Adrenocorticotropic 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, 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, Atsugi, 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.
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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), CRF (0.25 µg/100 g body weight), or AVP (0.25 µg/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 15 minutes after AVP administration 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.

All blood samples were collected in chilled plastic tubes and immediately centrifuged (1200 g) at 4°C for 15 minutes, and the serum samples were stored at −20°C until assay.

Duplication measurements of ACTH were made with 100-µl serum samples using commercially available radioimmunoassay kits (CEA-IRE-Sorin, Gif-sur-Yvette, France). According to information provided on the package insert, the cross-reactivity of the antiserum is 100% to human ACTH 1–39, 3% to fragment 1–16, 1.5% to fragment 11–24, 1% to β-endorphin, 0.3% to γ-lipotropin, and negligible in other ACTH-related peptides.

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-deoxycorticosterone, and less than 0.5% to other steroids. As cortisol and 11-deoxycorticosterone 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

<table>
<thead>
<tr>
<th>Table 1. Basal Blood Pressure, Weight, and Serum ACTH, Corticosterone, and Vasopressin Concentrations in Unanesthetized SHR and WKY</th>
<th>6-week-old</th>
<th>6-week-old</th>
<th>11-week-old</th>
<th>11-week-old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>SHR (n = 8)</td>
<td>WKY (n = 8)</td>
<td>SHR (n = 8)</td>
<td>WKY (n = 8)</td>
</tr>
<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.
and 121.7 ± 1.3 mm Hg (p<0.01). 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.

Figure 1. Serum ACTH response to saline, corticotropin releasing factor (CRF), and arginine vasopressin (AVP), 0.25 μg/100 g body weight (BW), in 6-week-old SHR (n = 20) and WKY (n = 18) pretreated with chlorpromazine, morphine, and sodium pentobarbital anesthesia. Blood samples in AVP group were collected 15 minutes after injection. Single (p<0.05) and double (p<0.01) asterisks indicate significant difference between groups. Values are means ± SEM.

Figure 2. Serum ACTH response to arginine vasopressin (AVP), 0.05 and 0.15 μg/100 g body weight (BW), in 6-week-old SHR (n = 20) and WKY (n = 18) pretreated with chlorpromazine, morphine, and sodium pentobarbital anesthesia. Single (p<0.05) and double (p<0.01) asterisks indicate significant difference between groups. Values are means ± SEM.

Figure 3. Serum ACTH response to corticotropin releasing factor (CRF) and arginine vasopressin (AVP), 0.25 μg/100 g body weight (BW), in 11-week-old SHR (n = 11) and WKY (n = 13) pretreated with chlorpromazine, morphine, and sodium pentobarbital anesthesia. Asterisk indicates significant difference between groups (p<0.05). Values are means ± SEM.

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 (chlorpromazine, 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 acetylsalicylic acid (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. Therefore, it was thought that endogenous CRF secretion was pharmacologically blocked in rats pretreated with anesthetics and that exogenous CRF and CRF-like substances stimulated the release of ACTH directly from the anterior pituitary gland. In the present study, the basal ACTH level was decreased in 6-week-old SHR and WKY pretreated with anesthetics. These results suggest that endogenous CRF may be elevated to sustain the ACTH level in 6-week-old SHR. The basal ACTH level in 6-week-old WKY pretreated with anesthetics seemed to be higher than that in unanesthetized age-matched WKY; however, this may be due to differences in blood collection methods.

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. Rivet et al. examined the ACTH response to intravenous administration of CRF and AVP in normal Sprague-Dawley rats at a range of 0.01 to 1.0 μg/rat and found a significant dose-dependent ACTH response to CRF in rats pretreated with chlorpromazine, morphine, and sodium pentobarbital. This dose may be high cardiovascularly. It is well known that various releasing hormones and some neuropeptides originate from the hypothalamus, reaching the anterior pituitary through hypothalamic portal veins, and have a regulatory effect on pituitary hormone secretion. Thus, the concentrations of these peptides are extremely high in portal blood; for example, the concentration of AVP in rat hypothalamic portal blood was reported to be nearly a thousand times higher (2.0 ± 0.4 ng/ml) than that found in peripheral blood. Therefore, to examine the hormone releasing activity of these peptides in vivo, high doses have been injected into the peripheral vein to attain a high level of these peptides in the pituitary gland.

Compared with responses in age-matched WKY, the response of ACTH to CRF was significantly impaired in 6-week-old SHR but recovered in 11-week-old SHR. This finding suggests that the anterior pituitary response to CRF was suppressed by elevated circulating corticosterone levels in 6-week-old SHR and that by 11 weeks of age, when hypertension was 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.

Vasopressin is a potent pressor agent that has both antiduretic and corticotropin releasing activities. Many reports have suggested that vasopressin is involved in the development of spontaneous hypertension. Crofton and Share and co-workers found that the plasma concentration and the 24-hour urinary secretion of AVP were elevated in SHR compared with those in WKY. In our study, there were no significant differences in basal serum AVP levels in SHR and WKY at 6 and 11 weeks of age. Infusion of CRF induced a significant increase in ACTH levels, while AVP infusion induced only a slight increase in normotensive WKY after pretreatment with anesthetics. On the other hand, SHR responded markedly to AVP infusion: peripheral ACTH level was rapidly elevated to more than 10 times the basal values. When AVP, 0.25 μg/100 g body weight, was injected in a preliminary study, some 6-week-old SHR expectorated pink spuva and died after unexpected difficulty in breathing. We presumed from these symptoms that there was pulmonary edema. An enhanced pressor response to AVP has been found in SHR. Montani et al. showed that even a physiological plasma concentration of AVP has hemodynamic effects in conscious dogs. These results suggest that an increased cardiovascular response might induce the enhanced ACTH secretion in SHR in spite of pretreatment with anesthetics. Because we did not measure blood pressure changes or peripheral AVP concentration after AVP injection, we cannot conclude whether the increased ACTH response to AVP in SHR was due to hypersensitivity in the anterior pituitary cells or to systemic stress secondary to the enhanced pressor response.

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.

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References


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