Effect of Captopril on Converting Enzyme Activity in Chemically Sympathectomized, Spontaneously Hypertensive Rats

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Accepted June 24, 1985

Abstract—Effect of subacute angiotensin converting enzyme (ACE) blockade on the converting enzyme activity (ACE activity) in plasma, aorta, lung, kidney and whole brain were evaluated in chemically-sympathectomized (with 6-hydroxydopamine) normotensive Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) using captopril given peripherally via the intraperitoneal (i.p) route and centrally through intracerebroventricular (i.c.v.) administration. Daily i.p. injection of 25 mg/kg for 8 days reduced the blood pressure of both WKY rats and SHR, and the ACE activity in the aorta, lung and plasma of both WKY rats and SHR were correspondingly depressed. The brain ACE activity remained unaltered in both strains of rats. The ACE activity in the kidney of WKY was depressed, while that of SHR remained unchanged. These observations are independent of peripheral sympathectomy with 6-hydroxydopamine (6-OHDA). Daily central captopril administration at a dose of 2 mg/kg, i.c.v., for 8 days significantly reduced the blood pressure of SHR but not WKY rats, whereas the ACE activity of the whole brain of both WKY and SHR were depressed. Central sympathectomy with 6-OHDA did not alter these responses. It is concluded that captopril exerts its antihypertensive effect not only via reduction of the ACE activity in the plasma and lungs as reported earlier, but also that of other organs, principally the aorta, and that these effects are independent of the sympathetic nervous system.

Angiotensin converting enzyme (ACE) was first isolated and purified from plasma in 1956 by Skeggs et al. (1). Subsequent studies confirmed the presence of this enzyme in a variety of tissues and organs in the body (2). Relatively high concentration of ACE is found in the lung (3). The presence of ACE has also been reported in the brain (4) and may constitute a component of a separate brain renin-angiotensin system.

ACE, the key enzyme in the renin-angiotensin system, is known to play an important role in the regulation of blood pressure and in the pathogenesis of hypertension. This is because of its ability to convert the inactive decapeptide angiotensin I to the potent vasopressor octapeptide angiotensin II, principally in the pulmonary circulation (5) and its ability to inactivate bradykinin, the hypotensive nonapeptide product of kallikrein (6). Specific inhibition of ACE by orally active captopril results in marked reduction in blood pressure (7) and captopril has proven to be an efficacious antihypertensive agent. Blockade of conversion of angiotensin I to angiotensin II cannot account for its antihypertensive efficacy (8); yet an antihypertensive mechanism for captopril unrelated to ACE activity has not been directly demonstrated (9). The present study is aimed at examining the effect of captopril on ACE activity in the aorta, lung, plasma, kidney and whole brain of chemically sympathectomized normotensive Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR). This is deemed important in view of the close relationship that exists between the renin-angiotensin system and the sympathetic nervous system in the regulation of normal blood pressure and in hypertensive
conditions (10, 11). Experiments were therefore conducted in central sympathectomized and peripheral sympathectomized WKY and SHR.

Materials and Methods

Male WKY and SHR weighing 200–300 g were used in the present investigation. Rats that were to receive peripheral administration of drugs were housed in groups of six in a cage and rats that were to receive central administration of drugs were housed individually. All rats were allowed free access to commercial food pellets and drank tap water ad libitum. Experimental sessions were conducted in the same room as the home cages were located, and the room was relatively quiet. All experiments were conducted at room temperature, and no attempts were made to control the humidity of the room.

Central administration: In central administration, injections were made via the i.c.v. route adapted from Hayden et al. (12). General anesthesia was achieved by intraperitoneal injection of sodium pentobarbitone (Nembutal, Abbot Laboratories), 40 mg/kg, and then aseptic surgery was performed. Implantation of cannula for i.c.v. injection was performed using a micro-manipulator in the stereotaxic instrument (Stoelting Co., U.S.A.). For this purpose, a 20 G needle cannula guide was fitted into the base of the plexiglass block that had a cavity filled with sealant. Dental cement was used to hold the needle in position. After surgery, the rats were given a recovery period of 7 days. All drugs used for central administration were dissolved in sterile saline. Central i.c.v. administration of captopril was made daily at a dose of 2 mg/kg for 8 days, whereas central sympathectomy was achieved by administration of 6-OHDA via the tail vein at a dose of 50 mg/kg on day 1, 100 mg/kg on day 2 and 500 mg/kg each on days 5 and 8 (13).

Blood pressure determination: Blood pressure was measured by tail plethysmography, connected to a physiograph in prewarmed (40°C for 15 min) rats, as reported by Krakoff et al. (14). Conscious systolic blood pressure was measured before and after treatment.

Experimental protocol: Experiments involved both central and peripheral treatment, and for these purposes, rats were divided into 4 groups:

- **Group 1**, serving as controls
- **Group 2**, captopril treated
- **Group 3**, 6-OHDA treated
- **Group 4**, both captopril and 6-OHDA treated

All animals were sacrificed after day 8, 24 hr after the last treatment by drug. For centrally treated rats, the whole brain was removed and angiotensin converting enzyme activity determined. For peripherally treated rats, the thoracic region was quickly opened to expose the heart directly after sacrificing the animal. Blood was obtained from the heart by a direct heart puncture using a 1 ml syringe fitted to size 26 needle which had been previously moistened with heparinized saline. The blood was then centrifuged to obtain the plasma. The aorta, lung, kidney and whole brain were immediately removed for the determination of converting enzyme activity.

Determination of converting enzyme activity: The converting enzyme activity was determined according to the method of Cushman and Cheung (15). One unit of ACE activity was defined as the amount that catalyzes the formation of 1 μmole of hippuric acid from hippuryl-L-histidyl-L-leucine (HHL) in 1 min at 37°C and atmospheric pressure. ACE activity was expressed as mU/mg protein. Total protein measurement was made using a Biorad Kit, a-gent (Abbott Laboratories).

Statistics: Student’s t-test was used to determine significant differences between the blood pressure before and after treat-
ment. In comparing the effects of captopril on the ACE activity in the various organs/tissues and plasma of the SHR and the WKY rats, Student’s t-test was also used to determine the significance between control and treated animals. All values are expressed as means ± S.E.M. An asterisk indicates values that are significantly different from the control group: *P<0.05, **P<0.01, ***P<0.001.

Results
Captopril, 6-OHDA, and combination of captopril and 6-OHDA given via the i.p. route significantly reduced the blood pressure of SHR and WKY rats as shown in Table 1. When similar experiments were repeated with central administration via the i.c.v. route, only the blood pressure of SHR was significantly reduced (Table 2). No significant changes in blood pressure were detected in WKY rats.

Figure 1 shows the ACE activity in the various tissues of WKY and SHR. It can be seen that there was no difference in the ACE activity both in the whole brain and in the aorta of the two strains of rat. However, in the plasma and in the kidney of SHR, the ACE

Table 1. Effect of captopril, 6-OHDA and both captopril and 6-OHDA on systolic blood pressure of SHR and WKY rats

<table>
<thead>
<tr>
<th></th>
<th>SHR</th>
<th>WKY rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Blood pressure (mmHg)</strong></td>
<td><strong>Blood pressure (mmHg)</strong></td>
</tr>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>Saline control</td>
<td>221±31 (14)</td>
<td>216±5 (14)</td>
</tr>
<tr>
<td>Captopril</td>
<td>230±30 (14)</td>
<td>191±25 (14)***</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>217±18 (15)</td>
<td>189±13 (15)***</td>
</tr>
<tr>
<td>Captopril+6-OHDA</td>
<td>243±27 (15)</td>
<td>179±36 (15)***</td>
</tr>
</tbody>
</table>

All injections were made via the i.p. route. Values are means ± S.E.M. for the number of animals indicated in parentheses, *P<0.05, **P<0.01, ***P<0.001.

Table 2. Effect of captopril, 6-OHDA and both captopril and 6-OHDA on systolic blood pressure of SHR and WKY rats

<table>
<thead>
<tr>
<th></th>
<th>SHR</th>
<th>WKY rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Blood pressure (mmHg)</strong></td>
<td><strong>Blood pressure (mmHg)</strong></td>
</tr>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>Saline control</td>
<td>187±17</td>
<td>182±5</td>
</tr>
<tr>
<td>Captopril</td>
<td>178±18</td>
<td>135±8**</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>165±8**</td>
<td>136±4***</td>
</tr>
<tr>
<td>Captopril+6-OHDA</td>
<td>185±37</td>
<td>127±1***</td>
</tr>
</tbody>
</table>

All injections were made via the i.c.v. route. Values are means ± S.E.M. n=6 in each case. **P<0.01, ***P<0.001.
activities were significantly lower, whereas in the lung, the ACE activity of SHR is higher in comparison with WKY rats.

Table 3. Effect of captopril, 6-OHDA and both captopril and 6-OHDA treatment on angiotensin converting enzyme activity in various tissues of SHR

<table>
<thead>
<tr>
<th></th>
<th>Brain</th>
<th>Plasma</th>
<th>Aorta</th>
<th>Lung</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline control</td>
<td>2.5±0.3(6)</td>
<td>0.7±0.1(6)</td>
<td>1.9±0.3(6)</td>
<td>18.5±0.9(6)</td>
<td>0.3±0.1(6)</td>
</tr>
<tr>
<td>Captopril</td>
<td>2.7±0.5(6)</td>
<td>0.2±0.1(6)**</td>
<td>0.4±0.1(6)**</td>
<td>1.1±0.1(6)**</td>
<td>0.3±0.1(6)</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>2.5±0.2(7)</td>
<td>0.7±0.3(7)</td>
<td>1.8±0.1(6)</td>
<td>17.9±0.3(7)</td>
<td>0.3±0.1(7)</td>
</tr>
<tr>
<td>Captopril+6-OHDA</td>
<td>3.8±0.5(7)</td>
<td>0.2±0.1(7)**</td>
<td>0.3±0.1(7)**</td>
<td>1.1±0.1(7)**</td>
<td>0.3±0.1(7)</td>
</tr>
</tbody>
</table>

Values are means±S.E.M. for the number of animals indicated in parentheses. **P<0.01 ***P<0.001.

Table 4. Effect of captopril, 6-OHDA and both captopril and 6-OHDA treatment on angiotensin converting enzyme activity in various tissues of the WKY rat

<table>
<thead>
<tr>
<th></th>
<th>Brain</th>
<th>Plasma</th>
<th>Aorta</th>
<th>Lung</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline control</td>
<td>1.6±0.5(6)</td>
<td>1.7±0.1(6)</td>
<td>1.4±0.1(6)</td>
<td>13.6±0.1(6)</td>
<td>1.2±0.1(6)</td>
</tr>
<tr>
<td>Captopril</td>
<td>1.7±0.4(6)</td>
<td>0.1±0.1(6)**</td>
<td>0.1±0.1(6)**</td>
<td>0.4±0.1(6)**</td>
<td>0.1±0.1(6)**</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>1.3±0.2(7)</td>
<td>1.6±0.1(7)</td>
<td>1.4±0.1(7)</td>
<td>13.2±0.2(7)</td>
<td>1.1±0.2(7)</td>
</tr>
<tr>
<td>Captopril+6-OHDA</td>
<td>1.7±0.1(7)</td>
<td>0.3±0.2(7)**</td>
<td>0.1±0.1(7)**</td>
<td>0.5±0.2(7)**</td>
<td>0.1±0.1(7)**</td>
</tr>
</tbody>
</table>

Values are means±S.E.M. for the number of animals indicated in parentheses. ***P<0.001.

Subacute intraperitoneal administration of captopril at 25 mg/kg daily for 8 days significantly reduced the ACE activity in the plasma, the aorta and the lungs of both the SHR (Table 3) and WKY rats (Table 4). Similarly, subacute intraperitoneal administration of captopril reduced the ACE activity in the kidney of WKY rats. However this effect is not seen in SHR where the ACE activity is unaffected by captopril. All these observations were independent of peripheral sympathectomy achieved through the administration of 6-OHDA via the tail vein.

Results involving central captopril treatment are shown in Fig. 2. Intracerebroventricular administration of captopril, 2 mg/kg daily for 8 days, significantly depressed the ACE activity measured in the whole brain of both SHR and WKY rats. In neither of these strains did central sympathectomy by 6-OHDA exert any effect on the ACE activity.

Discussion

The renin-angiotensin system in the SHR is generally known to be in a depressed state as can be seen from the lowered ACE activity in the plasma and the kidney of SHR. This is in spite of the higher ACE activity in the lung of SHR. These findings concur with
earlier observations (16). The present findings infer that there may be a functional renin-angiotensin system in these tissues that plays an important role in the regulation of normal blood pressure and in the hypertensive condition both in WKY and SHR.

The lung appeared to be the predominant site where ACE activity is found in the body and is certainly the main organ that is responsible for the conversion of inactive angiotensin I to active angiotensin II (5, 17). This is generally viewed to be the main mechanism through which blood pressure reduction is achieved following peripheral captopril administration in both the WKY and SHR. However the drastic reduction in the ACE activity both in the aorta of WKY and SHR following captopril treatment suggests that the blood pressure lowering effect of captopril could also be the consequence of this action. Evidence is available to suggest that the local renin-angiotensin system within the arterial walls may play a role in the maintenance of blood pressure (18, 19). Inhibition of angiotensin II production within the aortic wall and peripheral arteries will reduce the degree of vasoconstriction and thus peripheral resistance (20). Further evidence that the vascular tissue may be the target for angiotensin converting enzyme inhibitor is provided by Antonaccio et al. (21). They showed that the antihypertensive response after captopril treatment was associated with an increase in arterial wall renin which may indicate inhibition of angiotensin converting enzyme. Of particular interest is the present observation that this effect is independent of the sympathetic nervous system. It infers that the functional role of the renin-angiotensin system is operative even in the absence of the sympathetic nervous system and that the sympathetic nervous system is in no way affecting the converting enzyme inhibitory effect of captopril. This applies to both the lung and the aorta for both strain of rats.

It has long been established that the kidney as an organ by itself can generate angiotensin II through the local availability of renin, renin substrate and converting enzyme without extrarenal contribution (22, 23). An intrarenal role of the renin-angiotensin system had also been implicated (24, 25). The characteristically low ACE activity in the kidney of SHR as compared to that of WKY rats suggests a differentiating role of the renin-angiotensin system in the two strains of rat. The fact that the ACE activity is selectively depressed in the WKY rats further strengthened this claim and may suggest the existence of a mechanism for exerting control over the amount of ACE activity present in the SHR that differs from that of WKY particularly consequent to subacute captopril administration. How this difference arises is not known with certainty. It is of interest to note that no prerequisite of a functioning peripheral sympathetic nervous system is essential for the ACE activity in spite of the close relationship of angiotensin II with noradrenaline and the sympathetic nervous system, especially in relation to blood pressure regulation (10, 11).

Central subacute captopril administration reduced the ACE activity in the whole brain of WKY and SHR. Peripheral subacute administration of captopril exerts no influence on the ACE activity of the whole brain of both WKY and SHR, indicating that the peripherally administered captopril is not able to cross the blood-brain barrier. Alternatively, it may be that the amount of captopril reaching the brain following peripheral administration is negligible for affecting the brain ACE activity. The ability of peripheral captopril to cross the blood-brain barrier is a subject of much controversy. However, it is possible that the differences in the blood-brain barrier among the different species and strains of rats coupled with the relative instability of tissue captopril may account for the conflicting results on the ability of peripherally administered captopril entering the brain (8, 26). Whatever it is, the central reduction of ACE activity in the brain brought about by i.c.v. captopril is independent of the intact central sympathetic nervous system.

In summary, the present data suggest that captopril exerts its inhibition of ACE activity at least at two principle sites, the lung (also reflected through the plasma) and the aorta that may contribute towards its efficacious antihypertensive effect. Captopril is also able to inhibit the ACE activity in the brain.
However, the role of the brain renin-angiotensin system in the regulation of blood pressure remains to be elucidated as central inhibition of ACE activity is not a requirement for acute blood pressure regulation by captopril. This is in agreement with observations made by Cohen and Kurz (27). It is concluded that the inhibitory effect of captopril on ACE activity is independent of the sympathetic nervous system.

Acknowledgements: The authors are grateful to Professor K. Okamoto, Kinki University School of Medicine, Osaka, Japan for the generous gift of WKY and SHR. We would like to thank Squibb and Sons, Princeton, New Jersey, U.S.A. for the donation of captopril, and we thank Mrs. Alice Yap for typing the manuscript.

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