THE EFFECT OF ANGIOTENSIN II ANTAGONIST, SAR\textsuperscript{1}ILE\textsuperscript{8}ANGIOTENSIN II, ON FUROSEMIDE-INDUCED INCREASE IN PLASMA NORADRENALINE, RENIN ACTIVITY AND ALDOSTERONE IN UNANESTHETIZED DOGS

Yuji Ueno, Mikio Arita, Hidetoshi Suruda, Hideyo Ohtani, Tsuneo Minakata, Masato Kuchii, Ichiro Nishio, Yoshiaki Masuyama

In order to evaluate the role of the renin-angiotensin system and the sympathetic nervous system in the maintenance of blood pressure in the sodium-depleted state, the changes of plasma renin activity (PRA), plasma aldosterone concentration (PAC) and plasma noradrenaline (PNA) were examined in unanesthetized dogs after the administration of furosemide. Furthermore, the role of the renin-angiotensin system in the increased sympathetic nerve activity induced by furosemide was assessed by using Sar\textsuperscript{1}Ile\textsuperscript{8}angiotensin II, an angiotensin II antagonist.

When a dose of 0.8 mg/kg of furosemide was injected intravenously, 3 times every 15 minutes, PRA and PNA were significantly increased with a concomitant increase in PAC. Sar\textsuperscript{1}Ile\textsuperscript{8}angiotensin II induced a significant increase in PAC and a slight increase in PRA, while no changes were found in PNA and the mean blood pressure. The increase in PNA induced by furosemide was inhibited dose-dependently by Sar\textsuperscript{1}Ile\textsuperscript{8}angiotensin II, through PRA and PAC were further increased.

There results suggest that an administration of furosemide induced the increase in PNA and the increase in PNA by furosemide might be mediated by the renin-angiotensin system.

It has been shown by some investigators that the sympathetic nerve activity may be potentiated peripherally and centrally by angiotensin II\textsuperscript{1-7} and renin release from the kidney may be stimulated by an increase in the sympathetic nerve activity\textsuperscript{8,9} Therefore, it is possible that the interaction between the renin-angiotensin system and the sympathetic nervous system may affect blood pressure, especially in sodium and volume depleted states since these conditions activate the renin-angiotensin system.

In this study, the role of the renin-angiotensin system and the sympathetic nervous system during furosemide administration in the maintenance of blood pressure was assessed by using Sar\textsuperscript{1}Ile\textsuperscript{8}angiotensin II, an angiotensin II analogue. Plasma renin activity (PRA), plasma aldosterone concentration (PAC) and plasma noradrenaline (PNA) were measured as the indices for the activ-

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Plasma noradrenaline
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Furosemide

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TABLE I EFFECTS OF FUROSEMIDE (0.8 mg/kg, INTRAVENOUSLY 3 TIMES EVERY 15 MIN) ON MEAN BLOOD PRESSURE (MBP), HEART RATE (HR), HEMATOCRIT (Ht), PLASMA RENIN ACTIVITY (PRA), PLASMA ALDOSTERONE CONCENTRATION (PAC) AND PLASMA NORADRENALINE (PNA) IN 5 NORMAL, UNANESTHETIZED DOGS

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>furosemide (0.8 mg/kg x 3) iv</th>
<th>recovery ##</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
<td>30 min</td>
<td>45 min</td>
</tr>
<tr>
<td>MBP mmHg</td>
<td>95.2 ± 3.0</td>
<td>100.2 ± 6.4</td>
<td>102.2 ± 6.5</td>
</tr>
<tr>
<td>HR beat/min</td>
<td>114 ± 8 **</td>
<td>141 ± 8 **</td>
<td>156 ± 11 **</td>
</tr>
<tr>
<td>Ht %</td>
<td>34.4 ± 0.7</td>
<td>36.6 ± 0.9 *</td>
<td>38.8 ± 1.3 **</td>
</tr>
<tr>
<td>PRA ng/ml/hr</td>
<td>4.4 ± 0.3</td>
<td>7.5 ± 0.7 *</td>
<td>8.9 ± 0.9 **</td>
</tr>
<tr>
<td>PNA mg/ml</td>
<td>0.30 ± 0.05</td>
<td>0.45 ± 0.07 *</td>
<td>0.62 ± 0.11 *</td>
</tr>
<tr>
<td>PAC ng/dl</td>
<td>19.2 ± 3.6</td>
<td>21.2 ± 5.7</td>
<td>26.1 ± 5.2</td>
</tr>
</tbody>
</table>

##20 minutes after the last injection
*p < 0.05, **p < 0.01 compared to control values

ity of the renin-angiotensin system or the sympathetic nervous system.

MATERIALS AND METHODS

Healthy mongrel dogs of both sexes, weighing from 7 to 14 kg, were used in unanesthetized and unrestrained states. In order to avoid the effects of anesthetic agents, polyethylene catheters were inserted in advance into the aorta through the carotid artery and into the right atrium through the external jugular vein at least two days before the experiment. The dogs were fed a standard laboratory diet (Na: 35 mEq/day, K: 65 mEq/day), and were trained to lie quietly during the experiments. The carotid artery catheter was used for recording arterial blood pressure and heart rate. The jugular vein catheter was used for blood sampling and the administration of furosemide and Sar^I^Ile^S^angiotensin II.

A dose of 0.8 mg/kg of furosemide was injected three times, every 15 minutes. Concurrently, Sar^I^Ile^S^angiotensin II was infused at the rates of 2, 5 and 10 μg/kg/min for 45 minutes by Harvard infusion pump. The infusion rate was 0.76 ml/min. Blood sampling was carried out in the control period, 15, 30 and 45 minutes after the beginning of the infusion, and 20 minutes after the end of the infusion. During the experiment, the arterial blood pressure was recorded from the carotid catheter connected to a Statham P37b pressure transducer and heart rate was calculated from the blood pressure tracings. In the control groups, furosemide or Sar^I^Ile^S^angiotensin II alone was administered in the same way.

Both PRA and PAC were measured by radioimmunoassay, utilizing Dainabot RIA kits. PNA was measured by the radioenzymatic method of Henry et al.\textsuperscript{10} Blood samples were collected in the cold tubes containing EDTA(1 mg/ml) and centrifuged at 4°C, and the plasma was kept frozen at −40°C until the assay.

Statistical analysis of the data was performed by the Student's t-test for paired observations. The results are shown as mean ± SEM.

RESULTS

1: Effects of furosemide on PRA, PAC and PNA.

The changes of mean blood pressure (MBP), heart rate (HR), PRA, PAC and PNA in response to furosemide injections are shown in Table I. MBP was not significantly changed by furosemide injection. A significant increase in HR was observed from a mean value of 114 ± 8/min to 163 ± 14/min by furosemide injection (p < 0.01). Hematocrit (Ht) was also markedly increased from the control of 34.4 ± 0.7% to 40.4 ± 1.4% (p < 0.01).

PRA was increased from the control value of 4.4 ± 0.3 ng/ml/hr to 9.9 ± 1.1 ng/ml/hr following the furosemide injection. PNA was also significantly increased from 0.30 ± 0.05 ng/ml to 0.84 ± 0.19 ng/ml, 2.8 times of the control value (p < 0.01). A gradual increase in PAC was found, though the increase was not significant within 45 minutes after the first injection.

2: Effects of Sar^I^Ile^S^angiotensin II on PRA, PAC and PNA.

Sar^I^Ile^S^angiotensin II (4.8 μg/kg/min) was administered intravenously to five dogs. The changes of MBP, HR, Ht, PRA, PAC and PNA were measured by radioenzymatic method of Henry et al.\textsuperscript{10} Blood samples were collected in the cold tubes containing EDTA(1 mg/ml) and centrifuged at 4°C, and the plasma was kept frozen at −40°C until the assay.

Statistical analysis of the data was performed by the Student's t-test for paired observations. The results are shown as mean ± SEM.

\textsuperscript{10} Japanese Circulation Journal Vol. 44, December 1980
TABLE II EFFECTS OF Sar^1-Ile^8-ANGIOTENSIN II (4.8 μg/kg/min, INTRAVENOUSLY) ON MEAN BLOOD PRESSURE (MBP), HEART RATE (HR), HEMATOCRIT (Ht), PLASMA RENIN ACTIVITY (PRA), PLASMA ALDOSTERONE CONCENTRATION (PAC) AND PLASMA NORADRENALINE (PNA) IN 5 NORMAL, UNANESTHETIZED DOGS

<table>
<thead>
<tr>
<th>control</th>
<th>Sar^1-Ile^8-angiotensin II infusion</th>
<th>recovery##</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
<td>30 min</td>
</tr>
<tr>
<td>MBP mmHg</td>
<td>101.0 ± 3.2</td>
<td>102.0 ± 3.1</td>
</tr>
<tr>
<td>HR beat/min</td>
<td>118 ± 8</td>
<td>116 ± 7</td>
</tr>
<tr>
<td>Ht %</td>
<td>35.6 ± 1.5</td>
<td>35.6 ± 1.5</td>
</tr>
<tr>
<td>PRA ng/ml/hr</td>
<td>6.2 ± 1.1</td>
<td>7.1 ± 1.9</td>
</tr>
<tr>
<td>PNA ng/ml</td>
<td>0.38 ± 0.08</td>
<td>0.35 ± 0.09</td>
</tr>
<tr>
<td>PAC ng/dl</td>
<td>29.3 ± 7.3</td>
<td>50.4 ± 9.1*</td>
</tr>
</tbody>
</table>

##20 minutes after the last injection
*p < 0.05 compared to control values

are shown in Table II. Sar^1-Ile^8-angiotensin II did not affect the values of MBP, HR and Ht, 15 to 45 minutes after the injection. However, a transient rise in blood pressure was observed within five minutes after Sar^1-Ile^8-angiotensin II infusion in all dogs; by 10 minutes after the infusion the blood pressure returned to the control level as shown in Figure 1.

PRA was gradually increased by Sar^1-Ile^8-angiotensin II infusion, and by the end of 45 minutes-infusion, PRA rose significantly from the control value of 6.2 ± 1.1 ng/ml/hr to 9.4 ± 2.1 ng/ml/hr. The increase in PAC was observed throughout the period of the infusion (p < 0.01). Sar^1-Ile^8-angiotensin II failed to produce any significant effect on PNA by the intravenous infusion to the normotensive dogs.

Fig. 1. A transient rise in blood pressure induced by Sar^1-Ile^8-angiotensin II.

Fig. 2. Effects of furosemide and Sar^1-Ile^8-angiotensin II administration on mean blood pressure (MBP), as compared with that of furosemide alone.

Japanese Circulation Journal  Vol. 44, December 1980
3: Effects of Sar$^1$-Ile$^8$-angiotensin II on the increase in PRA, PAC and PNA induced by furosemide.

MBP was not significantly changed by a low dose (2 μg/kg/min) and a high dose (5 or 10 μg/kg/min) of Sar$^1$-Ile$^8$-angiotensin II combined with furosemide administrations, as shown in Figure 2. An increase in HR, comparable to that by furosemide alone, was found by 15 minute-infusion of a low dose of Sar$^1$-Ile$^8$-angiotensin II with furosemide injections, but not by a high dose infusion (Figure 3). Thus, an increase in HR by furosemide was suppressed following the high dose of Sar$^1$-Ile$^8$-angiotensin II infusion. However, further injection of furosemide induced the similar increase in HR in spite of the combined use of a high dose of Sar$^1$-Ile$^8$-angiotensin II. No significant difference in HR was found between the
groups in which furosemide was used alone and combined with Sar\textsuperscript{1}Ile\textsuperscript{8}-angiotensin II.

The effect of Sar\textsuperscript{1}Ile\textsuperscript{8}-angiotensin II on the furosemide induced increase in PNA is shown in Figure 4. The increase in PNA by furosemide was significantly suppressed by the high dose of Sar\textsuperscript{1}Ile\textsuperscript{8}-angiotensin II infusion (p < 0.01). The suppression was not significant by the low dose infusion, and seemed to be dose-dependent. As shown in Figure 5, both PRA and PAC were furthermore increased by Sar\textsuperscript{1}Ile\textsuperscript{8}-angiotensin II infusion combined with furosemide administration than the furosemide injection alone.

DISCUSSION

It has been suggested that the regulation of blood pressure might be performed by many factors, including the renin-angiotensin system and the sympathetic nervous system, but each system could not explain the pathogenesis of hypertension by itself\textsuperscript{11}. However, it has been shown that the renin-angiotensin system and the sympathetic nervous system may be functionally interrelated\textsuperscript{1-10} and plasma renin activity, one of the indices of the renin-angiotensin system, was activated by a low sodium diet or furosemide administration\textsuperscript{12}. Accordingly, the interaction between the renin-angiotensin system and the sympathetic nervous system may play some significant role in the regulation of blood pressure, especially in a sodium-depleted state which activates the renin-angiotensin system.

In order to evaluate the physiological significance of the effect of angiotensin II on the sympathetic nervous system, the changes in PNA, one of the indices of the sympathetic nerve activity, were determined under the activation of the renin-angiotensin system induced by furosemide, and the effect of angiotensin II antagonist was examined.

PRA, PAC and PNA were increased by furosemide injections. These increases in PRA, PAC and PNA by furosemide, indicating the activation of the renin-angiotensin and the sympathetic nervous system, were probably due to physiological responses to the fall of blood pressure by furosemide.

A transient rise of blood pressure by the infusion of Sar\textsuperscript{1}Ile\textsuperscript{8}-angiotensin II was found within five minutes after the infusion, which might be mediated not only by angiotensin-like action on the vascular receptors but also by an activation of the central and peripheral autonomic nervous system\textsuperscript{13,14}. Angiotensin II analogues have functionally different steroidogenic potencies, and Sar\textsuperscript{1}Ile\textsuperscript{8}-angiotensin II possesses a marked agonistic angiotensin-like potency on the kidney and the adrenal cortex,
but little vasopressor activity. In this experiment, Sar^4Ile^8-angiotensin II induced a significant increase in PAC, probably due to its agonistic action of Sar^4Ile^8-angiotensin II, but did not cause any significant change in PNA.

The increase in PNA by furosemide was suppressed by Sar^4Ile^8-angiotensin II (Fig. 5). While a low-dose (2 µg/kg/min) of Sar^4Ile^8-angiotensin II did not suppress significantly the furosemide-induced increase in PNA, a high-dose (5 or 10 µg/kg/min) of Sar^4Ile^8-angiotensin II significantly suppressed the increase in PNA by furosemide. The suppression of the furosemide-induced increase in PNA by Sar^4Ile^8-angiotensin II was dose-dependent. These results suggest that in acutely induced sodium and volume depletion, the increase in the sympathetic nerve activity appears to be mediated by the renin-angiotensin system.

Samuels, et al. reported that in sodium-depleted dogs, the renin-angiotensin system was required for the reflex sympathetic response to hypotension. Furthermore, Feuerstein, et al. recently reported that an acute hemorrhage in cats induced an immediate increase in adrenal catecholamine output, which was completely eliminated by angiotensin analogues. They also noticed that the denervated adrenal gland did not respond by an immediate adrenal catecholamine secretion to hemorrhage even in the high plasma angiotensin II concentrations. Our observation also confirmed that the increase in the sympathetic nerve activity by furosemide may be mediated by the action of angiotensin II to the sympathetic nervous system.

However, Sar^4Ile^8-angiotensin II could not completely suppress the increase in PNA induced by furosemide. It is possible that the furosemide-induced increase in PNA might be mediated partially by the renin-angiotensin system and partially by the other mechanisms including the volume receptors. On the other hand, it is also possible that the infusion-dose of Sar^4Ile^8-angiotensin II was insufficient as compared with the increase of angiotensin II induced by furosemide.

In relation to the suppression of the furosemide-induced increase in PNA by Sar^4Ile^8-angiotensin II, the increase in HR by furosemide was significantly suppressed by a high-dose of Sar^4Ile^8-angiotensin II 15 minutes after the infusion. The observed effects of Sar^4Ile^8-angiotensin II on HR and PNA suggest that the activation of the sympathetic nervous system including the baroreceptors may be attenuated by angiotensin II analogues.

Both PRA and PAC were further more increased by combined administrations of furosemide and Sar^4Ile^8-angiotensin II than by furosemide injection alone. This increase in PRA is probably due to inhibition of the short-loop negative feed-back mechanism, but the increase in PAC was unexpected. This change in PAC was not caused by elevated plasma angiotensin II, since the effects of angiotensin II were inhibited by Sar^4Ile^8-angiotensin II. The promising possibility is that the effect of Sar^4Ile^8-angiotensin II on the adrenal cortex might be agonistic rather than antagonistic independently of angiotensin II level.

It has been shown that the renin-angiotensin system played only a little role in the maintenance of blood pressure in sodium repleted animals or human, but it became increasingly more important with the pregressive hypovolemia. In this experiment, though Sar^4Ile^8-angiotensin II inhibited the action of intrinsic angiotensin II and the increase in the sympathetic nerve activity mediated by angiotensin II, Sar^4Ile^8-angiotensin II failed to lower blood pressure, indicating that the infusion-dose of Sar^4Ile^8-angiotensin II might be insufficient as compared with the furosemide-induced increase of angiotensin II. This possibility may be also supported by the finding that Sar^4Ile^8-angiotensin II could not completely suppressed the furosemide-induced increase in PNA or HR.

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Japanese Circulation Journal Vol. 44, December 1980

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