand, the DI rat also suffers from mineralocorticoid deficiency which might result in salt wasting. In addition it has recently been shown that in contrast to normal rats, there are no differences between superficial cortical and juxtamedullary nephrons of the DI rat with respect to single nephron filtration rate, glomerular volume, and proximal tubular length. This lack of internephron heterogeneity might also affect renal sodium handling in the DI rat. It thus seems of particular interest to evaluate the natriuretic response of DI rats to volume expansion.

In the present study, the natriuretic responses of DI and normal Long-Evans rats to acute and chronic volume expansion were compared. Other factors involved in sodium handling, namely mineralocorticoids and renal (Na+ + K+)-ATPase activity, were also studied.

MATERIALS AND METHODS

Male and female Long-Evans rats of the Brattleboro strain were used. They were either normal (LE) or homozygous for the hypothalamic diabetes insipidus trait (DI rats). Body weight ranged from 170–280 g. Control and experimental groups were matched for weight and sex.

Prior to any experiment, acute or chronic, rats were placed in individual metabolism cages for three to four days.

The effects of chronic volume expansion were studied in nine DI and eight LE rats on a high salt diet. The rats were first maintained on a normal diet during four days of balance study. The balance study consisted of daily measurements of food and water intake, body weight, urine volume and osmolality, and urinary excretion of sodium and potassium. The rats were then fed a high sodium diet (Purina Labchow plus 1% NaCl by weight). Five of the DI and four of the LE rats were injected daily with 0.5 mg/100 g BW of deoxycorticosterone acetate (DOCA, Upjohn). The remaining rats received an equivalent volume of sesame oil.
The effect of acute volume expansion was studied in six DI and six LE rats following a six-day equilibration period in metabolism cages. On the morning of the seventh day the rats were placed in clean metabolism cages without food but with water ad libitum. Two hours later each rat was induced to urinate by touching its nose with an ether-impregnated gauze. This sample was discarded. A control four-hour urine collection was then begun. At the end of this period (and all subsequent periods) urination was induced and this urine added to that which had been spontaneously voided during the collection period. The rats were then weighed and given by gavage a volume of isotonic saline equivalent to 5% of body weight in two doses, ten minutes apart. Urine was collected 30, 60, and 120 min after the second dose of saline. Sodium concentration and volume of all samples were measured.

One week later blood was obtained from the same rats following a four-hour control collection as previously described. The next day the animals were again saline loaded and blood was obtained at 60 minutes after loading, the time when the peak of natriuresis had been found to occur. In this manner, blood sampling did not interfere with the natriuretic response. Hematocrit and serum sodium concentration were measured.

A similar experiment was carried out in 12 DI and 12 LE rats pretreated with either DOCA (6 DI, 6 LE; 2 mg/day) or oil for three days before saline loading. Still another group (6 DI, 6 LE) received free deoxycorticosterone (DOC, Sigma Chemical Co.; 2 mg/day) for three days before the saline-loading study.

Plasma aldosterone concentration and renal (Na\(^+\)+K\(^+\))-ATPase activity were measured in another group of DI and Long-Evans rats of both sexes. Thirteen DI and nine Long-Evans rats weighing 201-338 g were housed in metabolism cages for more than one week. A balance study was carried out on the last two days. The conscious rats were then bled by tailcutting for determination of plasma aldosterone concentration (Abbott Labs).\(^{10}\) Eight of the DI and seven of the LE rats were then anesthetized with ether and the kidneys rapidly excised for determination of renal (Na\(^+\)+K\(^+\))-ATPase activity by methods previously described.\(^{11}\) The microsomal fraction of a whole kidney homogenate was separated by differential centrifugation. Total ATPase activity was determined by incubation of this fraction with a reaction mixture containing a final concentration of 100 mM NaCl, 10 mM KCl, 10 mM imidazole buffer, 6 mM MgCl\(_2\), and 6 mM disodium ATP at pH 7.4. Mg\(^{2+}\)-ATPase activity was determined by incubation with the same solution, from which sodium and potassium chloride had been omitted, and 2 mM ouabain added. The amount of inorganic phosphate produced in five minutes at 37° C was determined by the method of Fiske and Subbarow\(^{12}\) and the protein content by the method of Lowry et al.\(^{13}\) (Na\(^+\)+K\(^+\))-ATPase activity was expressed as the difference between total and Mg\(^{2+}\)-ATPase activity.

Sodium concentration of plasma and urine was determined by flame photometry (Radiometer, Model FLM 2E). Urine osmolality was determined by freezing-point depression (Advanced Instruments osmometer, Model 3W). Data was analyzed using paired-t and Student's t tests.

RESULTS

As shown in FIGURE 1, the urinary sodium excretion \((U_{Na}\cdot V)\) of both oil-treated and DOCA-treated Long-Evans rats was significantly increased on
Opava-Stitzer et al.: Natriuretic Response

A high sodium diet. Urine flow rate was unchanged but a significant increase in urine osmolality was seen (1827 ± 170 versus 2149 ± 158 mOsm/kg H₂O; p < 0.005). Body weight also increased significantly. The only differences between $U_{Na}^V$ of DOCA-treated and oil-treated LE rats occurred on days three and five of treatment. Sodium intake was greater than urinary sodium excretion on all days of study.

FIGURE 2 shows the response of DI rats to chronic volume expansion. Sodium excretion was significantly increased by a high salt diet with or without DOCA treatment. No differences were observed between $U_{Na}^V$ of DOCA-treated and oil-treated rats on any day of study. Urine flow rate was signifi-

cantly increased compared to values on a normal diet (95 ± 4 versus 72 ± 2 ml/24 h/100 g BW), while urine osmolality and body weight were unchanged. A negative sodium balance was observed in both oil- and DOCA-treated DI rats on the first day of a high salt diet. Urinary sodium excretion substantially exceeded sodium intake on this day. The urinary sodium excretion of DI and LE rats on the first day of a high salt diet is compared in FIGURE 3. DI rats had a significantly greater natriuresis on this day whether or not DOCA was also administered.

As shown in FIGURE 4, $U_{Na}^V$ of DI rats was significantly higher than that of LE rats (p < 0.01) during all periods following an oral saline load. Maximal
FIGURE 2. Urinary sodium excretion ($U_{Na}V$) and sodium intake of DI rats before and during a high salt diet plus DOCA or oil treatment. Values are means ± SEM. On Day 1 $U_{Na}V$ exceeded Na intake as shown by the shaded area.

$U_{Na}V$ was reached at 60 minutes after loading in both groups. No differences in $U_{Na}V$, hematocrit, or serum sodium concentration of DI and LE rats were observed in the period prior to saline loading. At 60 minutes after loading only serum sodium of DI rats differed from that of LE rats (147 ± 1 and 140 ± 2 mM/L, respectively).

Pretreatment with DOCA (FIGURE 5) significantly enhanced the natriuretic response of LE rats to saline loading but had no effect on the response of DI rats. Due to this enhanced response of LE rats, $U_{Na}V$ was higher in DI rats only at 90 and 120 min after loading. No difference was observed between DOCA-treated DI and LE rats with respect to $U_{Na}V$, hematocrit, and serum sodium concentration, either in the control period prior to loading or at 60 minutes after the saline load.

The effects of DOCA and DOC on the natriuretic response to saline loading are compared in FIGURE 6. These two forms of deoxycorticosterone were equally effective in augmenting the natriuretic response of normal rats, and neither affected the response of DI rats. Oil treatment had no effect in either DI or normal rats.

Plasma aldosterone concentration and renal ($Na^+ + K^+$)-ATPase activity of DI and Long-Evans rats are shown in TABLE 1. Plasma aldosterone concentration was significantly lower while renal ($Na^+ + K^+$)-ATPase activity was significantly higher in DI than in Long-Evans rats.
DI rats had an exaggerated natriuretic response to both chronic and acute volume expansion. The natriuretic response of DI rats to a high salt diet was so great as to result in negative sodium balance during the first 24 hours (Figure 2). In contrast, Long-Evans rats, even when DOCA-treated, always excreted less sodium than the amount ingested (Figure 1). The negative sodium balance observed in DI rats was transient, and urinary sodium excretion decreased on subsequent days of the high sodium diet (Figure 2). Nevertheless, throughout the period of high salt intake, the urinary sodium excretion of DI rats represented a greater fraction of sodium intake than did the UNaV of Long-Evans rats.

No significant difference was observed between UNaV of DOCA-treated and oil-treated DI rats on any day of treatment and in Long-Evans rats UNaV was higher in the DOCA-treated group on only two of seven days of treatment. This may be related to the reported resistance to DOCA in this strain of rats.14–16

There was also a clear difference in the natriuretic response of DI rats to an acute saline load (Figure 3). Pretreatment with either DOCA or DOC abolished this difference by enhancing the response of Long-Evans rats. Both forms of deoxycorticosterone were used since the development of hypertension in response to free DOC treatment has been reported to be greater than when DOCA is used in the Long-Evans strain.14

The increased response of LE rats to a salt load following deoxycorti-
costerone was no doubt due to volume expansion prior to saline loading. DOCA-treated LE rats had a higher $U_{Na^+}$ (0.78 ± 0.15 versus 0.42 ± 0.10 $\mu$Eq/min/100 g BW) and a lower hematocrit (45 ± 1 versus 47 ± 1) than oil-treated LE rats in the control period. In contrast, the hematocrit and urinary sodium excretion of DOCA-treated DI rats were not different from those of oil-treated DI rats in the control period. This suggests a lack of effect of DOCA in DI rats, possibly due to a reduction in the number and/or decreased affinity of tubular mineralocorticoid receptors, secondary to chronic mineralocorticoid deficiency.\textsuperscript{6-8} It also suggests that the natriuretic response of DI rats after DOCA treatment was not due to DOCA-induced volume expansion and therefore different in nature from the similar response in untreated DI rats. Furthermore, since there was no significant difference between the hematocrits of DI (41 ± 1) and LE (41 ± 1) rats after saline loading, the greater natriuresis of DI rats would not seem to be attributable to a greater degree of volume expansion.

It has been shown that the absence of aldosterone is associated with reduced

Figure 4. Urinary sodium excretion ($U_{Na^+}$) of DI and LE rats before and after an acute oral saline load. Values are means ± SEM. * Significantly different from LE rats at the same time, $p < 0.01$. 
Opava-Stitzer et al.: Natriuretic Response

(Na\textsuperscript{+} + K\textsuperscript{+})-ATPase activity\textsuperscript{17-19} and aldosterone replacement in adrenalectomized rats restores normal levels of enzyme activity.\textsuperscript{18, 19} It is not clear, however, whether the hormone has a direct effect on the enzyme or whether it affects activity indirectly by increasing the reabsorption of sodium across the luminal membrane of the tubule, thereby providing more substrate for previously dormant enzyme. Recent studies by Petty et al.\textsuperscript{20} have shown that restoration of normal enzyme activity in cortical collecting tubules of adrenalectomized rabbits, by aldosterone replacement, was prevented by simultaneous administration of amiloride, which blocks luminal sodium uptake in the collecting duct. These data support the hypothesis that mineralocorticoids affect (Na\textsuperscript{+} + K\textsuperscript{+})-ATPase activity indirectly, through changes in tubular sodium transport. In our studies renal (Na\textsuperscript{+} + K\textsuperscript{+})-ATPase activity was higher in the DI rat than the normal rat (TABLE 1), despite mineralocorticoid deficiency and an apparent resistance to deoxycorticosterone. This finding is consistent with the hypothesis that the level of plasma mineralocorticoids is not the primary

![Figure 5](image_url)

**Figure 5.** Urinary sodium excretion ($U_{\text{Na}}V$) of DOCA-treated DI and LE rats before and after an acute oral saline load. Values are means ± SEM. *Significantly different from LE rats at the same time, $p < 0.01$. 
regulator of renal (Na\(^+\) + K\(^+\))-ATPase activity. In addition the exaggerated natriuresis of the DI rat cannot be attributed to reduced renal (Na\(^+\) + K\(^+\))-ATPase activity. Even though we measured enzyme activity under basal conditions, the acute volume expansion induced in our experiments (either by oral saline loading or during the first 24 hours of a high salt diet) would not be expected to alter enzyme activity, as shown by others.\(^{21, 22}\)

An alternate explanation for both the exaggerated natriuretic response and increased (Na\(^+\) + K\(^+\))-ATPase activity in the DI rat could be reduced passive sodium reabsorption by the loops of Henle of juxtaglomerular nephrons in the absence of a high interstitial urea concentration. According to the Kokko model of sodium reabsorption in the inner medulla\(^{23}\) reabsorption of sodium

**TABLE 1**

<table>
<thead>
<tr>
<th>Aldosterone (ng%)</th>
<th>(Na(^+) + K(^+))-ATPase ((\mu)mol PO(_4)/mg protein/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long-Evans</td>
<td>12.4 + 1.6 (N=9)</td>
</tr>
<tr>
<td>DI</td>
<td>6.9 * + 1.2 (N=13)</td>
</tr>
</tbody>
</table>

* Significantly different from normal Long-Evans rats, \(p < 0.02\).
† \(p < 0.001\).
chloride by thin ascending limbs of juxtamedullary nephrons occurs passively down a concentration gradient established by the abstraction of water in the descending limb. This water abstraction occurs due to the presence of urea in the interstitium and the low permeability of this segment to urea and NaCl. This would not, however, be a significant means of sodium reabsorption in the DI rat with its extremely low inner medullary urea concentration. There might thus be a chronically increased delivery of sodium to the thick ascending limb resulting in an adaptive increase in \((\text{Na}^+ + \text{K}^+)\)-ATPase activity. This increased enzyme activity could then prevent salt wasting under basal conditions and no differences would be observed between DI and normal rats in the absence of salt loading. During volume expansion, however, when proximal tubular reabsorption is also depressed, the sodium not reabsorbed by the thin ascending limbs might appear in the urine, accounting for the augmented natriuretic response of the DI rat.

If this hypothesis is correct then there should be no difference between the sodium excretion of DI and normal rats during conditions of medullary urea washout. In fact during hypotonic mannitol infusion DI rats had CH2O curves that were not different from those of normal Wistar rats suggesting similar sodium handling under these conditions. In addition, in our studies, Long-Evans rats on a high salt diet increased their \(U_{\text{Na}}V\) by increasing urinary sodium concentration (and osmolality) rather than urine volume, suggesting that the interstitial sodium concentration was increased. In contrast, urine flow rate, but not osmolality, was increased in the DI rat.

One possible pitfall in our experiments is that we measured whole kidney microsomal enzyme activity. It remains to be seen whether the increased activity is due to a selective change in the medullary enzyme.

An alternative explanation for the enhanced natriuretic response of the DI rat to oral salt loading must also be considered. It has been shown in the rat that intraportal isotonic saline loading results in a greater natriuresis than a similar load given intravenously. It has been postulated that either a reflex involving a hepatic sodium receptor, or a hepatic humoral factor, may mediate this enhanced response. Since in our experiments saline was administered by gavage, we cannot rule out the possibility that the absence of ADH in some way facilitated the hepatic–mediated portion of the natriuresis in the DI rat.

In summary, the DI rat has been shown to have an exaggerated natriuretic response to both acute and chronic volume expansion. Despite aldosterone deficiency and an apparent unresponsiveness to mineralocorticoids, renal \((\text{Na}^+ + \text{K}^+)\)-ATPase activity was elevated compared to normal rats. These findings and other data suggest that in the absence of ADH there may be reduced sodium reabsorption by the thin ascending limbs of juxtamedullary nephrons, resulting in both the enhanced natriuretic response and the increased renal \((\text{Na}^+ + \text{K}^+)\)-ATPase activity. In addition, the absolute level of mineralocorticoids in the plasma would not seem to be the primary determinant of renal \((\text{Na}^+ + \text{K}^+)\)-ATPase activity in the rat.

REFERENCES


Opava-Stitzer et al.: Natriuretic Response


