Brain Corticotropin Releasing Factor in the Spontaneously Hypertensive Rat

TERUHIKO HATTORI, KOZO HASHIMOTO, AND ZENSUKE OTA

SUMMARY Corticotropin releasing factor and vasopressin were measured in major brain regions including the neurohypophysis in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY) during development of hypertension. The highest concentration of corticotropin releasing factor was found in the hypothalamus in both strains. Corticotropin releasing factor was decreased in most major brain regions of SHR. In the hypothalamus, corticotropin releasing factor was lower in 3- and 6-week-old SHR than in age-matched WKY (p<0.01), but was similar at 12 and 24 weeks of age. The content of corticotropin releasing factor did not differ in the neurohypophysis in 3-week-old rats but began to decrease at 6 weeks of age (p < 0.01) and continued to decrease during the development of hypertension (p< 0.01). Brain vasopressin concentration did not differ between SHR and WKY except in the hypothalamus. The level of hypothalamic vasopressin was consistently lower in SHR than in WKY (p < 0.01). These peptides are thought to be associated with autonomic nervous regulation, and our results may further strengthen the possibility that the deficit of the peptides may be involved in the development of spontaneous hypertension. (Hypertension 8: 1027-1031, 1986)

KEY WORDS • corticotropin releasing factor • vasopressin • adrenocorticotropic hormone • hypertension • spontaneously hypertensive rats • hypothalamus • neurohypophysis

SPONTANEOUSLY hypertensive rats (SHR) have a morphological abnormality in the hypothalamic-pituitary-adrenal system that appears to be related to the development of spontaneous hypertension. Corticotropin releasing factor (CRF) originates from the hypothalamus and is released into hypophyseal portal veins. This hypothalamic-pituitary system is mainly responsible for control of adrenocorticotropic hormone (ACTH) secretion. We previously found abnormal ACTH responses to CRF and vasopressin and a reduced hypothalamic CRF content in young SHR. These results suggest the possibility that these abnormalities might play some role in the development of spontaneous hypertension. This hypothesis is based on two additional observations. First, CRF neurons are widely distributed in brain regions outside the hypothalamus, such as the brainstem, an area thought to be involved in the regulation of the autonomic nervous system. Second, an intracerebroventricular injection of CRF in conscious rats resulted in an increase in mean arterial pressure and heart rate by stimulating sympathetic nervous activity.

Vasopressin is a potent pressor agent and may participate centrally and peripherally in cardiovascular regulation. Abnormal vasopressin content has been reported in the brain of SHR and stroke-prone SHR. In addition, vasopressin also has corticotropin releasing activity, and an immunohistochemical study has demonstrated that vasopressin and CRF were stained in the same neurons of adrenalectomized rats.

To evaluate the relationship between the central CRF system and spontaneous hypertension, we examined changes in brain CRF and vasopressin concentrations during the development of hypertension in SHR.

Materials and Methods

Male SHR and Wistar-Kyoto rats (WKY) obtained from Charles River Japan (Kanagawa, Japan) were used in the study. They were 3, 6, 12, and 24 weeks of age. All rats were housed in air-conditioned animal quarters with alternate 12-hour periods of light and dark, and they received food and water ad libitum.

Blood pressure was measured with the rats in a restrained, conscious condition the day before the experiment using tail-cuff plethysmography (Ueda Seisa-
kusho, Tokyo, Japan). On the day of the experiment, the animals were quickly decapitated (0900–1200) without anesthesia. The brain and neurohypophysis were quickly removed, and the medulla oblongata, midbrain, cerebellum, pons, cerebral cortex, hypothalamus, and thalamus were precisely dissected by the modified method of Glowinski and Iversen. The medulla oblongata was bound at the caudal end of the pons and sectioned transversely 2 mm below the obex. The hypothalamic tissue was bound by the optic chiasm and the rostral end of the mamillary bodies rostrocaudally. The horizontal section passed through the anterior commissure dorsally and contained the median eminence. The cerebral cortex was dissected from the frontal lobe. These tissue blocks were weighed and homogenized by a sonicator (Heat Systems-Ultrasonics, Plainview, NY, USA) in a 2-ml solution composed of acetone (80% vol/vol) and 0.5 N HCl (20% vol/vol). After centrifugation at 4000 g for 10 minutes at 4°C, the supernatant was transferred to another tube and 3 ml of petroleum ether was added to the samples. The samples were then mixed and centrifuged at 1200 g for 5 minutes at 4°C, and the lower layer then was transferred to another tube. The extracts were placed in a waterbath at 40 to 45°C, dried under a nitrogen stream, and stored at −20°C until assay. The mean recovery rates of CRF and vasopressin with this extraction method were 79.9 ± 4.2% and 73.3 ± 2.4% (mean ± SD), respectively. These procedures have been described previously.7

Tissue CRF and vasopressin were measured in duplicate by the radioimmunoassay method established in our laboratory.8,19 Dried extracts were reconstituted with 1 ml of radioimmunoassay buffer (0.02 M phosphate buffer, pH 7.4) containing 0.15 M NaCl, 0.5% bovine serum albumin, 1 mM ascorbic acid, and 25 mM ethylenediaminetetraacetic acid and 100 µl of this mixture was used for CRF radioimmunoassay. The remaining extracts were properly diluted, and 50 µl was used for vasopressin radioimmunoassay. The CRF was measured using the rat CRF antisem developed and characterized in our laboratory.18 This antisem showed no cross-reactivity with sauvagine, arginine vasopressin, oxytocin, ACTH, or other neuropeptides, except for ovine CRF (12.8%). Vasopressin also was measured with an antisem developed in our laboratory, and its characteristics and the assay procedure have been described previously.19 Synthetic rat CRF and vasopressin were used for iodination and as standards. Measurements of serially diluted tissue extracts revealed parallel to standard curves by CRF and vasopressin. The intra-assay coefficient of variation of CRF and vasopressin assays was 2.1% and 10.4%, respectively. The limits of detection were 20 and 0.5 pg per tube, respectively. Synthetic rat CRF and vasopressin were obtained from the Peptide Institute (Osaka, Japan).

Values are presented as means ± SEM. The CRF and vasopressin contents of neurohypophysis are expressed as nanograms per one hypophysis, and concentrations in other brain regions are expressed as nanograms per 100 mg wet weight of tissue sample. The difference between the values of two groups of rats was estimated by Student’s t test.

### Results

Systolic blood pressure was not increased in 3-week-old SHR (Table 1). Blood pressure began to rise in SHR at 6 weeks of age, and it was significantly higher in 6-, 12-, and 24-week-old SHR than in age-matched WKY. There were no significant differences in body weight at the four age periods.

The highest concentration of CRF immunoreactivity was found in hypothalamic tissue in both strains (Figure 1). There was a low level of CRF in other brain regions. Hypothalamic CRF concentration was lower in 3- and 6-week-old SHR (p<0.01), while it did not differ at 12 and 24 weeks of age. Thalamic CRF concentration was decreased in 3- and 24-week-old SHR (p<0.01 and p<0.05, respectively), but it was not different at 6 and 12 weeks of age. In the cerebral cortex, CRF concentration was consistently reduced in SHR at all tested periods. The CRF concentration in the midbrain was decreased only in 6-week-old SHR (p<0.01); no significant difference was found at other periods. In the cerebellum and pons, the CRF concentration was similar in SHR and WKY. In the medulla oblongata, CRF concentration was decreased in 3-, 6-, and 12-week-old SHR (p<0.01), but the difference disappeared at 24 weeks of age. Neurohypophyseal CRF contents did not differ at 3 weeks of age but began to decrease in SHR at 6 weeks of age (p<0.01) and was consistently lower thereafter (Figure 2).

The highest concentrations of vasopressin were found in the hypothalamus and neurohypophysis (Tables 2–5). Hypothalamic vasopressin was consistently reduced in SHR at the four age periods examined.

### Table 1. Blood Pressure and Body Weight of Age-matched SHR and WKY

<table>
<thead>
<tr>
<th>Variable</th>
<th>3 Weeks</th>
<th>6 Weeks</th>
<th>12 Weeks</th>
<th>24 Weeks</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>8</td>
<td>8</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>106.3±2.9</td>
<td>102.5±3.5</td>
<td>153.6±2.5</td>
<td>117.9±2.5*</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>38.9±0.7</td>
<td>42.6±1.2</td>
<td>144.9±2.2</td>
<td>148.3±2.0</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *p<0.01, compared with values in SHR.
CORTICOTROPIN RELEASING FACTOR AND VASOPRESSIN IN SHR/Hattori et al.

Figure 1. Concentrations of corticotropin releasing factor (CRF) in major brain regions of age-matched SHR and WKY. Vertical lines represent SEM. Hy = hypothalamus; Thl = thalamus; Mid = midbrain; Po = pons; Med = medulla oblongata; Cx = cerebral cortex; Cl = cerebellum. Single (p < 0.05) and double (p < 0.01) asterisks indicate significant difference compared with values in WKY.

Figure 2. Content of corticotropin releasing factor (CRF) in the neurohypophysis of age-matched SHR and WKY. Vertical lines represent SEM. Double asterisks indicate significant difference (p < 0.01) compared with values in WKY. (p < 0.01). At 6 weeks of age, however, vasopressin concentration of thalamic tissue was higher in WKY than in age-matched SHR (see Table 3).

Discussion

Immunohistochemical studies of CRF neurons in the rat brain have shown that CRF-stained cells are widely distributed in the central nervous system with strong localization in the hypothalamic paraventricular nucleus.8, 20, 21 These studies are generally consistent with CRF concentrations measured by the radiomunoassay method,22-24 although some results remain controversial. According to a recent report,8 large groups of parvocellular CRF neurons in the hypothalamic paraventricular nucleus project to the external lamina of the median eminence, and this system mainly controls ACTH secretion. Smaller, separate groups of CRF neurons project to the neurohypophysis. Distributions of CRF neurons were also found in the basal forebrain and brainstem and probably are associated with cardiovascular regulation. In the present study, the brain CRF concentration was reduced in SHR and differences between SHR and WKY varied with age as well as brain regions. The CRF concentration in the
TABLE 3. Vasopressin Concentrations in Major Brain Regions of 6-Week-Old SHR and WKY

<table>
<thead>
<tr>
<th>Region</th>
<th>Vasopressin immunoreactivity (ng/100 mg wet tissue weight)</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus</td>
<td>20.584 ± 1.777</td>
<td>40.038 ± 5.423*</td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.122 ± 0.026</td>
<td>0.103 ± 0.018</td>
<td></td>
</tr>
<tr>
<td>Midbrain</td>
<td>0.038 ± 0.003</td>
<td>0.040 ± 0.004</td>
<td></td>
</tr>
<tr>
<td>Pons</td>
<td>0.038 ± 0.004</td>
<td>0.039 ± 0.008</td>
<td></td>
</tr>
<tr>
<td>Medulla oblongata</td>
<td>0.075 ± 0.009</td>
<td>0.056 ± 0.005</td>
<td></td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>0.032 ± 0.003</td>
<td>0.030 ± 0.006</td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.024 ± 0.005</td>
<td>0.022 ± 0.005</td>
<td></td>
</tr>
<tr>
<td>Neurohypophysis</td>
<td>172.0 ± 14.0†</td>
<td>150.0 ± 7.7†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM. *p < 0.01, compared with values in SHR. †Values represent ng/one neurohypophysis.

TABLE 5. Vasopressin Concentrations in Major Brain Regions of 24-Week-Old SHR and WKY

<table>
<thead>
<tr>
<th>Region</th>
<th>Vasopressin immunoreactivity (ng/100 mg wet tissue weight)</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus</td>
<td>67.930 ± 6.190</td>
<td>96.380 ± 4.800*</td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>1.102 ± 0.110</td>
<td>1.154 ± 0.071</td>
<td></td>
</tr>
<tr>
<td>Midbrain</td>
<td>0.424 ± 0.021</td>
<td>0.505 ± 0.086</td>
<td></td>
</tr>
<tr>
<td>Pons</td>
<td>0.072 ± 0.007</td>
<td>0.085 ± 0.006</td>
<td></td>
</tr>
<tr>
<td>Medulla oblongata</td>
<td>0.068 ± 0.005</td>
<td>0.098 ± 0.019</td>
<td></td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>0.222 ± 0.027</td>
<td>0.189 ± 0.062</td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.032 ± 0.006</td>
<td>0.043 ± 0.009</td>
<td></td>
</tr>
<tr>
<td>Neurohypophysis</td>
<td>1500 ± 100†</td>
<td>1270 ± 70†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM. *p < 0.01, compared with values in SHR. †Values represent ng/one neurohypophysis.

With regard to vasopressin, there is enough evidence to show its participation in both central and hypothalamic and medulla oblongata was reduced even in the prehypertensive stage and in the early phase of hypertension, and the change disappeared when hypertension was established. The concentration in the neurohypophysis did not differ at the prehypertensive stage but continued to decrease with blood pressure changes.

A decrease in concentration could reflect a depletion caused by increased release of peptides, reduced biosynthesis, enhanced degradation, or transport to other areas. Therefore, an interpretation of changes in peptide concentrations is very difficult. Our previous study showed that the basal corticosterone level was higher in young SHR than in age-matched WKY while circulating ACTH level was similar, but pharmacological inhibition of hypothalamic CRF secretion resulted in a significant decrease in peripheral ACTH concentration. In addition, the ACTH response to exogenous CRF was blunted in young SHR. These data support the hypothesis that hypothalamic CRF secretion was elevated in young SHR to produce pituitary and adrenal hyperfunction. A high concentration of circulating corticosterone blunted the ACTH response to CRF. The normal plasma ACTH level in nontreated SHR may result from both CRF hypersecretion and the negative feedback effect of elevated corticosterone. A similar mechanism was postulated for an impaired ACTH response to CRF in depressed patients. Thus, reduced hypothalamic CRF concentration may be ascribed to enhanced CRF secretion. Our data support the results of some morphological studies.

It is interesting that the CRF content of the posterior pituitary decreased in SHR with age. The changes are difficult to interpret, since the function of CRF in the posterior pituitary remains unknown. A reduced CRF concentration in the cerebral cortex was also found in all age groups of SHR and might be implicated in the reported abnormal behavior found in SHR.

With regard to vasopressin, there is enough evidence to show its participation in both central and
peripheral cardiovascular regulation. Several reports have shown reduced brain vasopressin in SHR and stroke-prone SHR, and some reports have emphasized the importance of a reduction in the brainstem and neurohypophysis. Vasopressinergic pathways from the hypothalamus to the brainstem have been found in immunohistochemical studies. Our data are consistent with previous observations. In the present study, hypothalamic vasopressin was reduced in SHR but not in other regions, except in 6-week-old rats. The concentration in the thalamus of 6-week-old WKY apparently was higher than that in WKY of other ages. We cannot explain this result. A small amount of hypothalamic tissue with vasopressin might have contaminated the examined thalamic tissue of the 6-week-old WKY.

It is very interesting that CRF and vasopressin concentrations were reduced in the hypothalamus of SHR. We cannot conclude that this reduction in neuropeptides is directly related to hypertension, since the deficit may be due to a genetic factor that is independent of spontaneous hypertension. However, CRF concentration was reduced during the development of hypertension in the medulla oblongata, which is thought to be associated with central autonomic regulation. These data suggest that the central CRF system may be involved in the development of spontaneous hypertension.

Acknowledgments

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