Changes in Paraventricular Vasopressin and Oxytocin During the Development of Spontaneous Hypertension

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SUMMARY The potential role of central neuroendocrine changes in the development of spontaneous hypertension was evaluated. The developmental changes in blood pressure and hypothalamic and plasma levels of vasopressin (AVP) and oxytocin (OT) were determined in groups of SHR and WKY animals from 3 to 24 weeks of age. Hypothalamic OT content was significantly lower in 3-, 6-, and 12-week-old SHR rats compared to age-matched WKY animals. Hypothalamic AVP content was not different at 3 weeks of age, but was lower in the SHRs at 6 and 12 weeks. To localize strain differences in AVP and OT, specific hypothalamic nuclei were removed from 300 μm frozen brain sections, and hormone content measured. Paraventricular AVP and OT content was lower in the SHRs which had increased blood pressure (6, 12, and 24 weeks of age) but not in the prehypertensive groups (3 weeks of age). Neuropeptide content was unchanged in the supraoptic nucleus or median eminence. Plasma levels of AVP were increased in the SHR, while OT was unchanged. Thus, genetic hypertension is associated with specific and localized changes in hypothalamic AVP and OT. The fact that the peptide deficit occurred in the paraventricular nucleus, a region thought to be involved in the control of autonomic function, may have important implications in terms of the pathogenesis of hypertension.

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KEY WORDS • vasopressin • hypertension • oxytocin • paraventricular nucleus

CENTRAL peptidergic systems may be important in the regulation of blood pressure and possibly in the etiology of hypertension. Recent studies show that the neuropeptides, vasopressin (AVP) and oxytocin (OT) are localized in brain stem centers involved in cardiovascular regulation.1-4 Both peptides are present in the nucleus tractus solitarius, a region which receives afferent information from arterial baroreceptors.

In the spontaneously hypertensive rat (SHR), we observed a wide range of central neuroendocrine changes.5 In general, there was a decrease in hypothalamic peptide content, notably in AVP and OT. This was corroborated by another study which showed that the SHR had reduced levels of hypothalamic AVP.6 A localization study performed in our laboratory revealed that the deficit in AVP and OT occurred in the paraventricular (PVN) but not the supraoptic nucleus (SON).7 This is noteworthy since the PVN sends vasopressinergic and oxytocinergic projections to brain stem areas important in cardiovascular control while the SON’s primary input is to the neurohypophysis.1-4 Brain stem levels of AVP and OT were also reduced in the SHR,5 8-9 a finding which would be consistent with a reduction in PVN neuropeptides. Thus, the deficit in central AVP and OT occurs in regions which are important in the control of blood pressure.

To investigate the relationship between hypertension and the central peptidergic systems further, we carried out an ontogenetic study in which the emergence of central nervous system (CNS) neuropeptide changes and hypertension were compared.

Methods

Male SHR and their normotensive controls, Wistar-Kyoto (WKY) rats, were bred in our vivarium according to established procedures using sibling mating. New breeding stock is obtained from the National Institutes of Health (NIH) every seven generations. The animal colony is maintained in association with Dr. P.M. Hutchins and has been in existence since 1970. The rats used in these studies were 3, 6, 12, and 24 weeks of age. They were maintained on a 14 hrs light/10 hrs dark schedule with water and food ad libitum. Blood pressure was monitored using tail cuff plethysmography (Narco Systems, Houston, Texas) in restrained, conscious animals.

The animals were rapidly decapitated (0900 to 1100 hrs) and the blood collected in chilled heparinized
tubes. The brain and neurohypophysis were carefully removed. In the first study, a block of hypothalamic tissue was used for peptide measurement. The tissue consisted of an area bounded by the postchiasmatic recess and the premamillary recess with 1.5 mm cuts on either side of the median eminence crest; it contained both the paraventricular and supraoptic nuclei.

In the second study, a microdissection technique was used to remove specific hypothalamic nuclei. After removal of the brain, the median eminence was dissected out using iris scissors. The remainder of the brain was frozen on dry ice. Serial 300 μm sections of the hypothalamus were cut in the frontal plane at -15°C. The hypothalamic nuclei, the paraventricular, supraoptic and suprachiasmatic were removed with a chilled 300 μm stainless steel cannula.

All tissues were homogenized in 98% methanol/0.02 N acetic acid and centrifuged for 3 minutes at 8000 × g (1 ml for the hypothalamic block and neurohypophysis; 500 μl for the other tissues). The methanolic supernatant was aliquoted and dried under a stream of air for radioimmunoassay (RIA). The extracts were measured in duplicate and usually at two dilutions. Measurement of serial dilutions of tissue extracts revealed a parallelism with the AVP and OT standard curves. Protein was measured by the micro-method of Lowry. For tissue punches, the entire pellet was resuspended in water and assayed. Plasma samples (0.4 ml) for both vasopressin and oxytocin were extracted with acetone, placed in a water bath (42°C), dried under a stream of air, and stored at -20°C until assayed.

Common reagents and incubation protocols were used for the peptide RIAs. The buffer for dilution of standards, samples and 125I-hormone was phosphate buffered saline (0.5% BSA, pH 7.5), while the antisera were diluted in PBS-EDTA (1% normal rabbit serum).

Synthetic vasopressin (Ferring AB, Malmö, Sweden) and oxytocin (Vega Biochemicals, Tucson, Arizona) were used for iodination and standards. The final incubation volume was 500 μl with a 4- or 5-day incubation at 4°C. The bound hormone was separated using a second antibody. Two different types of assays were used: one for measurement of tissue extracts, and the other for measurement of plasma levels. In the standard protocol (tissue extracts), all of the reagents were added at the same time and equilibrium conditions used. Since measurement of plasma levels requires a more sensitive assay, a disequilibrium method was employed. In this case, standards or samples were preincubated with the antisera before the addition of the labeled hormone.

AVP was measured with an antiserum supplied by Dr. Tj. Van Wimersma Greidanus. Its characteristics have been previously described. In our laboratory it is routinely used at a final dilution of 1:100,000 with a sensitivity of 0.3 pg and a 50% displacement of 4.6 pg in the plasma RIA and values of 0.9 and 13.6 pg in the standard RIA. Oxytocin is measured with an antiserum developed in our laboratory. It is a highly specific antiserum with only 0.05% crossreactivity with AVP and no interaction with a wide range of other peptides. The plasma assay has a sensitivity of 0.3 pg and a 50% displacement of 5 pg; the standard assay shows a sensitivity of 0.7 pg and 50% displacement of 14.2 pg.

RIA data were analyzed using a logit transformation of the data. Statistical significance was determined using a two way analysis of variance followed by Duncan's multiple range test.

**Results**

Systolic blood pressure was first increased at 6 weeks of age in the SHR (fig. 1). It continued to rise,
TABLE 1. Comparison of the SHR to WKY in Body Weight, Heart Rate, Hematocrit, Plasma Vasopressin, and Plasma Oxytocin

<table>
<thead>
<tr>
<th>Measurement</th>
<th>3 weeks old</th>
<th>6 weeks old</th>
<th>12 weeks old</th>
<th>24 weeks old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
</tr>
<tr>
<td>No. of rats</td>
<td>13</td>
<td>10</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>23.3±0.9</td>
<td>32.5±3.9</td>
<td>103.1±9.8</td>
<td>85.6±3.9</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>424.4±6.1</td>
<td>424.7±14.6</td>
<td>516.8±15.3</td>
<td>500.1±13.5</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>22.0±0.4</td>
<td>25.5±1.8</td>
<td>37.4±0.9</td>
<td>37.7±1.0</td>
</tr>
<tr>
<td>Vasopressin* (ng/ml)</td>
<td>3.2±0.4</td>
<td>1.9±0.2</td>
<td>2.5±0.4</td>
<td>2.5±0.5</td>
</tr>
<tr>
<td>Oxytocin (pg/ml)</td>
<td>NM</td>
<td>NM</td>
<td>±2.0±2.5</td>
<td>±2.5±4.5</td>
</tr>
</tbody>
</table>

*p < 0.001, SHR vs WKY = by analysis of variance. NM = not measured because of insufficient sample.

reaching a level of 184 vs 126 mm Hg at 24 weeks. There was no significant difference in heart rate between the groups (table 1). The developmental changes in blood pressure in the SHR and WKY were inversely parallel to the changes in hypothalamic AVP (fig. 2). At 3 weeks of age there was no difference between the groups; while at 6 and 12 weeks of age, AVP levels were significantly lower in the SHRs. OT content was decreased in all of the age groups; however, the changes in OT were greatest in the older rats (p < 0.01) (6 and 12 weeks). Neurohypophysial AVP and OT were changed only in the 12-week-old animals (table 2). Vasopressin content was significantly increased in the SHR, while oxytocin was decreased.

A more detailed localization study revealed a parallel between the emergence of blood pressure differences and peptide content in the paraventricular nucleus (fig. 3). Paraventricular AVP and OT content were

![Figure 2](image-url)

**TABLE 2. Neurohypophysial Peptide Content**

<table>
<thead>
<tr>
<th>Age (wks)</th>
<th>Vasopressin (ng/hyp)*</th>
<th>Oxytocin (ng/hyp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>3 weeks</td>
<td>112.6±13.4</td>
<td>143.1±17.4</td>
</tr>
<tr>
<td>6 weeks</td>
<td>306.6±10.5</td>
<td>305.6±33.8</td>
</tr>
<tr>
<td>12 weeks</td>
<td>833.3±86.7</td>
<td>461.2±43.9</td>
</tr>
</tbody>
</table>

*ng hormone/neurohypophysis. **p < 0.01; n = 4 per group.

**TABLE 3. Median Eminence Vasopressin and Oxytocin**

<table>
<thead>
<tr>
<th>Age (wks)</th>
<th>Vasopressin (pg/µg)</th>
<th>Oxytocin (pg/µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>3</td>
<td>20.5±6.4</td>
<td>29.6±5.0</td>
</tr>
<tr>
<td>6</td>
<td>56.3±11.6</td>
<td>38.30±9.6</td>
</tr>
<tr>
<td>12</td>
<td>100.2±12.9</td>
<td>62.9±6.8</td>
</tr>
<tr>
<td>24</td>
<td>46.3±8.4</td>
<td>73.1±10.6</td>
</tr>
</tbody>
</table>
not different at 3 weeks of age; however, at 6, 12, and 24 weeks, the SHRs had significantly lower levels of both peptides. The neuroanatomical specificity of the differences was indicated by the absence of changes in the supraoptic nucleus (fig. 4) or the median eminence (table 3). With regard to the circulating levels of the neurohypophyseal hormones, AVP was increased in the SHR, while OT was not changed (table 1).

**Discussion**

The role of circulating neurohypophyseal peptides in the etiology of hypertension in both humans and animals is a subject of great controversy. Increases in plasma AVP were observed in rats with genetic, renal, and DOCA/salt hypertension and in humans with malignant hypertension. However, at least in the SHR, the changes are small, usually less than two-
fold, and not in the range of a vasopressor concentration.

Aside from the systemic effects of the neurohypophysical hormones, we must also consider their CNS role. Our work has focused on the study of central AVP and OT in the SHR. We find that genetic hypertension is associated with a reduction in hypothalamic peptide content, a result which has been corroborated by another laboratory. The present developmental study shows that the changes in neuropeptide levels were coincident or preceded the increase in blood pressure. The hypothalamic peptide deficits were most prominent in the mature animals, with a reduction in AVP and OT content of 1.6- and 2.3-fold, respectively, at 6 weeks of age.

To further localize changes in hypothalamic AVP and OT, peptide levels were measured in specific hypothalamic nuclei. Paraventricular AVP and OT concentrations were lower in the hypertensive animals, while no difference was observed in the young pre-hypertensive group (3 weeks age). There were no changes seen in other hypothalamic regions, such as the supraoptic nucleus or median eminence. The fact that the neuropeptides were specifically reduced in the PVN is interesting since immunohistochemical studies show that the paraventricular sends projections to the nucleus tractus solitarius, dorsal motor nucleus, locus coeruleus, and spinal cord, as well as to the neurohypophysis. These brain stem nuclei are important in blood pressure regulation, receiving and relaying information from the arterial baroreceptors. This pattern is quite different in the supraoptic nucleus in which the primary input is to the neurohypophysis.

Since PVN is the source of brain stem AVP and OT, one might also expect a reduction in neuropeptide concentrations in this region. Indeed, results from other laboratories show decreased levels of brain stem AVP and OT in the hypertensive rat. The developmental changes in brain stem neuropeptides were remarkably similar to the pattern observed in the PVN. The deficits in AVP and OT were most pronounced in the older hypertensive animals (7 and 22 weeks). Furthermore, a histological examination of the paraventricular nucleus of the SHR showed that it was significantly smaller in size and cellular density, while the SON was not changed.

We should emphasize that changes in tissue content are difficult to interpret. A decrease in neuropeptides could mean increased turnover resulting in a depletion or the converse, a reduction in biosynthetic activity. It could also be related to changes in axonal transport, degradation, and/or release. Further experiments which specifically address these questions will be required.

The next question is how these changes in central peptides may be related to the hypertension. Results suggest that AVP has different effects centrally and peripherally. It acts both to increase peripheral resistance and via a central interaction with the baroreceptor reflex to lower heart rate and cardiac output. This is illustrated by a study which compared the effect of intravenous versus intravertebral arterial infusion of AVP. The central infusion led to a greater decrease in heart rate with no increase in blood pressure even though the plasma hormone levels were the same. Experiments using baroreceptor denervation showed that this procedure greatly augments the pressor response to AVP. This was not the case for other pressor agents such as norepinephrine and angiotensin. An increased pressor responsiveness to AVP was also observed in patients with idiopathic orthostatic hypotension. Other studies suggest that AVP acts centrally to enhance baroreceptor reflex activity so that under normal conditions the increase in peripheral resistance is counterbalanced by a reduction in heart rate and cardiac output. The cardiovascular effects of AVP when given directly into the brain are somewhat contradictory. The intraventricular injection of the peptide is reported to cause both an increase and decrease in blood pressure and heart rate. However, the effective doses are rather high, ranging from 25 to 100 ng.

Genetic hypertension is associated with an impairment in cardiovascular reflex activity as well as a deficit in central neuropeptides. The SHR exhibits an attenuated bradycardia to various pressor stimuli. This is interesting in light of the possible interaction between AVP and baroreceptor reflex activity. It is still a matter of speculation whether the changes in central AVP are related to the alteration in reflex activity.

With regard to central oxytocin, there is little information on its functional role. It is interesting that oxytocin is present in brain stem cardiovascular centers at higher concentrations than AVP. However, its presence in these regions is somewhat of an enigma. The fact that central oxytocin is decreased in the hypertensive animal is intriguing. Further studies will be required in order to evaluate any role in cardiovascular function.

Our data do not establish a causal relationship between the reduction in neuropeptides and the development of the hypertension. It is possible that the alterations represent genetic differences which are independent of the high blood pressure. The results do demonstrate that the time course of the peptide changes is parallel to the changes in blood pressure. Furthermore, the fact that the deficit is localized in the paraventricular nucleus, a hypothalamic region thought to be important in autonomic function, provides evidence for specificity and suggests a possible role in hypertension.

Acknowledgments
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References
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