Inhibitory Effect of Cilazaprilat on Norepinephrine Release Induced by Renal Nerve Stimulation in Anesthetized Dogs

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Abstract—In pentobarbital-anesthetized dogs, the increase in the renal norepinephrine secretion rate elicited by renal nerve stimulation (1 Hz) during infusion of angiotensin I (15 ng/kg/min) was partially but significantly inhibited (by 21–37%) after dosing with cilazaprilat (0.1 mg/kg), an angiotensin converting enzyme inhibitor, accompanied by a decrease in the renal venous plasma norepinephrine concentration. These results may suggest that cilazaprilat exerts at least a part of its hypotensive effect through decreasing the facilitatory action of endogenous angiotensin II on adrenergic transmission.

It has been well-documented that exogenously administered angiotensin II (All) can facilitate sympathetic nerve function through multiple mechanisms (1-4). An attenuation of sympathetic nerve function would occur following any procedure which inhibits the formation of endogenous All.

It has been shown in dogs that exogenously administered All enhances the release of norepinephrine (NE) elicited by renal sympathetic nerve stimulation (RNS) and consequently potentiates the renal vascular vasoconstrictor response (5). However, the findings presented by Oliver et al. (6) demonstrated that there was no difference in the extent of NE release induced by RNS between two groups of dogs: one untreated and the other treated with captopril, an angiotensin converting enzyme (ACE) inhibitor. The apparently conflicting findings in the latter study can be considered to be due to the background level of endogenous All, which while contributing in a facilitatory manner to NE release, is insufficient to overcome the interanimal variation. Therefore, the present experiment was performed to determine NE release elicited by RNS with cilazaprilat (CIL, Ro 31-3113), which is an active metabolite of a newly developed ACE inhibitor, cilazapril (7), under experimental conditions where a sufficient level of All would be attained by intravenous infusion of angiotensin I (AI) in pentobarbital-anesthetized dogs.

Eight mongrel dogs of both sexes, weighing from 12.5 to 20 kg (15.4±1.0 kg), were anesthetized with intravenous pentobarbital sodium (30 mg/kg and supplemental infusion at 5 mg/kg/hr). The surgical procedures for preparation were similar to those previously described elsewhere (8). Briefly, the left kidney was exposed, and a platinum electrode was mounted on the distal cut end of the renal bundle as the means of delivering electrical RNS. Renal blood flow (RBF) was measured by a square wave-electromagnetic flow-meter through a non-cannulating flow-probe attached to the origin of the left renal artery. Simultaneous collection of renal venous and arterial blood was performed through cannulae inserted into the renal vein and the left brachial artery, respectively. Infusions of Al and injections of CIL were given into the right and left cephalic vein, respectively. Mean arterial blood pressure (ABP) was monitored at the right brachial artery. Heart rate (HR) was measured by an ECG (limb lead II). The plasma NE concentration (NEC) was determined with an amperometric detector after catecholamine separation by HPLC as previously described (9). After the
end of the perfusion experiment, the kidney weight was measured for the calculation of RBF, renal vascular resistance (RVR) and NE secretion rate (NE-SR) on the basis of kidney wet weight; NE-SR was calculated as the product of the renal venous-arterial plasma NEC difference and renal plasma flow. Renal plasma flow was determined as the product of the RBF and the value of one minus hematocrit.

Cilazaprilat (CIL) was kindly provided by Nippon Roche Co., Ltd.; it was dissolved in 0.1 N Na₂CO₃ and the pH adjusted to 7.0. Angiotensin I ([¹Asp-⁵Ile]-Al), purchased from Osaka Protein Foundation, was dissolved in 0.9% saline. All values are expressed as the mean±S.E. Analysis of variance was employed for the overall statistical analysis, and Tukey’s test was used for the statistical analysis of values at each blood sampling point. A P level of less than 0.05 was considered to be significant.

After completion of the preparatory surgical procedures, an interval of 60-90 min was allowed for the stabilization of cardiovascular variables. Control arterial and renal venous blood samples (3 ml each) were obtained and intravenous infusion of Al (15 ng/kg/min) was started and maintained throughout the experiment. From 15 min after the start of Al infusion, RNS (pulse rate 1 Hz, supramaximal voltage 10–15 V, pulse duration 1 msec) was carried out continuously for approximately 20 min. Ten minutes after the start of RNS during infusion of Al, an intravenous injection of CIL (0.1 mg/kg) was interposed. Periodic blood-sampling was repeated at the following times: just before and 5 min after the start of RNS and just before and 5 and 10 min after administration of CIL, respectively.

The results are shown in Table 1. Following intravenous infusion of Al, increases in ABP, RBF and RVR and a decrease in RBF were observed, but other variables were not altered. During infusion of Al, subsequent applications of RNS markedly increased NE-SR in parallel with increased renal venous plasma NEC (PNE-RV). After administration of CIL, the increase in NE-SR elicited by RNS was diminished by 21% and 37% (667±143 pg/min/g before CIL vs. 525±129 and 420±120 pg/min/g) at 5 min and 10 min after CIL, respectively, accompanying partial reduction in NEC-RV and a significant increase in NEC-A. The values in ABP, RBF and RVR during infusion of Al and subsequent RNS

<table>
<thead>
<tr>
<th>Periods</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
<th>B5</th>
<th>B6</th>
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</thead>
<tbody>
<tr>
<td>ABP (mmHg)</td>
<td>132±7</td>
<td>147±7*</td>
<td>150±7*</td>
<td>151±7*</td>
<td>132±8#</td>
<td>127±8#</td>
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<tr>
<td>HR (beats/min)</td>
<td>157±9</td>
<td>149±9</td>
<td>147±9</td>
<td>144±10</td>
<td>165±11#</td>
<td>165±12#</td>
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<tr>
<td>RBF (ml/min/g)</td>
<td>4.74±0.65</td>
<td>3.77±0.52*</td>
<td>3.53±0.48**</td>
<td>3.40±0.46**</td>
<td>4.00±0.47#</td>
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<td>RVR (mmHg/ml/min/g)</td>
<td>32.4±5.0</td>
<td>44.6±6.1*</td>
<td>48.1±6.3**</td>
<td>50.2±6.3**</td>
<td>35.8±4.2#</td>
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<td>NEC-A (pg/ml)</td>
<td>162±31</td>
<td>143±43</td>
<td>163±31*</td>
<td>157±29</td>
<td>237±45#</td>
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<td>NEC-RV (pg/ml)</td>
<td>124±24</td>
<td>85±16</td>
<td>531±64**</td>
<td>526±53**</td>
<td>488±35#</td>
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<td>NEC RV-A (pg/ml)</td>
<td>−38±27</td>
<td>−59±33</td>
<td>367±82**</td>
<td>369±71**</td>
<td>251±63#</td>
<td>194±58#</td>
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<td>NEC-SR (pg/ml)</td>
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<td>−75±48</td>
<td>712±182**</td>
<td>667±143#</td>
<td>525±129#</td>
<td>420±120#</td>
</tr>
</tbody>
</table>

Values are means±S.E. (8 dogs) at the blood sampling periods indicated in Fig. 1. P<0.05–0.01: *compared with the value before infusion of Al (vs. B1). *compared with the value before RNS (vs. B2) and #compared with the value before injection of CIL (vs. B4).

Table 1. Values of mean arterial blood pressure (ABP), heart rate (HR), renal blood flow (RBF), renal vascular resistance (RVR), arterial plasma norepinephrine concentration (NEC-A), renal venous plasma norepinephrine concentration (NEC-RV), renal venous-arterial plasma norepinephrine concentration (NEC RV-A) and norepinephrine secretion rate (NE-SR) during intravenous infusion of angiotensin I (Al), subsequent application of continued renal nerve stimulation (RNS) and intravenous injection of cilazaprilat (CIL) in anesthetized dogs.
were almost restored to the initial levels after CIL administration.

Increased endogenous All levels, caused by infusion of AI, may contribute to increment of vascular tone to a higher level through direct stimulation of peripheral vascular All receptors. In this situation, possible indirect facilitatory action of All on the sympathetic nervous system would also be involved. Therefore, the inhibitory effect of CIL on All-dependent vascular tone would appear mostly through the inhibition of ACE. The increase in NE-SR elicited by RNS was partially but significantly inhibited after administration of CIL. The partial inhibitory effect of CIL was unlikely to have been due to insufficient dosing, since our preliminary study showed the pressor response to injected AI was almost completely inhibited after intravenous injection of CIL at the same dose (0.1 mg/kg) as that used in the present experiment, thereby suggesting nearly full exertion of ACE inhibition. Although the study lacks the precise quantitative analysis, our preliminary experiment showed that the potency of the inhibitory effect of captopril (1 mg/kg) on the increase in NE-SR elicited by RNS was similar to that of CIL (0.3 mg/kg, data not shown). All has been shown to have a pre-junctional facilitatory action on NE release in peripheral sympathetic nerves, including those of dog kidney (5). Therefore, the inhibitory effect of CIL on the increase in NE-SR elicited by RNS would be ascribable to the decrease in background level of All resulting from ACE inhibition. The inhibitory effect of CIL observed in the kidney would also be exerted upon other peripheral tissues which are innervated by the sympathetic nervous system. Nevertheless, HR and NEC-A were increased after administration of CIL, and this was accompanied by a decrease in ABP. If one assumes that the extent of the inhibitory effect of CIL on NE release would be overcome by a possible stimulating effect on NE release, this may be ascribable to compensatory reflex activation of the sympathetic nervous system during the decrease in ABP. This study showed that CIL exerts a hypotensive effect associated with inhibition of endogenous All formation and also attenuates RNS-induced NE release from renal sympathetic nerve endings. These results may also suggest that CIL exerts at least a part of its hypotensive effect through decreasing the facilitatory action of endogenous All on adrenergic transmission.

References