This study was designed to investigate the central action of circulating angiotensin II on the regulation of blood pressure in sodium depleted states. The effects of intravertebral arterial infusion of angiotensin II and [Sar$^1$, Ala$^8$] angiotensin II (saralasin) on plasma norepinephrine (NE) were studied in α-chloralose anesthetized dogs. Intravertebral arterial infusion of angiotensin II (10 ng/kg/min) increased mean arterial pressure (MAP), heart rate (HR) and plasma NE. Plasma NE was decreased by intravertebral arterial infusion of saralasin (0.40 ± 0.05 to 0.28 ± 0.04 ng/ml, p < 0.05) in normal dogs. The administration of furosemide produced significant increases in plasma NE (142.4 ± 23.7%, p < 0.01), plasma renin activity (PRA) (158.6 ± 26.3%, p < 0.01) and HR (32.3 ± 6.0 beats/min, p < 0.01). A slight rise in mean blood pressure (3.9 ± 1.2 mmHg, p < 0.05) was observed during the furosemide administration. Saralasin infused into the vertebral artery significantly suppressed the furosemide-induced increases in plasma NE, HR and PRA, and lowered mean arterial blood pressure. Intravenous infusion of the same dose of saralasin produced no changes in arterial blood pressure, HR and plasma NE. These results suggest that the central sympathetic potentiation induced by circulating angiotensin II may contribute to the regulation of blood pressure in sodium and volume depleted states produced by furosemide.

Key Words:
[Sar$^1$, Ala$^8$] angiotensin II
Saralasin
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Division of Cardiology, Department of Medicine, Wakayama Medical College, Wakayama, Japan
Mailing address: Mikio Arita, M.D., Division of Cardiology, Department of Medicine, Wakayama Medical College, 1, 7-Bancho, Wakayama 640, Japan
TABLE I  EFFECTS OF INTRAVERTEREBRAL ARTERIAL INFUSION OF ANGIOTENSIN II (10 ng/kg/min) ON MEAN BLOOD PRESSURE (MBP), HEART RATE (HR) AND PLASMA NOREPINEPHRINE (NE) IN FIVE α-CHLORALOSE-ANESTHETIZED DOGS

<table>
<thead>
<tr>
<th></th>
<th>MBP</th>
<th>HR</th>
<th>Plasma NE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>117 ± 7</td>
<td>156 ± 12</td>
<td>0.48 ± 0.09</td>
</tr>
<tr>
<td>After infusion of angiotensin II</td>
<td>125 ± 8**</td>
<td>172 ± 12*</td>
<td>0.70 ± 0.15*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. *p < 0.05, **p < 0.01 as compared to controls.

central sympathetic nervous system, it should be possible to alter it by blocking the renin-angiotensin system using an angiotensin II antagonist. The present investigation was undertaken to study the effect of intravertebral arterial infusion of an antiantiotensin II antagonist (saralasin) on plasma NE, and the effect of sodium and volume depletion by furosemide on plasma NE levels.

MATERIALS AND METHODS

Mongrel dogs of both sexes, weighing 8–15 kg, were anesthetized with α-chloralose (100 mg/kg, i.v.). A cannula was inserted into a femoral artery for recording the arterial blood pressure using a Statham P37b transducer and a femoral vein was cannulated for blood sampling and systemic injection of drugs. While maintaining the blood flow, a polyethylene loop cannula was inserted into one vertebral artery for infusion of saralasin into the vertebro-basilar circulation. The other vertebral artery was left intact. In all experiments, arterial blood pressure was monitored continuously and heart rate (HR) was obtained from the arterial pressure tracing.

The effects of intravertebral arterial infusion of saralasin on arterial blood pressure, HR, plasma NE, PRA and plasma aldosterone concentration (PAC) were examined in normal and furosemide-treated dogs. Furosemide (1.0 mg/kg) was injected intravenously, 4 times, at 10-min intervals. Concurrently, saralasin (250 ng/kg/min) was infused into the vertebral artery for 30 min in the furosemide-treated dogs. In normal dogs, the same dose of saralasin was infused into the vertebral artery. Blood was collected 4 times, i.e., just before the infusion, 15, 30 and 60 min after the onset of the saralasin infusion. In addition, the effects of angiotensin II on mean blood pressure (MBP), HR and plasma NE were studied. Angiotensin II was infused into the vertebral artery at a dose of 10 ng/kg/min which was an ineffective dose when given intravenously. As controls, saline was infused intravertebrally in the same way, and the same dose of saralasin was

Fig.1. Blood pressure recording, which shows the effect of intravertebral arterial infusion of angiotensin II (10 ng/kg/min) on arterial blood pressure.

Fig.2. Effects of intravertebral arterial and intravenous infusions of saralasin (250 ng/kg/min) on mean blood pressure, heart rate and plasma norepinephrine in 8 dogs.
infused intravenously. Blood was collected in cold tubes containing ethylenediaminetetraacetate (EDTA) and centrifuged at 4°C, and then the plasma was frozen at −20°C until assay. Plasma NE was measured using the radioenzymatic method of Henry et al.12 PRA and PAC were measured by radioimmunoassay.

Statistical analysis of the data was performed using the Student’s t-test for paired observations. Differences and co-efficients were considered statistically significant when a p value was less than 0.05. The results are shown as mean ± SEM.

RESULTS

Effects of Intravertebral Arterial Infusion of Angiotensin II: Infusion of angiotensin II into the vertebral artery increased the mean blood pressure by an average of 8 mmHg, HR by 15 beats/min and plasma NE by 0.21 ng/ml. These increases were all statistically significant (Table I). A representative result is illustrated in Fig. 1. In contrast, the intravenous infusion of angiotensin II in the same dose was ineffective in producing changes in MBP, HR and plasma NE.

Effects of Saralasin on MBP, HR and Plasma NE in Anesthetized Dogs: To determine whether or not saralasin antagonizes the indirect effects of angiotensin II which are mediated through the central sympathetic nervous system, saralasin was injected into the vertebral artery. The effects of saralasin on MBP, HR and plasma NE are shown in Fig. 2. Intravertebral arterial infusion of saralasin (250 ng/kg/min) reduced blood pressure and HR by −6.9 ± 1.1 mmHg (p < 0.01) and −7.1 ± 2.9 beats/min (p < 0.05), respectively. Plasma NE was decreased significantly by −22.1 ± 8.1% (p < 0.05) during intravertebral arterial infusion of saralasin. Intravenous infusion of the same dose of saralasin produced no changes in arterial blood pressure, HR, plasma NE, PRA and PAC.

Effects of Furosemide Administration on Plasma NE, PRA, PAC, HR and MBP: When furosemide (1.0 mg/kg) was injected intravenously, 4 times at 10-min intervals, plasma NE increased significantly from a mean of 0.28 ± 0.04 to 0.64 ± 0.06 ng/ml (p < 0.01). Both PRA and PAC were also increased by furosemide (p < 0.05). During furosemide administration, HR increased from a mean of 118 ± 14 to 155 ± 13 beats/min (p < 0.01), and a slight but significant rise in blood pressure was observed. These results are summarized in Table II.

Effects of Intravertebral Arterial Infusion of Saralasin in Furosemide-treated Dogs: Blood pressure and heart rate responses to intravertebral arterial infusion of saralasin in furosemide treated dogs are presented in Fig. 3. A marked fall in blood pressure was observed during intravertebral arterial infusion of saralasin; moreover, the furosemide-induced increases in HR were suppressed significantly. The effect of intravertebral artery infusion of saralasin on the furosemide-induced increase in plasma NE is shown in Fig. 4. The increase in plasma NE produced by furosemide was suppressed from 142.4 ± 25.2 to 69.4 ± 12.3% by intravertebral arterial infusion of saralasin (p < 0.01). The increases in PRA and PAC caused by furosemide were also suppressed slightly during intravertebral arterial infusion of saralasin. During control experiments, when saline was infused intraventricularly for 30 min, there were no changes in MBP, HR, plasma NE, PRA and PAC.
DISCUSSION

Angiotensin II exerts a variety of effects on the sympathetic nervous system:13 1) enhancing the release and preventing the uptake of norepinephrine at nerve endings, 2) potentiating the release of adrenal catecholamines, 3) acting in the central nervous system to stimulate thirst, 4) augmenting central sympathetic activity. Although direct peripheral vasoconstriction is considered to be the major pressor mechanism in angiotensin II-induced hypertension, a central mechanism has also been demonstrated. Angiotensin exerts its effects on central mechanisms of blood pressure regulation by stimulating different brain structures, some of which are situated outside of the blood-brain barrier (area postrema, subfornical organ and others), while others are situated inside the blood-brain barrier (midbrain, subnucleus medialis, hypothalamus).14 Scoop et al3 have reported that the pressor response to angiotensin II infusion into the vertebral artery greatly exceeded the blood pressure increases following angiotensin II infusion into the carotid artery or into a peripheral vein. They have suggested that angiotensin can produce its effects on the circulation by a specific action in the area in which the vertebral arteries are distributed. In our experiment, angiotensin II (10 ng/kg/min) infused into the vertebral artery increased blood pressure by an average of 8 mmHg, and plasma NE by 0.21 ng/ml, whereas the same dose administered via the femoral vein produced no effect on blood pressure and plasma NE. It has been thought that angiotensin II dose not penetrate the blood brain barrier.15 These results indicate that a small dose of angiotensin II infused into the vertebral artery may act on the area postrema and produce a pressor effect.

It has been reported that plasma NE as well as PRA is increased by sodium depletion and/or diuretics9–11. Nicholls et al9 have reported that sodium intake is an important factor in the interpretation of plasma NE levels. Luft et al16 have also observed that sodium homeostasis has a significant effect on plasma and urinary NE values. The mechanism responsible for the increase in plasma NE produced by sodium depletion has not been investigated. In the present study, sodium and volume depletion induced by furosemide produced a significant increase in plasma NE, which probably reflects overactivity of the sympathetic nervous system. Brosniian et al17 have reported that chronic sodium depletion produced an increase of plasma NE in both conscious and anesthetized dogs. Samuels et al18 have found that when angiotensi-
sin II was administered via the vertebral artery, it led to an increase in HR and plasma catecholamines in sodium-depleted dogs. They concluded that a central action of angiotensin II is necessary for the expression of the reflex sympathetic response to hypotension in the salt deprived state. Recently, we have reported that the furosemide-induced increase in plasma NE was inhibited by an angiotensin II antagonist, [Sar¹, Ile⁸] angiotensin II, infused intravenously. These results suggest that the renin-angiotensin system may contribute to a furosemide-induced increase in plasma NE.

There have been several reports which clarified the role of the central action of angiotensin II by means of intraventricular infusion of angiotensin II antagonists. However, little has been done concerning intravertebral artery infusion of angiotensin II antagonists. Sweet et al. have shown that the cardiovascular response to angiotensin II infusion into the vertebral artery was abolished by vertebral artery infusion of [Sar¹, Ile⁸] angiotensin II. Our results demonstrate that intravertebral arterial infusion of saralasin (250 ng/kg/min) suppresses the furosemide-induced increases in plasma NE and HR, and lowers blood pressure, whereas no effect is observed after the intravenous infusion of the same dose of saralasin. These results suggest that angiotensin II may act on specific receptors in the central nervous system, and contribute to the regulation of blood pressure by affecting the central sympathetic nervous activity in sodium and volume depletion. Angiotensin II antagonist is known to cause an overactivity of the parasympathetic nervous system as well as an inhibition of the sympathetic nervous activity. This knowledge gives a basis for an inference that the decrease in mean blood pressure or HR induced by saralasin may be, in part, due to the overactivity of the parasympathetic nervous system.

In conclusion, sodium and volume depletion by furosemide increased plasma NE, reflecting overactivity of the sympathetic nervous system. Furthermore, furosemide-induced increases in plasma NE were inhibited by intravertebral arterial infusion of saralasin, resulting in a blood pressure fall. These results suggest that the central potentiation induced by circulating angiotensin II may contribute to blood pressure regulation in the sodium and volume depleted states produced by furosemide.

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Plasma Norepinephrine during Sodium Depletion

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