Neurohypophyseal Response to Dehydration in the Spontaneously Hypertensive Rat

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SUMMARY Experiments were performed to evaluate the effect of dehydration on neurohypophyseal hormone secretion, both vasopressin and oxytocin, fluid balance, and blood pressure in male spontaneously hypertensive rats (SHR) and their normotensive controls, Wistar Kyoto (WKY). Metabolic studies showed that the antidiuretic response to dehydration (24 and 48 hours of water deprivation) was significantly depressed ($p < 0.01$) in the hypertensive animals. They responded inappropriately to dehydration with a greater loss of water and sodium and a larger increase in hematocrit. In contrast, the vasopressin response (both urinary excretion and plasma levels) was increased. The peak plasma levels were 25.3 pg/ml (SHR) compared to 16.6 pg/ml (WKY), while the urinary excretion was 22.5 ng/24 hrs (SHR) vs 9.0 ng/24 hrs (WKY). Dehydration also elicited a stimulation of oxytocin secretion, with no differences observed in the responses of the groups. Blood pressure was significantly greater in the SHR and it did not change during dehydration. These results provide further support for the idea that hypertension is associated with abnormalities in the control of fluid/electrolyte balance. (Hypertension 4: 161-166, 1982)

KEY WORDS • vasopressin • dehydration • hypertension • oxytocin • fluid/electrolyte balance • spontaneously hypertensive rat

DISTURBANCES in the control of fluid/electrolyte homeostasis have often been associated with hypertension. Studies show that hypertensive humans and animals respond to a saline or water load with an exaggerated natriuresis and diuresis, and that they may have a defect in the kidney’s concentrating mechanism. Vasopressin (AVP), a hormone important in the control of blood volume and pressure, is implicated in the development of hypertension, since increases in plasma AVP have been observed in rats with genetic, renal, and DOCA-salt hypertension and in humans with malignant hypertension. In most cases the changes in AVP were not large, usually less than twofold, and not in the range of a vasopressor dose. Thus, results indicate that one of the problems associated with hypertension may be related to controlling mechanisms for salt and water excretion. These experiments investigate the regulation of fluid/electrolyte balance in the spontaneously hypertensive rat (SHR) by evaluating the changes in neurohypophyseal hormone secretion, both AVP and oxytocin (OT), fluid balance, and blood pressure in response to dehydration.

Methods

Male spontaneously hypertensive rats and their normotensive controls, Wistar-Kyoto (WKY) rats derived from the Okamoto-Aoki strain, were bred in our laboratory, and used for these experiments when they were 200 to 250 g and 10 weeks old. They were housed in group cages with a 14/10 hour light schedule, and given water and food ad libitum. Their blood pressure was measured before the experiments using tail cuff plethysmography.

Three types of experiments were carried out, metabolic, endocrine, and cardiovascular, on three separate groups of animals.
Metabolic Studies

Animals (n = 6) were placed in individual metabolic cages, and after an equilibration period of 3 days, underwent a three-stage study: 1) control (predehydration, Days 1 and 2); 2) dehydration (24 and 48 hours of water deprivation, Days 3 and 4); and 3) postdehydration (Days 5, 6, and 7). Water intake, urine volume, osmolality, and urinary excretion of sodium, AVP, and OT were measured daily.

Endocrine Studies

Control animals and those dehydrated for 24 and 48 hours were rapidly decapitated, and blood was collected in chilled heparinized tubes. Plasma levels of AVP and OT, hematocrit, and osmolality were determined. This experiment was repeated using control rats and those dehydrated for 48 hours.

Cardiovascular Studies

Blood pressure measurements using tail cuff plethysmography (Narco Systems) were made in control and dehydrated animals. For this procedure, the conscious animal was placed in a restraining device and warmed for 10 minutes (33°C) before systolic pressure was measured. All experimental procedures were conducted in the morning between 0900 and 1100.

Protocol

In the metabolic studies, urine was collected in graduated centrifuge tubes containing 100 μl of glacial acetic acid. The cages were washed daily and rinsed with distilled water. After measuring urine volume, urine samples were frozen (−20°C) and later osmolality (freezing point depression using a 200 μl sample), sodium (flame photometry), and hormone levels were determined. A correction was made for the effect of the acetic acid on urine osmolality. This was done by adding 100 μl of glacial acetic acid to varying amounts of pooled rat urine (0.5 to 5 ml). They were treated the same way as the experimental samples in that they were stored at room temperature for 24 hours, frozen, and osmolality determined. It was only necessary to correct osmolality on Day 4 when the mean urine volumes were 2.6 and 0.5 ml/24 hrs.

OT and AVP were measured by radioimmunoassay (RIA). All of the samples from an experiment were run in the same assay. Oxytocin was measured with an antiserum developed in our laboratory, with an antiserum with only 0.05% crossreactivity with another peptide. The assay was useful for measuring plasma levels of oxytocin since it had excellent sensitivity (0.3 pg/tube) and a 50% displacement level of 5 pg/tube.

Arginine vasopressin was assayed using an antiserum generously supplied by Dr. Tj Van Wimersma Greidanus (Utrecht, The Netherlands), whose characteristics have been previously described. Sensitivity of the assay is 0.3 pg/tube. At 1, 4, and 8 pg/tube, intraassay coefficients of variation were 2.7%, 3.6%, and 5.1%, respectively (n = 9 for each). At 4 and 16 pg/tube, interassay coefficients of variation were 12.3% and 15.6% respectively (n = 9 for each). Synthetic arginine vasopressin (1 μU = 2.2 pg) furnished by Ferring was used for iodination and standards.

Vasopressin was iodinated with 125I using chloramine t. The reaction was stopped with the addition of human serum albumin followed by Dowex AG 1 x 2 to remove free iodine, and the labeled peptide was purified further. The assay details as previously described. It is a specific column using 0.2 N acetic acid as an eluant.

Duplicate plasma samples (0.4 ml) for both AVP and OT were extracted with acetone, placed in a water bath (40°C), dried under a stream of air, and stored at −20°C until assayed. No correction was made for recovery, which was 81.0% ± 3.0% and 95.1% ± 2.6% when 4 pg of AVP or OT respectively was added to pools of rat plasma (n = 6 for each). The samples and standards were incubated with antiserum for 3 days at 4°C; then 125I-hormone was added and followed 24 hours later by the second antibody (sheep antirabbit gamma globulin). The following day the reaction mixture was centrifuged (1500 x g for 15 minutes), and the residual precipitate containing the bound hormone was counted. The assay buffer was 0.01 M phosphate, 0.15 M NaCl, 0.5% bovine serum albumin, pH 7.5.

Urine samples were extracted with 98% methanol/0.02 N acetic acid in order to remove protein material (1:10 urine/methanol). Samples were dried and stored at −20°C until measurement by RIA. In control animals only 10 μl of urine was required for measurement of AVP and 20 μl for OT. The recovery of immunoreactive AVP and OT from two pools of rat urine with normal and low urine osmolalities is shown in table 1. Recovery was essentially 100% after the addition of 4, 16, and 64 pg of

<table>
<thead>
<tr>
<th>Amount added (pg)</th>
<th>Normal</th>
<th>Diabetes insipidus</th>
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<tr>
<td>54.8 ± 2.3</td>
<td>0.8 ± 0.4</td>
<td>3.2 ± 0.2</td>
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<tr>
<td>50.0 ± 1.3</td>
<td>6.7 ± 0.3</td>
<td>7.8 ± 0.5</td>
</tr>
<tr>
<td>65.6 ± 2.1</td>
<td>65.6 ± 1.1</td>
<td>67.5 ± 2.3</td>
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Table 1. Recovery of Immunoreactive Vasopressin and Oxytocin from Urine

Vasopressin or oxytocin (0, 4, 16, and 64 pg) was added to a pool of urine from normal rats (osmolality = 1500 mOsm/kg) or Brattleboro rats homozygous for diabetes insipidus (osmolality = 300 mOsm/kg). Samples (n = 6) were extracted and measured in the RIA. Results are expressed as mean ± SEM.
hormone. Measurement of serial dilutions of urine extracts showed a correlation between volume of the urine extract and hormone concentration ($r = 0.996$ and 0.989 for AVP and OT respectively) and a parallelism with the assay standard curves. The intra-assay coefficients of variation for urine with 4 and 16 pg of hormone were 8.9% and 10.8% for AVP and 7.1% and 6.0% for OT ($n = 6$).

RIA results were calculated on a PDP-10 computer using a logit transformation of the raw data. All results are expressed as mean ± SEM. Statistical significance was determined using two-way analysis of variance followed by Duncan's multiple range test or Scheffe's test.

**Results**

Baseline measurements (pre-dehydration) of urinary parameters showed that the SHR generally produced more urine of a lower osmolality than the WKY control (fig. 1). This was significantly different ($p < 0.01$) on Day 2 for both parameters. Measurement of water intake supported these data, with a higher fluid intake in the hypertensive animal, 49 ± 1.5 vs 41.2 ± 2.2 ml/24 hrs. There were no differences in basal urinary excretion of the neurohypophyseal hormones (fig. 1, table 2).

As expected, dehydration produced significant increases in urine osmolality, AVP, and OT excretion, and a decrease in urine volume at 24 and 48 hrs (fig. 1 and table 2). More interesting was the difference in the responses in the two groups. The antidiuretic response was significantly greater in the WKY. This was especially evident in the case of urine osmolality in which the 48-hour response was significantly less in the SHR. Changes were also observed in urinary sodium excretion; however, interpretation is somewhat difficult without information on food intake. Results did show that the SHR exhibited a greater loss of sodium than the normotensive control (table 2). After 48 hours of dehydration, sodium excretion was 537 vs 88 mEq/24 hrs (SHR vs WKY). Thus, the SHR exhibited a significant deficit in its ability to conserve fluid and electrolytes in response to dehydration.

While the antidiuretic response was reduced in the SHR, the vasopressin response was greater (fig. 1). Urinary AVP excretion was significantly increased in the hypertensive animals (22.5 ± 2.0 vs 8.9 ± 1.1 ng/24 hrs after 24 hrs of water deprivation). Measurement of OT excretion showed that this was not a generalized neurohypophyseal phenomenon since the response was essentially identical in the two groups (table 2).

An investigation of the responses of plasma AVP and OT to dehydration supports the results found in the metabolic studies. In the first experiment, measurements showed that the hypertensive animals had a significantly higher basal level of AVP, 3.4 vs 2.2 pg/ml ($p < 0.01$), while plasma oxytocin was not different among the groups (figs. 2 and 3). Dehydration caused a stimulation of AVP secretion in both groups, but the effect was greater in the SHR ($p < 0.01$ at 24 and 48 hours). Plasma oxytocin was also stimulated by water deprivation, although the response was not different in the SHR and WKY. Similar results were obtained in a subsequent experiment in which animals were evaluated under control conditions and after 48 hours of water deprivation (table 3). In this case there was no difference in the basal levels of either AVP or OT. Dehydration elicited a stimulation of neurohypophyseal secretion with a greater AVP response observed in the hypertensive rats.

![Figure 1. Effect of dehydration on urine volume, osmolality and vasopressin excretion in the spontaneously hypertensive rats (SHR) and controls (WKY). Values are mean ± SEM. There was a significant difference ($p < 0.01$) in the responses of the SHR and WKY in all parameters measured.](http://hyper.ahajournals.org/doi/fig/10.1161/01.HYP.61.4.163)
TABLE 2.  Dehydration Response in the Spontaneously Hypertensive Rat

<table>
<thead>
<tr>
<th></th>
<th>SHR</th>
<th>WKY</th>
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<tbody>
<tr>
<td>Sodium excretion (μEq/24 hrs)</td>
<td>1421.0 ± 88.9 (6)</td>
<td>1205.6 ± 114.6 (6)</td>
</tr>
<tr>
<td>Urinary oxytocin (ng/24 hrs)</td>
<td>1.2 ± 0.1 (6)</td>
<td>1.4 ± 0.1 (6)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>41.2 ± 0.9 (6)</td>
<td>42.4 ± 0.9 (7)</td>
</tr>
<tr>
<td>Plasma osmolality (mOsm/kg)</td>
<td>292.2 ± 2.5 (10)</td>
<td>289.7 ± 1.3 (8)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>183.6 ± 1.9 (9)</td>
<td>125.3 ± 4.5 (9)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>SHR</th>
<th>WKY</th>
</tr>
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<tbody>
<tr>
<td>Sodium excretion (μEq/24 hrs)</td>
<td>1615.1 ± 100.4 (6)</td>
<td>824.5 ± 46.6 (6)</td>
</tr>
<tr>
<td>Urinary oxytocin (ng/24 hrs)</td>
<td>2.4 ± 0.2 (6)</td>
<td>3.2 ± 0.6 (6)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>49.3 ± 0.6 (9)</td>
<td>46.7 ± 0.4 (6)</td>
</tr>
<tr>
<td>Plasma osmolality (mOsm/kg)</td>
<td>300.2 ± 2.9 (10)</td>
<td>292.3 ± 2.8 (8)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>188.8 ± 3.7 (9)</td>
<td>124.9 ± 4.2 (9)</td>
</tr>
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</table>

Values given as mean ± SEM. Number of rats given in parentheses.

*p < 0.05 comparison within groups, control vs dehydration.
†p < 0.05 comparison between groups, SHR vs WKY.

The ability of the SHR to precisely regulate its volume/electrolyte composition was further questioned after measurement of plasma osmolality and hematocrit. Forty-eight hours of dehydration produced a significantly greater increase in hematocrit (53% vs 50%) and plasma osmolality (304.3 vs 294.3 mOsm/kg) in the SHR (table 2). When this experiment was repeated, the hypertensive animals again showed a greater rise in hematocrit (table 3). However, the rise in plasma osmolality in response to dehydration was similar in the SHR and WKY (increases of 14.4 and 14.9 mOsm respectively). Dehydration did not produce a significant change in blood pressure in either group (table 2).
EFFECTS OF DEHYDRATION IN THE SHR/Morris

TABLE 3. Effect of Dehydration on Neurohypophyseal Secretion, Plasma Osmolality, and Hematocrit (Experiment 2)

<table>
<thead>
<tr>
<th></th>
<th>Plasma osmolality (mOsm/kg)</th>
<th>Plasma vasopressin (pg/ml)</th>
<th>Plasma oxytocin (pg/ml)</th>
<th>Hematocrit (%)</th>
<th>Blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
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<tr>
<td>SHR (n = 5)</td>
<td>289.6 ± 3.4</td>
<td>2.9 ± 0.6</td>
<td>15.6 ± 1.3</td>
<td>42.5 ± 2.3</td>
<td>167.2 ± 2.0*</td>
</tr>
<tr>
<td>WKY (n = 5)</td>
<td>287.1 ± 3.5</td>
<td>2.8 ± 0.1</td>
<td>17.0 ± 2.2</td>
<td>37.0 ± 1.9</td>
<td>124.3 ± 3.0</td>
</tr>
<tr>
<td>Dehydration (48 hrs)</td>
<td></td>
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<tr>
<td>SHR (n = 5)</td>
<td>304.0 ± 3.0†</td>
<td>28.3 ± 2.0‡</td>
<td>37.6 ± 4.2†</td>
<td>63.7 ± 2.4‡</td>
<td>—</td>
</tr>
<tr>
<td>WKY (n = 5)</td>
<td>302.0 ± 2.2†</td>
<td>15.6 ± 1.6‡</td>
<td>45.3 ± 5.4‡</td>
<td>48.5 ± 1.9‡</td>
<td>—</td>
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*p < 0.01, SHR vs WKY.
†p < 0.05, control vs dehydration.
‡p < 0.01, control vs dehydration.

Discussion

To investigate the possible relationship between hypertension and control of volume/electrolyte balance, we evaluated the effects of dehydration in the genetically hypertensive rat. We found that the SHR exhibits a deficit in its antidiuretic response to dehydration in the face of an increased vasopressin response. There appears to be an impairment in the ability of the hypertensive animal to conserve fluids and electrolytes which is at least partially related to an inability to respond to increased circulating levels of vasopressin.

The results show that the SHR responds inappropriately to dehydration, with a greater loss of water and sodium and thus a larger blood volume depletion, as evidenced by the increased hematocrit. The estimated loss of blood volume was 33.3% in the SHR compared to 12.4% in the WKY (table 3). Measurement of plasma osmolality in two studies revealed either an increased osmotic response in the SHR or no difference between the groups. The reason for this discrepancy is not known except that in the first study the normotensive animals showed a smaller increase in osmolality than would be predicted on the basis of other investigations.11,22 As expected, these volume and osmotic stimuli resulted in an increase in neurohypophyseal secretion with a greater AVP response observed in the hypertensive animals.

A disturbance in concentrating ability was also observed in renal hypertensive rats subjected to dehydration and adminstration of vasopressin.10 In this study the clamped kidney did not excrete a maximally concentrated urine. This was thought to be due to an alteration in tubular function rather than changes in filtration or blood flow. Dehydration of hypertensive humans also produced an abnormal response upon comparison with normotensive controls.11 In agreement with our results, urinary excretion of AVP was markedly elevated in the dehydrated hypertensive patient. There also appeared to be a resistance to the antidiuretic effect of AVP since the free water clearance was the same in both groups.

Studies in which volume expansion is the experimental stimulus also suggest that hypertension is associated with disturbances in fluid balance. Infusion or ingestion of saline results in an exaggerated natriuresis and diuresis in the SHR,4,8,9 the salt-sensitive Dahl rat,7 and in rats with renal hypertension.6 Studies in human subjects reveal similar results, that is, an increased excretion of salt and water in response to volume expansion.1,4 The mechanism that causes this exaggerated natriuresis is suggested to be a reduction in the reabsorptive capacity of the ascending loop.5,6 This could perhaps account for the altered response to dehydration since a decrease in sodium reabsorption would reduce the osmotic gradient and diminish the concentrating ability. Thus, the vasopressin released would be less effective in eliciting an increase in urine osmolality. The reduced papillary blood flow that has been observed in the SHR3,4 might partially compensate for the deficit in sodium reabsorption by acting to conserve the medullary gradient. This would function to promote conservation and appears adequate unless the animal is challenged as in dehydration.

In addition to an impairment of renal function, hypertension also appears to be characterized by a partial resistance to the antidiuretic action of vasopressin. As in many forms of renal disease, the result is a type of nephrogenic diabetes insipidus. In the present study, AVP levels were increased in the SHR while its urinary concentrating ability was significantly less than the normotensive control. This was also observed in hypertensive patients who showed a greater AVP response to dehydration which was not associated with an increased antidiuretic effect.11 The idea that the SHR is partially unresponsive to the effects of AVP is in disagreement with a previous report.20 In this study a dose response to the pressor and antidiuretic effects of AVP was performed. Although there was no difference in the antidiuretic response of the SHR, the dose range was rather high, 0.5 to 12 ng, possibly obscuring any subtle change in urine flow.

Plasma osmolality and volume are important regulators of neurohypophyseal secretion.20,31,32 A previous investigation demonstrated that an increase in osmolality was equally potent in stimulating both AVP and OT secretion while volume depletion had a greater effect on vasopressin.20 Dehydration results in a hypertonicity of body fluids, a volume depletion and
consequently an increase in AVP and OT. In agreement with this, our results show that both the SHR and WKY respond to dehydration with an increase in osmolality, hematocrit, and AVP and OT, both plasma levels and urinary excretion. The increased AVP response which was observed in the hypertensive animal may be related to the differential effect of volume and osmotic stimuli on the neurohypophyseal hormones. Since the volume loss was greater in the SHR, one might expect a larger increase in AVP than OT.

Measurement of urinary levels of AVP and OT reveals that this may provide a noninvasive method for estimation of neurohypophyseal activity. In the SHR there was generally good agreement between the control period. This is consistent with other investigations in which urinary AVP was used as an index of secretion. However, it is the first study to report concomitant measurement of urinary oxytocin and vasopressin.

Results in the WKY animals reveal that there is not always a perfect correlation between plasma and urinary levels. Under conditions of profound antidiuresis as observed in the WKY, plasma concentrations were elevated while urinary excretion was not different from the control period. This is not too surprising since plasma levels represent an instantaneous measurement while urinary excretion represents a cumulative response. Excretion is based on a combination of factors such as hormone degradation, clearance, renal function, rather than a simple expression of posterior pituitary secretion. This must be taken into consideration when urinary excretion is used as the only index of neurohypophyseal secretion.

The results of this study lend support to the idea that the disease of hypertension is characterized by problems in the ability to precisely regulate blood volume and electrolyte composition. The spontaneously hypertensive rat demonstrated a deficit in its antidiuretic response to dehydration, showing a greater loss of salt and water and a larger increase in vasopressin secretion. This appeared to be caused by an impairment of renal function coupled with a partial resistance to the action of vasopressin.

Acknowledgments
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References
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