Adrenocorticotropin Responses to Corticotropin Releasing Factor and Vasopressin in Spontaneously Hypertensive Rats

TERUHIKO HATTORI, KOZO HASHIMOTO, AND ZENSUKE OTA

SUMMARY The effects of exogenous corticotropin releasing factor and arginine vasopressin were evaluated in 6- and 11-week-old spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). Basal adrenocorticotropic hormone (ACTH) and vasopressin levels did not differ between SHR and WKY, but basal corticosterone level was higher in 6-week-old SHR (p<0.01). To block endogenous corticotropin releasing factor secretion and nonspecific systemic responses, both groups were pretreated with chlorpromazine, morphine, and sodium pentobarbital anesthesia before measurement of ACTH responses to corticotropin releasing factor and vasopressin infusion. Basal ACTH level was lower in anesthetized 6-week-old SHR than in age-matched WKY (p<0.01), but no difference was seen between 11-week-old WKY and SHR. The ACTH response to corticotropin releasing factor in 6-week-old WKY was significantly greater than that in age-matched SHR (p<0.01), whereas in 11-week-old SHR and WKY the response was similar. Compared with responses in WKY, SHR showed an increased ACTH response to high doses of vasopressin (0.25 μg/100 g body weight) at both ages (p<0.05). These results indicate that the ACTH response to corticotropin releasing factor is blunted in the early stages of hypertension in SHR but later recovers. These abnormal responses to corticotropin releasing factor and vasopressin may be related to the development of spontaneous hypertension. (Hypertension 8: 386-390, 1986)

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

MORPHOLOGICAL abnormalities have been reported in the pituitary and adrenal glands of spontaneously hypertensive rats (SHR), indicating that the hypertension may be related to abnormal hypothalamic-pituitary-adrenal function. Many investigators have examined basal corticosterone level and corticosterone response to stress in SHR, but the results have not been consistent. Some neuropeptides regulate pituitary hormone secretion and may also aid in regulating blood pressure. Elevated plasma vasopressin levels and a high 24-hour urinary secretion of vasopressin have been reported in SHR, and as have changes in hypothalamic vasopressin and oxytocin content. Vale et al. recently purified corticotropin releasing factor (CRF) from ovine hypothalamic extract. Intracerebroventricular administration of CRF raised blood pressure in conscious rats, whereas intravenous injection decreased blood pressure.

The present study was undertaken to examine the serum adrenocorticotropic hormone (ACTH) responses to CRF and arginine vasopressin (AVP) to determine their role in the development of hypertension in SHR.

Materials and Methods
Male 6- and 11-week-old SHR and normotensive Wistar-Kyoto rats (WKY) (Charles River Japan, Aichi, Japan) were used because 6 weeks represents an early stage of hypertension whereas hypertension is nearly established in 11-week-old SHR. All rats were housed in the animal quarters of our laboratory and received food and water ad libitum. Blood pressure was monitored the day before the experiment using tail cuff plethysmography (Ueda Seisakusho, Tokyo, Japan) in restrained, conscious animals.
ACTH RESPONSES TO CORTICOTROPIN RELEASING FACTOR AND VASOPRESSIN/Hattori et al. 387

For the measurement of basal hormone levels, 8 untreated animals from each age group were decapitated at 1000 to 1200 and blood samples were collected in chilled plastic tubes. The blood samples were immediately centrifuged (1200 g) at 4°C for 15 minutes, and serum samples were stored at −20°C until assay.

The serum ACTH response to CRF and AVP was examined in 20 SHR and 18 WKY at 6 weeks of age and in 11 SHR and 13 WKY at 11 weeks of age. Experiments were conducted from 0900 to 1400. The animals were anesthetized with chlorpromazine (1 mg/100 g body weight s.c.) at time zero, morphine HCl (2 mg/100 g body weight s.c.) at 3 hours, and sodium pentobarbital (Nembutal; 2.5 mg/100 g body weight i.p.) at 3 hours, 15 minutes to eliminate endogenous CRF secretion and secondary, nonspecific systemic responses.18

All drugs were dissolved in saline, and the total injection volume was 1 ml/100 g body weight. The anesthetized rats were placed in the supine position, and a small, longitudinal incision was made just over the jugular vein. At 4 hours, the jugular vein was punctured with a 24-gauge needle, 1 ml of blood was aspirated, and 0.9% saline (1 ml/100 g body weight) was injected into the vein. Twenty minutes later (4 hours, 20 minutes), 1 ml of blood was again collected from the jugular vein to measure the ACTH concentration. Blood was collected after injection of CRF and AVP in 6-week-old SHR and WKY, because we found in a preliminary study that some 6-week-old SHR died within 20 minutes of injection, presumably because of acute pulmonary edema. Thus, a lower, nonfatal dose (0.05 and 0.15 μg/100 g body weight) of AVP was given to the 6-week-old SHR and WKY.

Table 1. Basal Blood Pressure, Weight, and Serum ACTH, Corticosterone, and Vasopressin Concentrations in Unanesthetized SHR and WKY

<table>
<thead>
<tr>
<th>Variable</th>
<th>SHR</th>
<th>WKY</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>147.5±2.9*</td>
<td>113.7±7.4</td>
<td>187.3±3.0*</td>
<td>138.5±3.7</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>126.3±2.6</td>
<td>132.9±2.1</td>
<td>261.0±3.8</td>
<td>273.5±3.9</td>
</tr>
<tr>
<td>ACTH (pg/ml)</td>
<td>45.0±11.8</td>
<td>34.6±5.9</td>
<td>70.7±14.6</td>
<td>90.0±5.0</td>
</tr>
<tr>
<td>Corticosterone (μg/dl)</td>
<td>16.8±1.6*</td>
<td>9.2±1.4</td>
<td>24.4±3.2</td>
<td>20.0±2.4</td>
</tr>
<tr>
<td>Vasopressin (pg/ml)</td>
<td>6.0±4.1</td>
<td>7.8±4.5</td>
<td>4.6±2.4</td>
<td>2.2±0.8</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

*p < 0.01, compared with values for WKY.

The systolic blood pressure of 6-week-old SHR and WKY pretreated with chlorpromazine, morphine, and sodium pentobarbital anesthesia was 148.0 ± 3.0

Corticosterone also was measured in duplicate with 50-μl serum samples with a cortisol radioimmunoassay kit (Daichi Radioisotope Labs, Tokyo, Japan). Corticosterone was serially diluted with hormone-free rat serum and used to make the standard curve. Nakane et al.19 have reported that the cross-reactivity of this antiserum (using 5 μl of sample) is 7.2% to corticosterone, 3.5% to 11-deoxycortic, and less than 0.5% to other steroids. As cortisol and 11-deoxycortic are present at very low levels in rat blood, the measured corticosterone values reported herein were ascribed mostly to corticosterone. When 50 μl of diluted standard and sample were used for the corticosterone assay, a good standard curve and reasonable corticosterone values were obtained. The values obtained from this method were compared with those obtained with the fluorescent method used by other investigators, and the correlation was high (r = 0.8554, p < 0.01).

The AVP was assayed by a radioimmunoassay established in our laboratory.20 The AVP was extracted from a 600-μl serum sample with acetone-petroleum ether. The mean recovery rate of extraction was 71.9 ± 1.7% (mean ± SD), and the intraassay coefficient of variation was 11.5%.

Synthetic ovine CRF and synthetic arginine vasopressin were obtained from the Peptide Institute (Osaka, Japan). Statistical analysis was performed with the Student's t test. Results are expressed as means ± SEM.

Results

Basal systolic blood pressure in the unanesthetized group was significantly higher in SHR than in WKY at both ages (p < 0.01; Table 1). The basal ACTH and AVP levels were not different between strains. The corticosterone concentration was higher in 6-week-old SHR than in age-matched WKY (p < 0.01), but no between-group difference was found in 11-week-old rats.

The systolic blood pressure of 6-week-old SHR and WKY pretreated with chlorpromazine, morphine, and sodium pentobarbital anesthesia was 148.0 ± 3.0
Corresponding values at 11 weeks were 175.5 ± 3.3 and 137.4 ± 2.4 mm Hg, respectively (p < 0.01). When the endogenous CRF secretion was blocked with anesthetics, the basal ACTH level was significantly lower in 6-week-old SHR (39.1 ± 2.1 pg/ml) than in age-matched WKY (71.4 ± 4.1 pg/ml; Figure 1). In both strains, serum ACTH showed no response to saline infusion. The WKY showed a good response to CRF, but it was impaired in 6-week-old SHR (p < 0.01). When AVP, 0.25 µg/100 g body weight, was injected, the serum ACTH level increased 10-fold from the basal level in SHR in both age groups, whereas levels in WKY showed only a twofold increase. Six-week-old SHR responded markedly to this dose of AVP; all animals died of acute pulmonary edema within 20 minutes of injection. When lower doses of AVP were administered in 6-week-old SHR, however, all rats survived. Six-week-old SHR also showed a strong response to AVP, 0.15 µg/100 g body weight, which was similar to that seen with AVP, 0.25 µg/100 g body weight. No response was elicited by AVP, 0.05 µg/100 g body weight (Figure 2). Age-matched WKY showed no ACTH response to either of these lower dosages.

Figure 3 shows the results of CRF and AVP infusions in 11-week-old rats. No significant between-group difference in the basal ACTH level was seen after pretreatment with anesthetics (39.1 ± 3.5 pg/ml vs 51.4 ± 5.5 pg/ml). In both strains, serum ACTH was similarly increased by CRF, 0.25 µg/100 g body weight. The ACTH response to AVP, 0.25 µg/100 g body weight, was stronger in SHR than in WKY (p < 0.05), similar to the findings in 6-week-old rats. All 11-week-old rats survived AVP administration.

Discussion

The specific mechanisms for causing hypertension in SHR are unknown. Abnormalities in the hypothalamic-pituitary-adrenal systems may play some role in the development of this hypertension. 1, 2 Sowers et al. 4 reported that basal corticosterone concentration was increased in 2-month-old SHR. Yamori et al. 3 found no significant difference in plasma corticosterone level...
between 6-month-old SHR and WKY. In contrast, other investigators have reported decreased corticosterone levels in SHR as compared with WKY. Basal ACTH levels were reported to be similar in 12- to 13-week-old SHR and WKY on high and low sodium diets. In the present study, the basal corticosterone level in 6-week-old SHR was elevated, but the basal ACTH level was not. The discrepancies in these results may be due to different methods of blood collection, assays, and animal care used in the various studies.

The anesthetic preparation (chloropromazine, morphine, sodium pentobarbital) used in the present study is an established method in the bioassay of CRF. Arimura et al. reported that epinephrine (20 ng/rat s.c.), angiotensin II (1 μg/rat i.v.), or acetylcholine (20 ng/rat i.v.), or histamine (20 ng/rat i.v.), which might be expected to evoke a great systemic stress response, caused no significant increase in peripheral corticosterone concentration in normal rats pretreated with anesthetics. Therefore, it was thought that endogenous CRF secretion was pharmacologically blocked in rats pretreated with anesthetics. These results suggest that endogenous CRF may be elevated to sustain the ACTH level when hypertension is nearly established, the anterior pituitary response to CRF recovered. This theory is consistent with the abnormal morphological changes noted in the pituitary gland of SHR. A similar phenomenon has also been reported in adrenal steroidogenesis in SHR.

Intracerebroventricular administration of CRF caused elevations in mean arterial pressure and heart rate in conscious, freely moving rats, while an intravenous injection produced a significant reduction of blood pressure. This pressor response to central CRF injection was thought to be mediated by the sympathetic nervous system in unanesthetized rats. Brown et al. found that intravenously administered CRF increased the superior mesenteric artery flow in proportion to the reduction noted in blood pressure. However, the dose required to decrease blood pressure significantly was nearly 10-fold higher than that used in the present study. This depressor effect might be due to pharmacological action.

In summary, the present results indicate that young SHR show an impaired ACTH response to CRF and an enhanced response to AVP, which may be related to the development of hypertension in this strain.

Acknowledgments

We thank Dr. Kazuhiro Murakami, Dr. Kazuyuki Fujino, and Miss Reiko Akiyama for technical assistance.
References


Adrenocorticotropic responses to corticotropin releasing factor and vasopressin in spontaneously hypertensive rats.
T Hattori, K Hashimoto and Z Ota

Hypertension. 1986;8:386-390
doi: 10.1161/01.HYP.8.5.386

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/8/5/386