Attenuation of the Development of Spontaneous Hypertension in Rats by Chronic Central Administration of Captopril

TETSUJI OKUNO, M.D., SHUSAKU NAGAHAMA, M.D., MARSHALL D. LINDHEIMER, M.D., AND SUZANNE OPARIL, M.D.

SUMMARY Captopril infused into the lateral ventricle (ICV) of adult spontaneously hypertensive rats (SHR) decreases blood pressure. The current study was designed to explore the effects of brain converting-enzyme inhibition in young animals before the development of established hypertension and to characterize changes induced by captopril in a variety of pressor systems that might be responsible for the development of hypertension in this strain. Captopril (1.25 μg/0.5 μl/hr) was infused into male SHR starting at 7 weeks of age. Four weeks later systolic blood pressure was only 157 ± 3.3 compared to 181 ± 3.9 mm Hg in vehicle-infused controls, and the pressor effect of ICV-injected angiotensin I was attenuated by 50%. When the same dose of captopril was infused intravenously, hypertension progressed as in vehicle-treated rats. Serum angiotensin-converting enzyme activity (SACE) and plasma arginine vasopressin (AVP) concentration were significantly higher (p < 0.001 and 0.05, respectively), in the ICV captopril group than in the ICV vehicle group, while plasma aldosterone concentration and renin activity, fluid intake, urine volume, and urinary sodium excretion were similar in the two groups. Peripheral sympathetic nervous system activity assessed in the resting state was not altered by captopril treatment. In addition, AVP content of the telencephalon, diencephalon, mesencephalon, and pons medulla were not altered by ICV captopril. Renin activity was elevated in the telencephalon of ICV captopril-treated animals but unaltered in the other brain regions examined. These data demonstrate that ICV administration of captopril attenuates the development of hypertension in young SHR by mechanisms apparently independent of altered fluid and sodium balance and the sympathoadrenal system. The effect on blood pressure occurs in the absence of changes in renin activity or AVP content of plasma or those brain regions most often associated with blood pressure control. (Hypertension 5: 653–662, 1983)

Key Words • captopril • chronic central infusion • blood pressure • spontaneously hypertensive rats • renin-angiotensin-aldosterone system • catecholamines • vasopressin

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The brain renin-angiotensin system is altered in spontaneously hypertensive rats of the Okamoto strain (SHR). These animals demonstrate increments in brain renin activity1 and angiotensin-like material in cerebrospinal fluid2 and enhanced pressor responses to centrally administered angiotensin II.3 Moreover, intracerebroventricular (ICV) administration of the angiotensin II antagonist saralasin4 or the converting enzyme inhibitor captopril5 lowers blood pressure in adult SHR. The mechanisms by which the brain renin-angiotensin system contributes to, and centrally administered captopril decreases, established hypertension in adult SHR are not known. In this regard, studies implicating sympathetic and arginine vasopressin (AVP) pressor systems in the response to centrally administered captopril are of interest. Centrally infused 6-hydroxydo-
pamine or beta-adrenoreceptor blocking agents, peripherally administered alpha-adrenoreceptor blocking drugs or the AVP antagonist d(CH2)5 Tyr(Me)AVP, and renal denervation inhibit or attenuate the pressor effect of centrally administered angiotensin II.9,10 The present study was designed to: 1) determine whether ICV captopril influences the development and/or progression of hypertension in young SHR; and 2) explore the effects of central administration of this drug on several central and peripheral components of the sympathetic nervous system and AVP in this model.

Methods

Six-week-old male SHR (Charles River Breeding Laboratories, Wilmington, Massachusetts) housed under constant conditions of temperature (24°C), humidity (60% ± 5%) and photoperiod (12 hours on, 12 hours off) were fed normal rat chow and allowed tap water ad libitum. In all protocols, systolic blood pressure was measured twice weekly in conscious restrained rats by the tail cuff method, using an electrophysymomanometer and physiograph recorder (Narco Biosystems, Incorporated, Houston, Texas).

Surgical Preparation

Animals were anesthetized with ether and placed in a Kopf stereotaxic holder. A PE 10 cannula was filled with test solution, placed into the lateral ventricle ventral to the skull surface and fixed to the skull with screws and dental cement. The test solution was infused twice weekly in conscious restrained rats by the tail cuff method, using an electrophygmonanometer and physiograph recorder (Narco Biosystems, Incorporated, Houston, Texas).

Dose and Distribution of Intracerebroventricular Captopril

In all experiments, captopril was dissolved in saline, resulting in an infusion of pH 3, osmolality 294 to 296 mOsm/kg. Control animals received saline of similar toxicity adjusted to pH 3 with 1 N HCl. In preliminary studies, captopril infused ICV at a rate of 25 µg/hr from the 7th to 11th week of age prevented the development of hypertension in SHR. Systolic blood pressure in animals receiving captopril was 156 ± 5 mm Hg (mean ± SEM) (n = 9) compared to 182 ± 4 mm Hg in controls receiving ICV infusions of vehicle (n = 9) (p < 0.001).

Three doses of 14C-captopril (kindly supplied by Dr. Bruce Migdalof, Squibb Institute of Medical Research, New Brunswick, New Jersey) were dissolved in normal saline to final concentrations of 0.625, 2.5, and 10 mg/ml, injected into minipumps, and infused intraventricularly for 2 weeks beginning at 7 weeks of age. The infusion rate of 14C-captopril was 5, 1.25, and 0.31 µg/hr, respectively. At the end of 2 weeks, animals were sacrificed by decapitation under ether anesthesia. During sacrifice, care was taken not to contaminate blood and peripheral organs with 14C-captopril from the cannula. Blood was collected and lung, kidney, and brain removed. The plasma was separated and the organs extracted with distilled water for determination of total 14C-labeled material by liquid scintillation counting.

Protocols Assessing the Effects of ICV Captopril

Brain Angiotensin-Converting Enzyme Inhibition

In these studies the pressor effects of ICV angiotensin I and II were examined in both captopril-treated (1.25 µg/hr) and vehicle-treated rats. At 10 to 14 days after the start of captopril infusion, rats were anesthetized with pentobarbital (50 mg/kg intraperitoneally) and the cannulas that had been placed in the lateral ventricles were removed. Three doses of angiotensin I (50, 200, and 800 ng) or angiotensin II (12.5, 50, and 200 ng) were then injected into the lateral ventricle through a new cannula, and pressor responses were compared. Each injectate volume was 5 µl.

Assessment of Central and Peripheral Actions of Captopril

The effects of captopril (1.25 µg/hr) infused into the jugular vein of SHR were compared to those of the same dose of drug given ICV. Animals were treated at 7 to 11 weeks of age. Two days before sacrifice, 0.5 ml of blood was collected through a tail artery under light ether anesthesia for determination of serum angiotensin-converting enzyme (SACE) activity. Blood removed for sampling was immediately replaced with an equal volume of 0.9% saline. Animals were sacrificed rapidly by decapitation without anesthesia, and blood was collected in iced tubes containing ethylenediaminetetraacetic acid (EDTA, 1 mg/ml) for determination of plasma renin activity (PRA).

Brain Renin, Water and Sodium Intake

Two groups of rats receiving either captopril (1.25 µg/hr) or vehicle ICV, housed in individual metabolic cages, drinking distilled water and eating a basal purified diet (0.29% sodium) (Ralston Purina Company, St. Louis, Missouri) were studied for 9 days beginning 5 days after commencement of the ICV infusions. Fluid intake, urine volume, and sodium excretion were measured over this period. At the end of the 4-week study, awake animals were sacrificed by guillotine, and blood was collected into chilled heparinized tubes for determination of plasma aldosterone concentration (PAC). The brain was removed immediately and dissected into the telencephalon, diencephalon, mesencephalon, and pons medulla, frozen in liquid nitrogen, and stored at −80°C for subsequent analysis of renin and acid protease (cathepsin D) activities.
Peripheral Sympathetic Nervous System Activity

To examine the hypothesis that centrally administered captopril prevents the development of hypertension in SHR by interfering with sympathetic outflow, plasma norepinephrine and epinephrine concentrations and blood pressure response to ganglionic blockade were measured as indices of peripheral sympathetic nervous system activity. Cannulas (Micro-Line, Thermoplastic Sciences Inc., Warren, New Jersey) pre-treated with graphite to prevent coagulation were inserted into the femoral artery 3 weeks after initiating ICV captopril or vehicle treatment. The cannulas were tunneled subcutaneously to the back, externalized between the scapulae, and fixed in position. After an interval of 48 hours, tubing was connected to the cannula and at least 1 hour was allowed to pass before 0.5 ml of blood was sampled from the conscious, unrestrained resting animal. Only resting animals were sampled. All animals were sampled at the same time of the day under the same environmental conditions to avoid diurnal variation or ambient temperature influences on plasma catecholamines. Blood for catecholamines was collected in iced tubes containing EDTA (90 mg/ml) and glutathione (60 mg/ml). The amount of blood withdrawn was immediately replaced with an equal volume of 0.9% saline. After a rest period of 3 hours or more, each animal’s cannula was connected to a Statham P 50 pressure transducer (Statham Instruments, Oxnard, California) coupled to a Grass Model 50 polygraph. After a stable mean blood pressure was recorded, 30 mg/kg of hexamethonium bromide was injected intraarterially, and the maximum decrease in mean blood pressure was recorded. This dose of hexamethonium bromide has been shown to interrupt sympathetic transmission controlling the cardiovascular system in the rat.15

Brain and Plasma Arginine Vasopressin Levels

The hypothesis that centrally administered captopril prevents the development of hypertension in SHR in part by interfering with the synthesis and/or release of AVP was examined. In these studies awake animals receiving ICV captopril or vehicle were guillotined 4 weeks after the start of the infusion. The blood issuing from the trunk was collected into chilled heparinized tubes for determination of plasma AVP levels. During sacrifice, care was taken not to excite the animals and not to squeeze their thoraces, since both of these insults are known to stimulate AVP release.18 Blood was immediately centrifuged at 4°C. Osmolality was determined on 250 μl of plasma by freezing-point depression, and the remaining sample was stored at −10°C until used. The brain was removed immediately and dissected into telencephalon, diencephalon, mesencephalon, and pons medulla, frozen in liquid nitrogen, and stored at −80°C for subsequent AVP analysis.

Response to Dehydration

The ability of rats to concentrate their urine in response to alterations in water intake was examined in captopril-treated (1.25 μg/hr, ICV) and vehicle-treat-
ed animals. Such studies constitute an indirect assessment of the osmotic control of AVP release. Animals were housed individually in metabolic cages, and basal 24-hour water intake and urine volume were measured after a 2-day period of acclimatization. Then water was withheld for 48 hours while daily urine collections continued. All urines were collected under mineral oil to prevent evaporation.

Biochemical Determinations

PRA19 and PAC20 were measured by radioimmunoassay, and ACE was measured by a modification of the spectrophotometric assay of Cushman and Cheung.21 Renin activity in brain regions was measured by radioimmunoassay using a modification of the technique of Schelling et al.1 Brain regions were homogenized in 0.1 M sodium phosphate buffer, pH 6.0, and centrifuged for 30 minutes at 4°C, 10,000 rpm, in a Sorvall RC-5 centrifuge (SM-24 head) (Dupont Sorvall, Wilmington, Delaware). The pellet was discarded and the supernatant stored at −20°C for subsequent renin analysis. For determination of renin activity, the supernatant was thawed at 4°C and an aliquot (50 μl) was added to enough partially purified rat renin substrate to generate 300 ng of angiotensin I. The substrate used in these experiments was prepared by the method of Boucher et al.22 and standardized, as previously reported,23 from the plasma of rats that had undergone bilateral nephrectomy 48 hours before decapitation. Rat renin was purified from rat kidneys by the method of Haas (personal communication) as previously reported.24 Renin substrate was dissolved in 0.5 ml of 0.1 M sodium phosphate buffer, pH 6.0, containing 5 μl of 10% EDTA (2.6 mM, final concentration), 5 μl of a 50 mg/ml solution of 8-hydroxyquinoline (6.8 mM, final concentration), 1 μl of dimercaptopropanol (3.2 mM, final concentration), and 50 μl of dithiothreitol (Cleland’s reagent) (5 mM, final concentration). After a zero-time sample was removed, the mixture was incubated for 3 hours at 37°C. The reaction was stopped by freezing in a dry ice/acetone bath. Samples of the reaction mixture and the zero-time sample were then subjected to radioimmunoassay for angiotensin I.16 The zero-time value was subtracted from the generated value and the final results expressed as nanograms of angiotensin I generated per milligrams of protein per hour (ng Al/mg protein/hr). Protein concentrations of the brain extracts were determined by the method of Lowry et al.25 Aliquots of the brain extracts were adjusted to pH 3.5 and subjected to assay for acid protease (cathepsin D) activity by hydrolysis of hemoglobin using a modification of the method of Anson.26 The assay mixture consisted of 0.4 ml of a 2% bovine hemoglobin (Sigma) solution made fresh before the assay, containing 0.27 M acetic acid and 0.004 M ammonium sulfate. The hemoglobin solution was incubated with 100 μl brain extract at 37°C for 1 hour. The reaction was stopped by the addition of 0.5 ml 10% trichloroacetic acid, and the samples were centrifuged and the supernatant used for the assay. Final
results were expressed as µg BSA equivalents/µg brain protein.

Plasma norepinephrine and epinephrine concentrations were measured by a modification of the radioenzymatic assay of Peuler and Johnson.27 Plasma and tissues for AVP determinations were extracted with acetone and assayed with a highly specific antiserum (10169) which has virtually no cross reactivity with oxytocin, vasotocin, or angiotensin II. Antiserum 10169, utilized at a final dilution of 1:3,000,000 in a nonequilibrium assay, in which bound and free AVP are separated by charcoal, has a sensitivity of 0.1 pg/assay tube. All measurements were in triplicate and each tissue extract was assayed at three different dilutions to assess parallelism to the standard curve. Our AVP assay is described in detail elsewhere.28 Plasma and urine osmolality was determined using an Advanced Osmometer (Advanced Instruments, Inc., Needham Heights, Massachusetts); sodium, using an IL flame photometer (Instrumentation Laboratory, Inc., Lexington, Massachusetts).

Statistics

Results were expressed as means ± SEM. Statistical analyses of data over time were made using a split plot in time analysis of variance design. Tests for group X time interaction were performed, comparisons between groups at each time point were done, and changes across time within each group were analyzed. One-way analysis of variance was used to compare groups with Duncan’s New Multiple Range Test for comparing group means. In all cases, p values of < 0.05 were considered significant.

Table 1. Distribution of [14C]-Labeled Material Following Intracerebroventricular Infusion of [14C]-Captopril (DPM/organ or total plasma volume)

<table>
<thead>
<tr>
<th>Site</th>
<th>5 µg/hr (n = 7)</th>
<th>1.25 µg/hr (n = 8)</th>
<th>0.31 µg/hr (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>3493</td>
<td>1054</td>
<td>385</td>
</tr>
<tr>
<td>Serum</td>
<td>411</td>
<td>139</td>
<td>111</td>
</tr>
<tr>
<td>Lung</td>
<td>3330</td>
<td>1654</td>
<td>2880</td>
</tr>
<tr>
<td>Kidney</td>
<td>1129</td>
<td>398</td>
<td>379</td>
</tr>
</tbody>
</table>

Table 2. Effects of Captopril (1.25 µg/hr ICV) on Plasma Renin Activity (PRA), Serum Angiotensin-converting Enzyme Activity (SACE), and Plasma Aldosterone Concentration (PAC)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PRA (ng/ml/hr)</th>
<th>SACE (U/ml)</th>
<th>PAC (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICV Captopril (10)</td>
<td>2.2±0.2</td>
<td>78.3±2.3</td>
<td>320.3±31.3</td>
</tr>
<tr>
<td>IV Captopril (9)</td>
<td>1.9±0.2</td>
<td>87.1±2.0*</td>
<td>52.6±1.8t</td>
</tr>
<tr>
<td>IV Captopril (9)</td>
<td>1.8±0.1</td>
<td>52.6±1.8*</td>
<td>53.7±2.3§</td>
</tr>
<tr>
<td>Sham (11)</td>
<td>1.6±0.1*</td>
<td>53.7±2.3§</td>
<td>53.7±2.3§</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Number of animals is indicated in parentheses.

*p < 0.02.
†p < 0.01.
‡p < 0.001.
§p < 0.0001.

Results

Dose and Distribution of Intracerebroventricular Captopril

Figure 1 summarizes systolic blood pressures in SHR receiving ICV [14C]-captopril at doses of 5, 1.25, or 0.31 µg/hr, or vehicle for 2 weeks. Pretreatment systolic pressures were similar in all groups. Table 1 demonstrates the distribution of [14C] label expressed as total disintegrations per minute (DPM) less background (40 DPM). These data suggest that there was leakage of captopril from the brain into the periphery even at doses that did not alter the course of the hypertension. Since the 1.25 and 5 µg/hr dose schedules had similar effects on blood pressure (fig. 1), the former regimen was used in all subsequent studies.

Brain Angiotensin-Converting Enzyme Inhibition

Intracerebroventricular injection of angiotensin I (50, 200, and 800 ng) and angiotensin II (12.5, 50, and 200 ng) induced dose-dependent increases in blood pressure of SHR (11.8 ± 4.0, 16.8 ± 5.3, and 24.3 ± 5.7 mm Hg after angiotensin I, and 10.8 ± 4.1, 17.1 ± 6.6, and 22.5 ± 4.2 mm Hg after angiotensin II). The pressor effects of ICV angiotensin I were decreased in ICV captopril-treated rats by about 50% (p < 0.01), but the pressor effects of angiotensin II were not altered by captopril treatment (fig. 2). These results indicate that the conversion of angiotensin I to angiotensin II in the brain of SHR is partially blocked by the injection of this dose of captopril.
Assessment of Central and Peripheral Actions of Captopril

Captopril infused centrally at a dose of 1.25 μg/hr significantly attenuated the development of hypertension in SHR, whereas the same dose of the converting enzyme inhibitor infused intravenously did not alter blood pressure (fig. 3). At sacrifice, mean systolic blood pressure of the ICV captopril group (n = 10) was 157 ± 3.3 mm Hg compared with 181 ± 3.9 mm Hg for the ICV vehicle group (n = 9) (p < 0.001). Pressures of the IV captopril group (n = 10) and the sham-operated group (n = 9) (not shown) were 180 ± 4.5 and 183 ± 4.5 mm Hg, respectively. Serum angiotensin-converting enzyme activity, PRA, and PAC are summarized in table 2. Serum angiotensin-converting enzyme activity in both ICV and IV captopril groups was significantly greater than in the ICV vehicle or sham-operated animals. There were no significant differences in PRA among the three treatment groups, but values were lower in the sham-operated rats compared to the ICV captopril animals (p < 0.02). Finally, PAC in the ICV captopril and ICV vehicle groups was similar. These data indicate that ICV infusion of captopril attenuates the development of hypertension in SHR via a central effect. The attenuation of the blood pressure rise appears unrelated to inhibition of the peripheral renin-angiotensin-aldosterone system.
Brain Renin, Fluid and Sodium Intake

Central administration of captopril had no significant effect on fluid intake, urine volume, or urinary sodium excretion (fig. 4). Weight gain was also similar in ICV captopril and ICV vehicle-treated animals. At sacrifice, captopril-treated rats weighed 205 ± 3.6 g compared to 211 ± 4.7 g in animals infused with vehicle (NS). As shown in table 3, ICV captopril treatment did not alter the activities of renin or cathepsin D in the diencephalon, mesencephalon, or pons medulla. Renin activity but not cathepsin D activity was significantly (p < 0.02) increased in the telencephalon of animals treated with ICV captopril compared to ICV vehicle controls.

Peripheral Sympathetic Nervous System Activity

In the ICV captopril group (n = 11), plasma norepinephrine was 179.1 ± 12.6 pg/ml and epinephrine was 76.3 ± 12.6 pg/ml, not significantly different from those in the ICV vehicle group (norepinephrine, 177.0 ± 14.0 pg/ml, and epinephrine, 80.8 ± 21.4 pg/ml, n = 9) (fig. 5). Basal mean blood pressure of ICV captopril rats was significantly (p < 0.01) lower than that of ICV vehicle rats (131 ± 4.3 mm Hg, n = 10, vs 154 ± 6.6 mm Hg, n = 8). However, the depressor response to hexamethonium bromide in the ICV captopril group (43.4% ± 2.0%) was similar to that in the ICV vehicle group (42.4% ± 1.3%), as expressed by percent decrease in mean blood pressure (fig. 6). These results suggest that the depressor effect of centrally administered captopril is not due to decreased sympathetic nervous system activity.

Brain and Plasma Arginine Vasopressin Levels

Table 4 summarizes AVP levels in plasma and brain regions of animals receiving ICV or IV captopril and vehicle controls. Plasma osmolality and AVP levels were slightly but significantly lower in vehicle-treated rats compared with both groups receiving captopril. There were no significant differences among groups in AVP content of any of the brain regions examined.

Response to Dehydration

When fluid was withheld for 2 days, urine osmolality rose from a basal value of 1559 ± 103.1 to 2619 ± 99.0 mOsm/kg at 24 hours and to 3079 ± 103.1 mOsm/kg at 48 hours in the ICV captopril group (n = 9) compared to increases from 1569 ± 143.7 to 2156 ± 134.3 mOsm/kg at 24 hours and 2704 ± 146.3 mOsm/kg at 48 hours in the ICV vehicle group (n = 9) (the comparative value at 24 hours was p < 0.02 and at 0 hour and 48 hours was not significant) (table 5). Urine volumes at 24 and 48 hours were significantly lower in the ICV captopril group compared with the ICV vehicle group (4.1 ± .2 vs 5.5 ± .6 ml/day at 24 hours, p < 0.05 and 3.1 ± .07 vs 4.1 ± .4 ml/day at 48 hours, p < 0.02). These findings indicate that AVP release in the face of dehydration is unimpaired and may even be slightly enhanced in SHR treated with ICV captopril.
TABLE 3. Renin and Cathepsin D Activities of the Telencephalon, Diencephalon, Mesencephalon, and Pons Medulla in Intracerebroventricular (ICV) Captopril and ICV Vehicle-Treated Rats

<table>
<thead>
<tr>
<th>Activity</th>
<th>Telencephalon</th>
<th>Diencephalon</th>
<th>Mesencephalon</th>
<th>Pons-Medulla</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin activity (ng/ml/hr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Captopril (10)</td>
<td>17.0 ± 1.1</td>
<td>18.9 ± 0.9</td>
<td>8.0 ± 0.8</td>
<td>14.1 ± 0.9</td>
</tr>
<tr>
<td>Vehicle (11)</td>
<td>13.0 ± 1.0*</td>
<td>16.9 ± 1.5</td>
<td>7.0 ± 0.6</td>
<td>13.1 ± 1.0</td>
</tr>
<tr>
<td>Cathepsin D activity (mg BSA equivalents/mg brain protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Captopril (10)</td>
<td>0.36 ± 0.02</td>
<td>0.56 ± 0.04</td>
<td>0.38 ± 0.02</td>
<td>0.39 ± 0.02</td>
</tr>
<tr>
<td>Vehicle (11)</td>
<td>0.35 ± 0.01</td>
<td>0.56 ± 0.04</td>
<td>0.35 ± 0.02</td>
<td>0.36 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Number of animals is indicated in parenthesis.

*P < 0.02.

TABLE 4. Plasma Adenosine Vasopressin (AVP) Concentration and AVP Contents of the Telencephalon, Diencephalon, Mesencephalon, and Pons Medulla in ICV Captopril, IV Captopril, ICV Vehicle, and Sham-Treated Rats

<table>
<thead>
<tr>
<th></th>
<th>Plasma AVP concentration (pg/ml)</th>
<th>Plasma osmolality (mOsm/kg)</th>
<th>Telencephalon (ng/mg protein)</th>
<th>Diencephalon (ng/mg protein)</th>
<th>Mesencephalon (ng/mg protein)</th>
<th>Pons medulla (ng/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICV Captopril (12)</td>
<td>3.09 ± 0.89</td>
<td>296.3 ± 0.84</td>
<td>0.08 ± 0.02</td>
<td>52.0 ± 4.49</td>
<td>7.3 ± 0.95</td>
<td>22.9 ± 1.39</td>
</tr>
<tr>
<td>IV Captopril (10)</td>
<td>2.70 ± 0.28</td>
<td>295.7 ± 1.08</td>
<td>0.10 ± 0.01</td>
<td>43.0 ± 3.07</td>
<td>8.1 ± 0.81</td>
<td>21.0 ± 2.00</td>
</tr>
<tr>
<td>ICV Vehicle (11)</td>
<td>2.13 ± 0.15*</td>
<td>292.6 ± 0.85</td>
<td>0.09 ± 0.01</td>
<td>46.3 ± 2.90</td>
<td>9.4 ± 0.51</td>
<td>19.6 ± 1.27</td>
</tr>
<tr>
<td>Sham (9)</td>
<td>2.69 ± 0.42</td>
<td>296.3 ± 1.01</td>
<td>0.08 ± 0.02</td>
<td>48.5 ± 5.92</td>
<td>6.6 ± 0.70</td>
<td>23.8 ± 2.00</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Number of animals is indicated in parentheses. The p values refer to comparisons with the ICV captopril group.

*P < 0.05.
†P < 0.01.

TABLE 5. Changes in Urine Volume, Urine Osmolality, and Urine Sodium Excretion in ICV Vehicle Rats During 48 Hours of Water Deprivation

<table>
<thead>
<tr>
<th></th>
<th>Hours deprived</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Water intake (ml/day)</td>
<td></td>
</tr>
<tr>
<td>Vehicle (n = 9)</td>
<td>13 ± 1.4</td>
</tr>
<tr>
<td>Captopril (n = 9)</td>
<td>13 ± 0.8</td>
</tr>
<tr>
<td>Urine volume (ml/day)</td>
<td></td>
</tr>
<tr>
<td>Vehicle (n = 9)</td>
<td>7.3 ± 1.0</td>
</tr>
<tr>
<td>Captopril (n = 9)</td>
<td>6.6 ± 0.5</td>
</tr>
<tr>
<td>Urine osmolality (mOsm/kg)</td>
<td></td>
</tr>
<tr>
<td>Vehicle (n = 9)</td>
<td>1559 ± 143.7</td>
</tr>
<tr>
<td>Captopril (n = 9)</td>
<td>1559 ± 103.1</td>
</tr>
<tr>
<td>Urine sodium excretion (mEq/day)</td>
<td></td>
</tr>
<tr>
<td>Vehicle (n = 9)</td>
<td>0.972 ± 0.044</td>
</tr>
<tr>
<td>Captopril (n = 9)</td>
<td>0.872 ± 0.058</td>
</tr>
</tbody>
</table>

Values are means ± SEM.
*P < 0.05.
†P < 0.02.
‡P < 0.01.

Discussion

Intracerebroventricular administration of captopril decreases blood pressure in adult SHR with established hypertension.5-8 Our study extends such observations by demonstrating that chronic ICV captopril attenuates the development of hypertension in young SHR by a central mechanism. In addition, we tested a number of hypotheses concerning the development and maintenance of hypertension in this strain. Our results suggest that the action of ICV captopril could be due to the ability of the drug to inhibit conversion of angiotensin I to II within the central nervous system but is not related to alterations in fluid or sodium balance, brain or plas-
ma renin activity, or AVP content or plasma aldosterone levels.

When $^{14}$C captopril was infused ICV, label was detected in the peripheral circulation even when doses too low to affect blood pressure were administered. These data suggest that centrally administered captopril might leak out of the brain and exert its hypotensive effect, at least in part, via a peripheral action. However, the findings from our study and that of others that captopril lowers blood pressure of SHR to a greater extent following ICV than peripheral administration and that peripheral administration of saralasin does not alter the blood pressure of SHR suggest that the depressor effects of these substances following ICV administration are mainly dependent on their central action.

There is increasing evidence that the brain isorenin-angiotensin system is more active in SHR than in normotensive WKY. Ganten et al. found elevated levels of angiotensin-like material in the cerebrospinal fluid of hypertensive rats. Stamler et al. reported that specific binding of $^{125}$I-angiotensin II in the area of forebrain that mediates the pressor response to ICV angiotensin II is higher in SHR than in WKY. Further, Schelling et al. found that the renin content of catecholaminergic nuclei of the brain stem and neurohypophysis is elevated during the development of hypertension in stroke-prone SHR compared to normotensive WKY. More recently, Weyhenmeyer and Philips demonstrated over twice as many cells and fibers that had angiotensin II-like immunoreactivity in the brains of SHR as in control WKY brains. It is known that the pressor effects of ICV angiotensin II are greater in SHR than in normotensive WKY, and, as noted above, central administration of both converting enzyme inhibitors and angiotensin II antagonists lowers blood pressure in the adult SHR but is without effect in normotensive WKY. In our own experiments, captopril administered ICV at a rate of 1.25 µg/hr attenuated the pressor effects of ICV angiotensin I by 50% but did not alter the pressor response to central infusion of angiotensin II. Such observations are consistent with the hypothesis that the brain isorenin-angiotensin system plays an important role in the development and maintenance of hypertension in SHR and with the interpretation that centrally administered captopril can prevent the rise in blood pressure in young SHR by inhibiting the formation of angiotensin II in the brain.

In the current study, renin activity was increased in the telencephalon but not the diencephalon, mesencephalon, orpons medulla of animals treated with ICV captopril compared to ICV vehicle controls. We interpret the increase in tissue renin activity as evidence of converting enzyme blockade and inhibition of AII generation with consequent stimulation of renin release and/or formation by interruption of the negative feedback loop. The absence of concomitant alterations in cathepsin D activity provides evidence that the increases in enzyme activity that were observed are specific to renin and not related to stimulation of acid protease activity in brain. Our results contrast with the data of Unger et al., who found significant increases in renin activity in the neurohypophysis, hypothalamus, and medulla oblongata of SHR treated with oral captopril for 6 months beginning at weaning. These animals were the offspring of female rats that had been maintained on oral captopril since 2 weeks before mating. In contrast, we administered captopril in much smaller doses and by a restricted route of distribution for only 3 weeks in rats beginning at 7 weeks of age, a time when blood pressure was already beginning to rise. The difference between the two studies in the extent of converting enzyme blockade and secondary release of inhibition of renin formation may relate to the dose, route, and duration of the administration of captopril.

The possibility that ICV captopril could prevent the development of hypertension in young SHR by inhibiting sympathetic outflow from the brain is suggested by the observation that pressor effects of centrally administered angiotensin II are partially dependent on the stimulation of the sympathetic nervous system and that the sympathetic nervous system plays an important role in the development and maintenance of hypertension in SHR. The indices of peripheral sympathetic nervous system activity used in this study were plasma norepinephrine levels and the mean arterial pressure response to ganglionic blockade with hexamethonium bromide. Plasma norepinephrine in the rat is principally derived from noradrenergic nerve endings and appears to correlate well with other indices of sympathetic function. The observations that central captopril treatment did not change resting plasma norepinephrine concentration or the depressor effect of hexamethonium bromide suggest that the depressor effect of centrally administered captopril in SHR is not dependent on decreased sympathetic nervous system activity. Our data do not completely rule out any contribution of the sympathetic nervous system to the antihypertensive effect of centrally administered captopril, however, since the indices of sympathetic activity that were employed are imperfect ones and the rats were studied in the resting state only. Studies employing more sensitive indices of sympathetic outflow following chemical or electrical stimulation of the brain are needed before this issue can be fully resolved.

Several lines of evidence suggest that the pressor effect of the brain isorenin-angiotensin system is mediated, at least in part, by AVP release. The pressor effect of centrally administered angiotensin II is associated with an increase in plasma AVP and can be partially attenuated by pretreatment with an AVP antagonist. Further, Crofton et al. have reported that AVP excretion is elevated in SHR and may be partially responsible for the elevated blood pressure in that strain. Interestingly, the same group has demonstrated decreased AVP excretion and attenuated hypertension in young SHR during oral administration of captopril. Accordingly, they hypothesized that one of the depressor mechanisms of orally administered captopril may be direct central inhibition of vasopressin release.
Contrary to this hypothesis, Crofton et al. 41 have reported that acute ICV administration of captopril in high doses increased blood pressure and AVP secretion in SHR. In our study, plasma AVP levels in ICV captopril-treated SHR were slightly (+0.86 pg/ml) elevated compared to ICV vehicle-treated SHR. This slight difference seemed related to plasma osmolality and was certainly inadequate to account for the major differences in blood pressure between the two experimental groups. The observations that regional brain AVP concentrations were not altered in ICV captopril-treated animals and that these animals were able to concentrate their urine when dehydrated suggest that AVP synthesis and release were not impaired. Taken together, these findings suggest that the depressor effects of centrally administered captopril in SHR are not due to inhibition of AVP synthesis or release. Our data do not completely rule out any contribution of AVP to the antihypertensive effect of centrally administered captopril, however, since the concentrations and turnover of AVP in specific brain nuclei known to be involved in blood pressure control were not assessed and the animals were not studied in the stressed state. Further studies are needed before this issue can be fully resolved.

In this study, we were unable to identify the biochemical mechanism(s) by which centrally administered captopril prevents hypertension in young SHR. Experiments recently performed in our laboratory indicate that central treatment with captopril in young SHR decreases vascular reactivity to vasoconstrictors and increases the sensitivity of the baroreflex. 42 Further studies are necessary to fully elucidate the importance of these mechanisms in the prevention of genetic hypertension by centrally administered captopril.

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