Captopril and the Response to Stress in the Spontaneously Hypertensive Rat

KATHLEEN H. BERECEK, GINA COSHATT, ANNIE JO NARKATES, SUZANNE OPARIL, KAREN M. WILSON, AND JOEL ROBERTSON

SUMMARY The purpose of this study was to determine the effect of chronic blockade of the brain renin-angiotensin system on the hormonal response to stress in spontaneously hypertensive rats (SHR). To this end, we measured changes in plasma corticosterone, vasopressin, plasma renin activity, aldosterone, norepinephrine, and epinephrine in SHR treated with a 4-week intracerebroventricular infusion of captopril (osmotic minipump, 1.25 μg/hr) or vehicle in response to cold stress (4 °C x 4 hours) or ether stress (5 minutes). Within the fourth week of treatment, the average systolic blood pressure of captopril-treated SHR was significantly lower than that of vehicle-treated rats. Basal plasma levels of corticosterone, but not vasopressin, were significantly lower in SHR treated with captopril. In response to cold stress, captopril-treated SHR showed significantly lesser increases in both corticosterone and vasopressin than did vehicle-treated SHR. There were no differences in basal plasma levels of norepinephrine, epinephrine, plasma renin activity, or aldosterone between captopril-treated and vehicle-treated SHR, and both groups showed elevations of a similar magnitude after exposure to cold. In response to ether stress, captopril-treated SHR also showed significantly smaller increases in corticosterone and vasopressin than did vehicle-treated SHR. These results suggest that chronic intracerebroventricular administration of captopril, through blockade of the brain renin-angiotensin system, alters the hormonal response of SHR to stress.

Key Words - corticosterone • vasopressin • catecholamines • cold stress • ether stress • captopril

UD laboratory, as well as others, has shown that acute or chronic intracerebroventricular (i.c.v.) administration of a competitive inhibitor of angiotensin II, saralasin, or an angiotensin converting enzyme inhibitor, captopril, in doses that were ineffective or much less effective when given by the intravenous route, substantially lower blood pressure in spontaneously hypertensive rats (SHR), and attenuate the development of hypertension in this model. While evidence suggests participation of the brain renin-angiotensin system in the development and maintenance of spontaneous hypertension, the mechanism by which this occurs is unknown. The purpose of the present study was to test the hypothesis that brain angiotensin II may participate in cardiovascular regulation and the pathogenesis of hypertension by altering the release of vasopressin or adrenocorticotropic hormone (ACTH)/corticosterone (or both). To this end, we measured changes in plasma corticosterone and vasopressin in response to either cold or ether stress in SHR treated with a chronic i.c.v. infusion of captopril or vehicle. In addition, we measured plasma renin activity, and aldosterone, norepinephrine, and epinephrine levels.

Materials and Methods

Six-week-old SHR from a virus-free colony (Charles River Breeding Laboratories, Wilmington, MA, USA) were used. The animals were housed in groups of four per cage under identical conditions of temperature (24 °C), humidity (60 ± 5%), and light cycle (12 hours on, 12 hours off). They were allowed normal rat chow and water ad libitum. After three determinations of baseline blood pressure and heart rate, SHR were divided into two groups: Group 1 received i.c.v. infusion of captopril and Group 2 received i.c.v. infusion of vehicle. Beginning at 7 weeks of age, rats were given an infusion of captopril for 4 weeks at a dosage of 1.25 μg/hr into the left lateral cerebral hemisphere.
ventricle according to a previously published technique. Captopril was infused by osmotic minipump (Alzet Model 2002, Alza, Palo Alto, CA, USA). The nominal reservoir volume and pumping rate were 200 and 0.5 μl/hr, respectively. Pumps were changed 2 weeks after initiation of the infusion. Captopril was dissolved in a 0.9% sterile saline solution. Since the pH of the captopril solution was 3.0, control rats received vehicle solution of 0.9% saline adjusted to a pH of 3.0 with 1 N HCl. After i.c.v. catheterization, rats were given 100,000 U of penicillin G (Squibb Institute, Princeton, NJ, USA) intramuscularly.

Systolic blood pressure was measured twice weekly using an indirect tail-cuff method (Narco Biosystems, Houston, TX, USA) in conscious, restrained rats prewarmed at 37°C for 5 to 10 minutes. Rats were conditioned to the restraining device and cuff inflations before baseline values were determined.

Stress studies were performed within the fourth week of captopril or vehicle administration. Blood samples from all rats were taken between 1700 and 1800. One day prior to application of cold stress, each rat received a femoral artery catheter and was placed in a separate cage. On the day of experimentation, stress was induced in half of the rats from the captopril- and vehicle-treated groups by placing the animals in a cold room (4°C). The remaining captopril-treated and vehicle-treated animals were left undisturbed at room temperature (23°C) to serve as unstressed controls.

During the fourth hour of cold exposure, 0.6 ml of blood was drawn from the arterial catheters and collected into chilled polystyrene tubes containing 10 μl of EGTA and glutathione preservative for the measurement of catecholamines. After removal of 0.6 ml of blood, the same volume of heparinized saline was infused back into the animal. Blood for catecholamines was immediately centrifuged (2000 rpm, 4°C, 20 minutes), and plasma was removed and stored at −70°C until assay. One hour after catecholamine sampling, all rats were sacrificed by decapitation within 30 seconds of initiating contact with the cage. Trunk blood was collected into chilled, heparinized tubes for measurement of vasopressin, corticosterone, and aldosterone, and in EDTA for measurement of plasma renin activity.

For the ether stress experiments, rats were exposed to the anesthetic for 5 minutes and then killed by decapitation.

Plasma vasopressin was measured by radioimmunoassay according to previously published methods. 

The standard used was synthetic (Arg8)vasopressin (Bachem, Torrance, CA, USA). Rabbit antibody to vasopressin was raised using male New Zealand white rabbits and arginine vasopressin–bovine thyroglobulin conjugates. The final dilution in the assay tube was 1:400,000, in a total volume of 0.4 ml. The ED50 was 11.4 ± 1.6 pg vasopressin with the least detectable dose of 0.3 ± 0.07 pg assay tube. Recovery of radioiodinated vasopressin from plasma was 88 ± 8%. The intra-assay and interassay coefficients of variations were 10.9% and 12.5%, respectively.

Plasma corticosterone was measured by a competitive protein binding assay according to modifications of the method of Murphy. 

Plasma proteins were extracted with methylene chloride. All standards and samples were run in duplicate. All samples were corrected for fractional recovery, which averaged 85 ± 6.6%. Corticosterone (Calbiochem-Behring Diagnostics, La Jolla, CA, USA) standard stock solution was prepared at a concentration of 1 mg/ml ethanol and stored at 4°C. Serial dilutions of the stock were made to yield standard concentrations that ranged from 0.25 to 16 ng/100 μl. To each test tube (standards and extracted samples), 0.5 ml corticosteroid-binding globulin isotope solution was added. The isotope solution contained [H]corticosterone ([1,2,6,7-3H] corticosterone, Amersham, Arlington Heights, IL, USA), stripped rat plasma (devoid of endogenous corticoid), and phosphate-buffered saline. All tubes were incubated in a water bath at 45°C for 5 minutes and then placed in an ice bath (−5°C) for 10 minutes. Dextran-coated charcoal (0.25%, 0.5 ml) was used to separate bound from free corticosterone. After centrifugation (2000 rpm, 4°C × 15 minutes), 0.9 ml of supernatant was removed from each tube and placed in a vial containing 5 ml scintillation cocktail that consisted of Toluene/Triton X-100 (Amersham), 2:1 with 6-g Omnifluor/L (New England Nuclear, Boston, MA, USA). Samples were counted in a liquid scintillation counter for 10 minutes. The intra-assay and interassay coefficients of variation for the assay were 5.1% and 9.5%, respectively.

Plasma renin activity was measured by modifications of the radioimmunoassay procedures of Haber et al. and Page et al. Plasma aldosterone was measured by the “Coat-a-Count” radioimmunoassay kit (Diagnostic Products, Los Angeles, CA, USA). Plasma norepinephrine and epinephrine were measured by radioenzymatic assay (CAT-A-KIT radioenzymatic kit, Upjohn Diagnostics, Kalamazoo, MI, USA).

Data from the vasopressin radioimmunoassay and the corticosterone competitive binding assay were analyzed using the Four-Parameter Logistic Program developed in the laboratory of Dr. David Rodbard at the National Institutes of Health and adapted for the IBM-XT personal computer by M. L. Jaffe. All results are presented as group means ± SE. Statistical analysis of the data was done using two-way analysis of variance.

**Results**

Systolic blood pressures (in mm Hg) in vehicle and captopril-treated rats were as follows before treatment: vehicle, 137 ± 2; captopril, 141 ± 1.8; after captopril or vehicle treatment: 1 week: vehicle, 147 ± 2; captopril, 137 ± 2 (p < 0.05); 2 weeks: vehicle, 154 ± 2; captopril, 140 ± 1.4 (p < 0.01); 3 weeks: vehicle, 170 ± 3; captopril, 143 ± 2 (p < 0.01); 4 weeks: vehicle, 165 ± 3; captopril, 139 ± 3 (p < 0.01).

Figure 1 shows basal (at 1800) and cold stress levels of corticosterone and vasopressin in SHR treated with vehicle or captopril. Captopril-treated SHR showed...
significantly lower basal serum corticosterone levels than control SHR. Both groups showed a significant rise in corticosterone in response to cold stress; however, the rise was significantly less in captopril-treated rats. No significant between-group differences were noted in basal levels of plasma vasopressin. Vehicle-treated SHR responded to cold stress with an approximate fourfold increase in plasma vasopressin level, whereas captopril-treated SHR showed no significant increase in plasma vasopressin. In contrast to the findings with vasopressin and corticosterone, there were no differences in basal or cold stress levels of norepinephrine, epinephrine, plasma renin activity, or plasma aldosterone in SHR treated with either captopril or vehicle (Table 1). Both groups showed significant ($p<0.001$) increases in norepinephrine and epinephrine in response to cold stress, but the magnitude of the increases was not different between captopril-treated and vehicle-treated groups.

In response to ether stress, SHR treated with captopril also showed significantly smaller increases in corticosterone and vasopressin than did SHR treated with vehicle. Levels of corticosterone (in $\mu$g/dl) in unstressed SHR were as follows: vehicle, $12.1 \pm 2.1 \, (n = 15)$; captopril, $12.5 \pm 2.6 \, (n = 16)$; levels in the ether-stressed group: vehicle, $26.6 \pm 1.0 \, (n = 15)$;

**TABLE 1. Basal and Cold Stress Levels of Catecholamines, Plasma Renin Activity, and Aldosterone**

<table>
<thead>
<tr>
<th>SHR</th>
<th>Norepinephrine (pg/ml)</th>
<th>Epinephrine (pg/ml)</th>
<th>Plasma renin activity (ng Ang I/ml/hr)</th>
<th>Plasma aldosterone (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle-treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>$248 \pm 34 , (13)$</td>
<td>$100 \pm 27 , (13)$</td>
<td>$0.98 \pm 0.48 , (7)$</td>
<td>$444 \pm 120 , (7)$</td>
</tr>
<tr>
<td>Cold stress</td>
<td>$927 \pm 108 , (11)^*$</td>
<td>$318 \pm 95 , (11)$</td>
<td>$1.29 \pm 0.56 , (7)$</td>
<td>$389 \pm 57 , (7)$</td>
</tr>
<tr>
<td>Captopril-treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>$258 \pm 32 , (16)$</td>
<td>$95 \pm 28 , (16)$</td>
<td>$1.44 \pm 0.63 , (8)$</td>
<td>$539 \pm 259 , (8)$</td>
</tr>
<tr>
<td>Cold stress</td>
<td>$1151 \pm 234 , (17)^*$</td>
<td>$334 \pm 66 , (17)$</td>
<td>$2.42 \pm 0.95 , (9)$</td>
<td>$507 \pm 121 , (9)$</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE. The numbers in parenthesis represent the numbers of animals tested.

$^*p<0.001$, statistical analysis by two-way analysis of variance, basal vs cold stress levels.
captopril, 21.7 ± 0.9 (n = 15); p < 0.05). The attenuation in hormonal response was particularly striking with vasopressin. The SHR treated with vehicle showed a sixfold increase in vasopressin levels (in pg/ml) in response to ether stress (unstressed, 4.2 ± 0.5; stressed, 29.6 ± 7.9) whereas captopril-treated rats showed only a twofold increase (unstressed, 3.5 ± 0.5; stressed, 8.1 ± 2.1; p < 0.001).

Discussion

The current study examined the effect of chronic i.c.v. administration of captopril on the hormonal response of SHR to stress. In agreement with previous studies, we found that in young SHR it attenuated the development of hypertension. The mechanism by which captopril produced this attenuation is unknown, but our previous studies suggested that it was by a central action, because the same dose infused intravenously had no effect on the development of hypertension in SHR. The current study suggests that the antihypertensive effect of i.c.v. infusion of captopril may be due to blockade of the brain-renin angiotensin system with secondary depression in vasopressin or ACTH/corticosterone mechanisms (or both).

Angiotensin II is known to stimulate the release of ACTH and vasopressin. Injection of renin into the brain of rat, dog, and monkey produced a rise in plasma corticosterone and vasopressin mediated by the central effects of angiotensin II. Moreover, the central effects of renin on ACTH/corticosterone and vasopressin release were inhibited by simultaneous i.c.v. injections of captopril. The renin-induced increases in plasma corticosterone and vasopressin are of particular interest in that both hormones have been reported to be elevated in SHR. Moreover, SHR show greater increases in the levels of these hormones and greater increases in arterial pressure in response to various stresses than normotensive Wistar-Kyoto rats do. Our findings that increases in corticosterone and vasopressin release in response to stress were attenuated in captopril-treated SHR suggest that the enhanced hormonal response to stress in SHR may be due to brain angiotensin II. Furthermore, the attenuated vasopressin and ACTH/corticosterone release may be associated with the antihypertensive effect of chronic i.c.v. captopril infusion.

The SHR demonstrate numerous functional abnormalities that could participate in the pathogenesis of hypertension, including increased vascular reactivity, alterations in baroreceptor reflex function, and increased sympathetic activity. We previously showed that the antihypertensive effect of chronic i.c.v. administration of captopril was associated with decreased vascular reactivity, enhanced baroreceptor reflex sensitivity, and decreased sympathetic activity. Corticosterone and vasopressin have been reported to enhance vascular responsiveness and sympathetic activity and decrease baroreceptor reflex sensitivity by producing alterations in release of vasopressin or ACTH/corticosterone (or both). Furthermore, the antihypertensive effect of captopril may be due to blockade of the central effects of angiotensin II on corticosterone or vasopressin (or both).

Acknowledgments

We are grateful to Dr. Zola P. Horovitz, the Squibb Institute for Medical Research (Princeton, NJ, USA) for generously supplying the captopril, and to Deborah Clark for typing the manuscript.

References

5. Murphy BEP. Some studies on the protein binding of steroids and their application to the routine micro and ultra micro measurement of various steroids in body fluids by competitive protein binding radioassay. J Clin Endocrinol 1967;27:973–990
Captopril and the response to stress in the spontaneously hypertensive rat.
K H Berecek, G Coshatt, A J Narkates, S Oparil, K M Wilson and J Robertson

*Hypertension*. 1988;11:I144
doi: 10.1161/01.HYP.11.2_Pt_2.I144

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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/11/2_Pt_2/I144