Acute Effects of Captopril on the Baroreflex of Normotensive and Spontaneously Hypertensive Rats

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SUMMARY

When captopril was injected intravenously in urethane anesthetized rats, a hypotensive effect accompanied by bradycardia was obtained, while an intravenous (i.v.) injection of prostaglandin I₂ (PGI₂), which induced hypotension of the same magnitude as the hypotensive effect obtained with captopril, caused a marked tachycardia. Simultaneously, sympathetic nerve activity recorded from abdominal sympathetic nerves was unchanged following injection of captopril, while it was significantly increased during hypotension induced by PGI₂. The bradycardia, but not the hypotensive effects induced by captopril was abolished by i.v. pretreatment with atropine. Intracisternal injection of a small dose of captopril inhibited reflex tachycardia during hypotension induced by PGI₂ and prolonged the hypotensive effect, while intravenous administration of this dose did not inhibit the reflex tachycardia induced by PGI₂. In spontaneously hypertensive rats (SHR), the hypotensive effect of captopril was increased partly, however, the accompanying bradycardia was significantly reduced. These findings suggest that captopril inhibits the baroreflex and centrally activates the cardiac vagal nerve. Moreover in SHR, the effect of captopril on cardiac vagal activity was disturbed.

Additional Indexing Words:
Captopril  Baroreflex  Blood pressure  Heart rate  Sympathetic nerve activity  SHR

Since the angiotensin converting enzyme inhibitor (CEI), teprotide, was reported to reduce blood pressure in hypertensives, CEIs have been popular antihypertensive drugs, and widely used in clinical and experimental
hypertension, especially since the development of captopril which is a potent and specific orally active converting enzyme inhibitor. Captopril prevents the production of circulating angiotensin II (A II) and also the inactivation of bradykinin by inhibition of angiotensin converting enzyme (ACE; Kininase II). However, recently, evidence has accumulated that the antihypertensive effect of captopril cannot be explained solely by these actions. In the complex mechanisms of action of captopril, the effect of captopril on the baroreflex mechanism might contribute in part to the reduction of blood pressure. This hypothesis is based on reports that captopril does not elicit reflex tachycardia in humans and animals, while hypotension induced by peripherally acting antihypertensive drugs usually increases heart rate via the baroreflex. The purpose of the present study was to determine whether captopril could inhibit the baroreceptor reflex system during hypotension and thus contribute to the hypotensive effect of captopril in normotensive and spontaneously hypertensive rats.

**Methods**

Normotensive Wistar and spontaneously hypertensive rats weighing 200-250 g were used in all experiments. These rats were anesthetized with urethane (1.2 g/kg, i.p.), and catheters were inserted into the right jugular vein for the injection of drugs and into the right common carotid artery for recording arterial blood pressure. Phasic blood pressure was recorded through a cannula connected to a low volume-displacement pressure transducer (MPV-290, NEC-Sanei Sokki). Heart rate was measured from the phasic pressure signals by a heart rate meter (NEC-Sanei Sokki). Sympathetic nerve activity was recorded principally as described previously. For intracisternal injection, rats were mounted on a stereotaxic apparatus. After dissecting the neck muscles, a 24 gauge needle was inserted into the cisterna magna and a microsyringe (Terumo) used for injection (10 µl). The abdominal sympathetic nerve plexus was exposed, and with the aid of a stereoscopic microscope, a bipolar stainless-steel electrode (uni-inserted tips 1 mm apart) was placed on the major bundle between the cardiac and celiac ganglia. Nerves and electrode tips were immersed in salad oil to reduce tissue drying. Spike potentials were amplified (Grass P15 AC amplifier) and monitored on a storage oscilloscope (Kikusui 5516 ST). To reduce noise during these recordings, spontaneous respiration was abolished by paralyzing the skeletal muscles with decamethonium bromide (0.2 mg/100 g, i.v.) and connecting the rats to a small animal respirator (Ealing) ventilated with room air. Analog signals were recorded and simultaneously fed into
a spike counter (PSE 332P, Biomedical System), whose output was recorded separately as a histogram on the recorder and printed out digitally. The low level control of the window discriminator was routinely set to filter background noise persisting after the nerve bundle was crushed.

PGI₂ (Ono Pharmaceutical Co.) 0.3 µg/kg, nitroprusside (Nakarai Chemical Co.) 25 µg/kg and atropine (Sankyo Pharmaceutical Co.) 75 µg/kg were injected intravenously.

Data (average±SEM) from rat groups were analyzed using the t-test for comparing means of independent samples, and differences at a 5% level (p<0.05) were considered significant.

RESULTS

Captopril reduced blood pressure and heart rate in urethane anesthetized rats

After the rats were anesthetized with urethane, basal blood pressure was 98±4 mmHg and heart rate 359±15 beats/min in normotensive outbred Wistar rats. Following i.v. injection of captopril (2 mg/kg), blood pressure was lowered and bradycardia induced in normotensive Wistar rats. This hypotension was continuous for at least 30 min. Heart rate slowed gradually after injection with the maximum effect at 10 min. Thereafter, the bradycardia was continuous (Table I). On the contrary, heart rate was always increased during hypotension (Fig. 1-A) when other hypotensive drugs (PGI₂, 0.3 µg/kg, nitroprusside, 25 µg/kg) adjusted to get the same reduction in blood pressure (as with captopril, 2 mg/kg) were injected intravenously (Fig. 1-B). To determine the role of the vagal nerve in the bradycardia induced by captopril, atropine was injected intravenously before i.v. administration of captopril. Five min after i.v. injection of atropine, blood pressure and heart rate were slightly elevated (BP: from 105±5 to 107±4 mmHg, HR: from 348±14 to 380±13 beats/min). Pretreatment with atropine abolished the bradycardia following i.v. injection of captopril without affecting the hypotensive effect of captopril (Table II).

Table I. Cardiovascular Responses to Intravenous Injection of Captopril in Urethane Anesthetized Normotensive Rats

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>−25±4</td>
<td>−29±4</td>
<td>−30±4</td>
<td>−27±4</td>
<td>−26±5</td>
<td>−25±4</td>
<td>−21±4</td>
<td>−19±4</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>−19±8</td>
<td>−32±10</td>
<td>−34±11</td>
<td>−36±12</td>
<td>−36±12</td>
<td>−56±14</td>
<td>−53±14</td>
<td>−58±8</td>
</tr>
</tbody>
</table>

All values represent average±SEM changes from baselines before injection of drug in normotensive rats. MAP=mean arterial pressure; HR=heart rate.
Fig. 1. A: Heart rate change following i.v. injections of captopril (×) at 2 mg/kg, PGI₂ (●) at 0.3 μg/kg and nitroprusside (○) at 25 μg/kg. Values are mean ± SEM for 6 anesthetized normotensive rats. Each point on the abscissa indicates 1 min. * indicates p<0.01. B: Hypotensive effect of i.v. injection of captopril (C), PGI₂ (P) and nitroprusside (N).

**Table II. Cardiovascular Responses to Intravenous Injection of Captopril in Atropine Injected Rats**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>-31±5*</td>
<td>-35±3*</td>
<td>-36±3*</td>
<td>-32±4*</td>
<td>-25±4*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>+1±7</td>
<td>-7±11</td>
<td>-1±8</td>
<td>-4±12</td>
<td>-7±13</td>
</tr>
</tbody>
</table>

All values represent average ± SEM changes from baselines before injection of captopril in normotensive rats. * indicates p<0.001, compared with basal blood pressure.

**Sympathetic nerve activity during hypotension induced by captopril**

Following intravenous injection of captopril, sympathetic nerve activity was not altered, while injection of PGI₂ elicited by increases in sympathetic nerve activity during hypotension was accompanied by tachycardia. These differences between the 2 groups were significant for 2 min after administra-

**Table III. Sympathetic Nerve Activity Following Intravenous Injection of PGI₂ and Captopril in Urethane Anesthetized Rats**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>n</th>
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<th>3</th>
<th>4</th>
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</tr>
</thead>
<tbody>
<tr>
<td>PGI₂</td>
<td>6</td>
<td>163±11</td>
<td>172±11</td>
<td>181±18</td>
<td>144±22</td>
<td>137±22</td>
<td>142±23</td>
</tr>
<tr>
<td>Cap</td>
<td>6</td>
<td>92±8*</td>
<td>101±8</td>
<td>99±7*</td>
<td>96±7</td>
<td>111±5</td>
<td>110±5</td>
</tr>
</tbody>
</table>

All values represent average ± SEM % changes from baseline before injection of drugs. * indicates significant differences (p<0.025).
Intracisternal treatment of captopril reduced reflex tachycardia induced by i.v. injections of PGI₂

To determine whether captopril acts centrally to inhibit the baroreflex, tachycardia induced by PGI₂ was recorded after intracisternal treatment with captopril. As a control, saline was injected intracisternally in other rats. Reflex tachycardia during hypotension induced by i.v. injection of PGI₂ was significantly inhibited in captopril treated rats, while tachycardia was not affected in the saline treated rats. To eliminate the possibility that captopril leaking from the brain might act peripherally to inhibit tachycardia, the same dose of captopril was administered intravenously. However, it did not reduce the reflex tachycardia induced by PGI₂ (Table IV). Moreover, to determine if this inhibition of reflex tachycardia contributes to the hypotensive effect of captopril, the hypotension induced by i.v. injection of PGI₂ was compared in 2 groups with or without intracisternal administration of captopril.

### Table IV. Tachycardia Induced by PGI₂ in IC and IV Pretreatment with a Small Dose of Captopril in Urethane Anesthetized Rats

<table>
<thead>
<tr>
<th></th>
<th>n</th>
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<th>3</th>
<th>4</th>
<th>5</th>
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</thead>
<tbody>
<tr>
<td>Veh (IC)</td>
<td>6</td>
<td>45±4</td>
<td>76±5</td>
<td>72±4</td>
<td>70±6</td>
<td>62±5</td>
<td>58±5</td>
</tr>
<tr>
<td>CP (IC)</td>
<td>6</td>
<td>35±3</td>
<td>38±6</td>
<td>43±9</td>
<td>43±10</td>
<td>40±10</td>
<td>36±8</td>
</tr>
<tr>
<td>CP (IV)</td>
<td>5</td>
<td>43±4</td>
<td>57±9</td>
<td>78±8</td>
<td>78±8</td>
<td>80±8</td>
<td>78±4</td>
</tr>
</tbody>
</table>

The values are mean±SEM (beats/min) changes from basal line obtained following intravenous injection of PGI₂. Veh=vehicle treated rats (intracisternal injection); CP (IC)=captopril treated rats (intracisternal injection); CP (IV)=captopril treated rats (intravenous injection). Veh/CP (IC) comparison difference *** p<0.001, ** p<0.025, * p<0.05. CP (IC)/CP (IV) comparison difference p<0.001, p<0.025. CP (IV)/Veh comparison difference p<0.025.

### Table V. Enhanced Hypotensive Effect of PGI₂ after Intracisternal Treatment with Captopril in Urethane Anesthetized Rats

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veh</td>
<td>6</td>
<td>-44±4</td>
<td>-41±5</td>
<td>-35±4</td>
<td>-28±4</td>
<td>-21±2</td>
<td>-19±1</td>
</tr>
<tr>
<td>Cap</td>
<td>6</td>
<td>-41±4</td>
<td>-43±3</td>
<td>-42±4</td>
<td>-40±4*</td>
<td>-40±4*</td>
<td>-36±4*</td>
</tr>
</tbody>
</table>

All values represent average±SEM changes from basal line (mmHg) before intravenous injection of PGI₂. * indicates significant difference p<0.025.
In rats treated with captopril, hypotension continued for significantly longer than it did in rats treated with saline only (Table V). These findings suggest that the inhibitory effect of captopril on the baroreflex might occur in the brain.

Cardiovascular responses to intracisternal injection of captopril (2 mg/kg) in urethane anesthetized rats

Captopril (2 mg/kg) was injected intracisternally in urethane anesthetized rats. Basal blood pressure and heart rate were not different between the saline and captopril treated rats (BP: 104 ± 9 vs 94 ± 3 mmHg, HR: 410 ± 8 vs 394 ± 10 beats/min). Blood pressure was lowered after intracisternal injection (Table VI), however, these hypotensive effects were significantly smaller than those (Table I) following intravenous injection (3 min: p<0.001, 10 min: p<0.005). Bradycardiac responses (Table VI B) were markedly larger following intracisternal injection than after i.v. injection (3 min: p<0.005, 5 min p<0.001). These results suggest that the hypotensive effect might be primarily due to non-central mechanisms, while the bradycardiac responses might depend upon central mechanisms.

Table VI. Cardiovascular Responses to Intracisternal Injections of Captopril (2mg/kg)

(A) Blood Pressure

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>3</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>6</td>
<td>0.6±1.1</td>
<td>2±2</td>
<td>4±2</td>
<td>5±2</td>
<td>6±2</td>
</tr>
<tr>
<td>Cap</td>
<td>6</td>
<td>-9±5</td>
<td>-13±4*</td>
<td>-10±1***</td>
<td>-12±3**</td>
<td>-10±3***</td>
</tr>
</tbody>
</table>

mean±SEM mmHg. * p<0.025, ** p<0.005, *** p<0.001.

(B) Heart Rate

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>3</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>6</td>
<td>0±3</td>
<td>0±5</td>
<td>-1±4</td>
<td>-8±6</td>
<td>-10±6</td>
</tr>
<tr>
<td>Cap</td>
<td>6</td>
<td>-120±21***</td>
<td>-110±19***</td>
<td>-94±10**</td>
<td>-96±16**</td>
<td>-96±21**</td>
</tr>
</tbody>
</table>

mean±SEM beats/min. Others are same as above.

Captopril did not reduce the heart rate in SHR

Hypotensive responses were also obtained in urethane anesthetized SHR when captopril was administered intravenously. The early phase of hypotensive responses were significantly augmented in SHR than those of Kyoto Wistar rat (WKY) and normotensive outbred rats (Fig. 2). The reduction in heart rate was significantly smaller in SHR than in WKY following i.v. injection of captopril. The reduction in heart rate was somewhat less in
BAROREFLEX RESPONSE TO CAPTOPRIL

Fig. 2. Depressor responses to i.v. injection of captopril (2 mg/kg) in 6 urethane anesthetized SHR (●), 5 WKY (○) and 6 NT = normotensive outbred Wistar rats (△). * indicates p<0.05.

Fig. 3. Heart rate change following i.v. injection of captopril in 6 urethane anesthetized SHR (●), 5 WKY (○) and 6 NT (△).

WKY than in the controls (Fig. 3). These findings suggest that cardiac vagal activity in Kyoto Wistar rats may be changed and it may be most obvious in SHR.

DISCUSSION

I.V. injection of captopril lowered the blood pressure in urethane anesthetized normotensive and spontaneously hypertensive rats. In normotensive rats, the hypotensive effect of captopril was accompanied by bradycardia and sympathetic nerve activity was not altered following i.v. injection of captopril. The bradycardia induced by captopril disappeared after pretreatment with
atropine. These results indicate that i.v. captopril not only inhibits
the baroreflex, but also activates the cardiac vagal nerve in normotensive rats.
However, the activation of the vagal nerve does not contribute to the hypo-
tensive effect of captopril, because atropine did not affect the depressor
responses induced by captopril. Since intracisternal injection of captopril
(2 mg/kg) elicited an enhanced bradycardiac response, the activation of the
cardiac vagal nerve might be central. The activation of the vagal component
has also been seen clinically after treatment with captopril.15) The blunted
baroreflex which was seen in the cardiovascular responses to captopril was
confirmed by the lack of an increase in sympathetic nerve activity during the
hypotension induced by captopril, while PG\textsubscript{I}\textsubscript{2} increased sympathetic nerve
activity following the hypotension. There are three possible sites where
captopril could act to inhibit the baroreflex. First, at baro-receptors located
in the aortic arch, carotid sinus and a few others, second, centrally and third
at the efferent nerve endings. Because reflex increases of efferent sympathetic
nerve activity were depressed after treatment with captopril in the present
study, and it is reported that captopril did not reduce the release of norepine-
phrine in WKY and SHR,16) the third possibility is not likely. Central
administration of captopril inhibited reflex tachycardia which was elicited
following hypotension induced by i.v. injection of PG\textsubscript{I}\textsubscript{2}. This finding suggests
that captopril inhibits the baroreflex centrally. Bradycardia is due to the
activation of the cardiac vagal nerve, since atropine treatment abolished
bradycardia. However, after atropine injection, reflex tachycardia did not
reappear. The lack of tachycardia showed the lack of an increase in cardiac
sympathetic nerve activity. In the present study, because i.v. injection of the
same dose as the intracisternal injection of captopril did not reduce the reflex
tachycardia induced by PG\textsubscript{I}\textsubscript{2}, the possibility that captopril could directly
affect baro-receptors is small. Recently, it has been reported17) that captopril
can penetrate the brain blood barrier. But even if it cannot,18) the area
postrema is not covered by the barrier near the brain stem. Therefore, capto-
pril administered i.v. could easily reach the brain stem where the baroreflex
center is located.19) In our study, we could not determine whether the
central inhibition of the baroreflex by captopril resulted from a reduction in
central angiotensin II. However, our results confirmed that this inhibition
of the baroreflex contributed in part to the hypotensive effect of captopril,
since central administration of captopril prolonged the hypotension induced
by i.v. injection of PG\textsubscript{I}\textsubscript{2}.

There have been many theories as to the hypotensive mechanisms of this
drug, including the reduction of plasma angiotensin II,3) the activation of
bradykinin,4,5) the inhibition of brain20,21) or vascular angiotensin9,22) and
the activation of the prostaglandin system. However, since no one of these by itself can explain fully the hypotensive effect of captopril, it is probable that a number of these factors act in concert to reduce the blood pressure. The inhibitory effect of captopril on the baroreflex is possibly one of multiple factors. The lack of reflex tachycardia has also been reported by others in humans and animals. This modulation of the baroreflex by captopril was reported as a potentiation of the baroreflex, since the bradycardia response during pressor responses to i.v. injection of phenylephrine was augmented in captopril treated rats. The present study did not examine the baroreflex responses during pressor responses, however, it is possible that captopril blunted the baroreflex in the lower portion of the stimulus-response curve of the baroreflex. This difference in baroreflex response following treatment with captopril was observed in essential hypertension after treatment with captopril. Present experiments were undertaken in anesthetized rats. However, the hypotensive effect of captopril is not accompanied by reflex tachycardia in awake rats as well. Therefore, the cardiovascular responses elicited by captopril are not specific to anesthetized rats.

In addition to its effect on normotensive rats, captopril also reduces the blood pressure in urethane anesthetized SHR. The hypotensive effect of captopril was significantly more enhanced in the early phase in SHR than in WKY. This enhancement of the hypotensive effect of captopril occurred only within the first minute, but the present study did not clarify the mechanism of enhancement. Heart rate responses to i.v. injection of captopril in SHR were significantly different from those of normotensive rats. The differences in cardiovascular responses to captopril between WKY and SHR might have been due to the effect of anesthesia on plasma renin activity (PRA), but this was not measured in the present study. However, this is unlikely, since the enhanced hypotensive effect in SHR was only transient in the early phase. If activated PRA in SHR induced enhanced hypotension, the hypotensive effects should have continued for as long as captopril was effective. The lack of bradycardiac responses in SHR could not have been due to the reduction of angiotensin II, because angiotensin II itself diminishes or does not alter baroreflex sensitivity. These findings suggest that the baro-receptor system in SHR is deranged. However, WKY which is the mother strain of SHR also demonstrated small bradycardia responses to captopril. This indicates that cardiac vagal activity in these strains is different from that in normotensive rats.
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