Central Actions of Circulating Angiotensin II on the Sympathetic Nervous System and Blood Pressure Control

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SUMMARY
The effects of intravertebral artery infusions of [Sar1, Ile8] and [Sar1, Thr8] angiotensin II on the central nervous system were studied in furosemide-treated dogs anesthetized with α-chloralose. Acute administration of furosemide led to a significant increase in plasma renin activity, plasma noradrenaline levels and heart rate, and also to a slight rise of blood pressure. In the furosemide-treated dogs, intravertebral artery infusion of either angiotensin II antagonist (250 ng/Kg/min, for 30 min) suppressed the furosemide-induced increases in plasma noradrenaline, heart rate and arterial blood pressure. The effects of [Sar1, Thr8] angiotensin II on the last two parameters were more pronounced than those of [Sar1, Ile8] angiotensin II. Intravenous infusion of the same dose of each antagonist had little influence on the furosemide-induced increases in arterial blood pressure, heart rate and plasma noradrenaline levels. These results suggest that the central actions of angiotensin II contribute to the regulation of blood pressure through the sympathetic nervous system.

Additional Indexing Words:
Central nervous system Intravertebral artery infusion Angiotensin II Angiotensin antagonist Noradrenaline

Angiotensin II has been shown to stimulate sympathetic neural activity by affecting the central and the peripheral sympathetic nervous system.1)-3) Moreover, it has been demonstrated that renin release from the kidney is mediated at least partially through sympathetic neural activation.4)
Consequently, the interaction between the renin-angiotensin system and the sympathetic nervous system may play a significant role in the regulation of blood pressure. However, any hemodynamic and humoral effects attributed to changes in one system may also be affected by concomitant alterations in the other system. Previously, we observed that the increase in plasma noradrenaline (NA) concentration induced by furosemide administration in normotensive dogs was significantly suppressed by the intravenous infusion of \([\text{Sar}^1, \text{Ile}^8]\) angiotensin II, an angiotensin II antagonist.\(^5\) This raised possibility that the renin-angiotensin system may play a significant role in the enhancement of sympathetic neural activity.

In the present study, the role of central nervous system activation induced by circulating angiotensin II in the regulation of blood pressure was studied by intravertebral artery infusion of an angiotensin II antagonist in furosemide-treated dogs. In addition, the ability of two angiotensin II antagonists (\([\text{Sar}^1, \text{Ile}^8]\)- and \([\text{Sar}^1, \text{Thr}^8]\)-angiotensin II) to elicit hemodynamic responses and alter plasma NA was compared.

**Materials and Methods**

Nineteen healthy mongrel dogs of both sexes, weighing 9–18 Kg, were fed standard dog chow containing 0.25 Gm of sodium/100 Gm of diet, and anesthetized with \(\alpha\)-chloralose (100 mg/Kg, iv). Under sterile conditions, the femoral artery was catheterized for recording arterial blood pressure by use of a Statham p37b pressure transducer and the femoral vein was cannulated for blood sampling and furosemide administration. A polyethylene cannula with an external diameter of 0.9 mm was inserted into one of the vertebral arteries, without appreciably affecting the natural blood flow, to permit infusion of an angiotensin II antagonist into the vertebrobasilar circulation. The other vertebral artery was left intact. The arterial blood pressure was recorded continuously and the heart rate was derived from the arterial pressure trace. \([\text{Sar}^1, \text{Ile}^8]\) angiotensin II (Protein Research Foundation, Osaka, Japan) or \([\text{Sar}^1, \text{Thr}^8]\) angiotensin II (The Cleveland Clinic, Ohio, USA) was diluted with 0.9% saline and administered via either the vertebral artery or the femoral vein at the rate of 250 ng/Kg/min. The infusion rate of saline was 0.49 ml/min. The following experiments were performed in anesthetized dogs.

To examine the arterial blood pressure and plasma NA responses to acute furosemide administration, furosemide (1.0 mg/Kg) was injected intravenously 4 times at intervals of 10 min in 5 dogs. Blood was sampled during the control period, 15 and 30 min after the first injection of furosemide, and during the recovery period (30 min after the last injection of furosemide). In
another 5 dogs, [Sar\textsuperscript{1}, Ile\textsuperscript{8}] or [Sar\textsuperscript{1}, Thr\textsuperscript{8}]-angiotensin II was infused into the vertebral artery at a rate of 250 ng/Kg/min for 30 min concurrently with the first injection of furosemide. Blood sampling was carried out in the same manner.

To examine whether the central dose (250 ng/Kg/min) of an angiotensin II antagonist has peripheral actions, each antagonist was infused intravenously with injections of furosemide (1.0 mg/Kg, 4 times at 10 min-intervals) in dogs.

The plasma renin activity (PRA) was measured by a modified radioimmunoassay method and expressed as nanograms per milliliter per hour of angiotensin I, according to the method of Haber et al.\textsuperscript{6}) Plasma NA concentration was measured using the radioenzymatic method described by Henry et al.\textsuperscript{7}) Blood samples were collected in cold tubes containing EDTA (1.0 mg/ml) and centrifuged at 4°C. The plasma was kept frozen at −20°C until assay.

Statistical analysis was performed in accordance with recommendations in the literature.\textsuperscript{8}) In analysing the differences within a time series, “time-trend” analysis was used. In analysing the differences between groups, “multi-group repeated measurements design” was used. Any difference was considered significant when a p value was smaller than 0.05. Data were represented as mean ± SEM.

**RESULTS**

1. Effects of furosemide on arterial pressure, heart rate, PRA and plasma NA

When furosemide (1.0 mg/Kg) was administered intravenously 4 times at 10 min-intervals to 5 anesthetized dogs, the mean arterial pressure (MAP)

<table>
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<th>Control</th>
<th>Furosemide administration</th>
<th>Test for trend</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>15 min</td>
<td>30 min</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>107.7 ± 4.2</td>
<td>113.0 ± 4.8</td>
<td>112.0 ± 4.3</td>
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<tr>
<td>HR (beats/min)</td>
<td>117 ± 14</td>
<td>132</td>
<td>146 ± 15</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>7.1 ± 1.0</td>
<td>15.9 ± 2.1</td>
<td>17.6 ± 2.5</td>
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<tr>
<td>Plasma NA (ng/ml)</td>
<td>0.50 ± 0.02</td>
<td>0.53 ± 0.07</td>
<td>0.73 ± 0.08</td>
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Values are mean ± SEM.
increased slightly and the heart rate was markedly increased. The PRA gradually rose about 2.5 fold 30 min after the onset of furosemide injection. The plasma NA concentration was also elevated from 0.30±0.02 to 0.73±0.08 ng/ml (p<0.01). These changes are summarized in Table I.

2. Intravertebral artery infusion of an angiotensin II antagonist in furosemide-treated dogs

The effects of intravertebral artery infusion of both angiotensin II antagonists on the furosemide-induced changes in MAP and heart rate are displayed in Fig. 1. [Sar¹, Ile⁸] and [Sar¹, Thr⁸]-angiotensin II significantly reduced the MAP by −2.3 and −11.0 mmHg, respectively, 30 min after starting the infusion. The fall in MAP induced by the latter antagonist was significantly greater than that by the former (p<0.05). Moreover, [Sar¹, Thr⁸]-angiotensin II significantly reduced the furosemide-induced increase in heart rate 30 min after the infusion. [Sar¹, Ile⁸]-angiotensin II appeared to reduce the furosemide-induced increase in heart rate, but it was not significant by one-way analysis of variance. Therefore, the reduction of heart rate by [Sar¹, Thr⁸]-angiotensin II was more marked than that by [Sar¹, Ile⁸]-angiotensin II.
II. As shown in Fig. 2, the furosemide-induced increase of plasma NA was significantly suppressed by concomitant infusion of either antagonist. The magnitude of suppression of plasma NA due to intravertebral artery infusion of [Sar\(^1\), Thr\(^8\)]-angiotensin II was equivalent to that due to [Sar\(^1\), Ile\(^8\)]-angiotensin II in furosemide-treated dogs.

3. Intravenous infusion of an angiotensin II antagonist in furosemide-treated dogs

In control experiments, intravenous infusion of [Sar\(^1\), Ile\(^8\)]- or [Sar\(^1\), Ile\(^8\)]-angiotensin II antagonists on the furosemide-induced increase in plasma noradrenaline (NA) concentration. Values are mean±SEM. * p<0.05, ** p<0.01 as compared with furosemide alone group.

![Graph showing effects of intravertebral artery (250 ng/Kg/min) infusions of angiotensin II antagonists on the furosemide-induced increase in plasma noradrenaline (NA) concentration. Values are mean±SEM.](image)

![Bar graphs showing changes in mean arterial pressure (MAP), heart rate (HR) and plasma noradrenaline (NA) concentration 30 min after intravenous infusion of [Sar\(^1\), Ile\(^8\)]- (shaded column, n=4) and [Sar\(^1\), Thr\(^8\)]- (dotted column, n=4) angiotensin II (250 ng/Kg/min) in furosemide-treated dogs. The open bar (n=5) shows data from dogs given furosemide alone. Values are mean±SEM.](image)
Thr⁸]-angiotensin II at a rate of 250 ng/Kg/min for 30 min did not affect the furosemide-induced increase in MAP and heart rate. The furosemide-induced increase in plasma NA level was also not affected by intravenous infusion of the same dose of either antagonist. These results are shown in Fig. 3.

**DISCUSSION**

It has been well documented that angiotensin II affects the central nervous system.¹ Its central actions may be mediated through stimulation of brain vasomotor centers, because the blood pressure elevation induced by angiotensin II can be blocked by adrenergic neuron blocking agents.³ However, since angiotensin II does not freely cross the blood-brain barrier,¹⁰,¹¹ brain sites outside the blood-brain barrier are most likely to be candidates for mediators of the action of circulating angiotensin II on the brain.

In the present study, we administered furosemide to increase plasma angiotensin II levels, and infused two angiotensin II antagonists, [Sar¹, Thr⁸]- and [Sar¹, Ile⁸]-angiotensin II, into a vertebral artery to examine the role of the central sympathetic activation induced by circulating angiotensin II in blood pressure regulation. In addition, the responses of arterial blood pressure and plasma NA to the antagonists were compared to test for possible differences in catecholamine release and cardiac effects.¹²,¹³

The administration of furosemide led to a significant increase in plasma NA levels and PRA. In addition, a marked increase in heart rate was observed. The influence of acute administration of furosemide on plasma NA is complex and may be affected by many factors. Decreased plasma volume, the baroreceptor reflex and increased circulating angiotensin II levels may all contribute to the furosemide-induced increase in plasma NA. We previously reported that the furosemide-induced increase in plasma NA was inhibited by a large dose of an angiotensin II antagonist, indicating a significant role of the renin-angiotensin system on increased plasma NA levels.⁵

However, since angiotensin II stimulates sympathetic neural activity by affecting various sites of the sympathetic nervous system, an attempt was made in this study to inhibit the central action of circulating angiotensin II by intravertebral artery infusion of an angiotensin II antagonist. The furosemide-induced increase in plasma NA was significantly suppressed by intravertebral artery infusion of [Sar¹, Thr⁸]- as well as [Sar¹, Ile⁸]-angiotensin II at the rate of 250 ng/Kg/min, though both were ineffective when infused intravenously. In addition, infusion of these antagonists into the vertebral artery suppressed the furosemide-induced increase in heart rate. These results suggest that circulating angiotensin II may stimulate sympathetic neural activity in-
directly through the central nervous system when the renin-angiotensin system is activated.

Although intravenous infusion of angiotensin II antagonists at a rate of 250 ng/Kg/min did not produce a significant fall in arterial blood pressure, the central infusions resulted in a significant blood pressure fall. This is compatible with the observation of Sweet et al that the hypertensive response to angiotensin II infused into the vertebral artery is completely antagonized by concomitant infusion of [Sar¹, Ile⁸]-angiotensin II.¹⁴ The fall in blood pressure induced by [Sar¹, Thr⁸]-angiotensin II was greater than that by [Sar¹, Ile⁸]-angiotensin II. In addition, the suppression of heart rate by [Sar¹, Thr⁸]-angiotensin II seemed to be more pronounced than that by [Sar¹, Ile⁸]-angiotensin II. Since angiotensin II can inhibit vagal tone,¹⁵ any antagonist will apparently increase vagal tone in conditions when the angiotensin II level is high. Bravo et al have shown in salt-deprived dogs that intravenous infusion of [Sar¹, Thr⁸]-angiotensin II reduces blood pressure, cardiac output and heart rate; these decreases are reversed by atropine.¹⁶ This observation gives a basis for an influence that the marked decrease in MAP or heart rate induced by [Sar¹, Thr⁸]-angiotensin II may be due, in part, to the overactivity of the parasympathetic nervous system.

REFERENCES

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