The Role of Brain Angiotensin II in the Mechanism of the Pressor Responses to Intracisternal Injection of Hypertonic NaCl in Urethane Anesthetized Rats

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Intracisternal injections of hypertonic NaCl elicited the pressor responses in urethane anesthetized spontaneously hypertensive and normotensive Wistar rats. The intracisternal pretreatment of 1-Sar-8-Ile-angiotensin II, angiotensin II analog, could abolish the pressor responses to intracisternal injections of 5% NaCl in urethane anesthetized spontaneously hypertensive rats, while basal blood pressure was not affected by these pretreatment.

Both intracisternal and intravenous administration of captopril, an angiotensin I converting enzyme inhibitor, did not alter the pressor effect of hypertonic NaCl in normotensive rats. Basal blood pressure was lowered by intravenous injection of captopril. The involvement of angiotensin II in the pressor mechanism of hypertonic NaCl was confirmed by the enhanced pressor responses to intracisternal injections of 5% NaCl in urethane anesthetized rats pretreated intracisternally with angiotensin II. These findings suggest that angiotensin II itself could play an important role in the pressor responses to intracisternal injections of hypertonic NaCl without involving a converting enzyme system.

IT has been widely accepted that sodium can accelerate blood pressure elevation in hypertensive men¹ and animals^{2,3} but the underlying mechanism has not yet been clarified. Aside from this effect of sodium, an important role of the sympathetic nervous system in the development and maintenance of hypertension has also been reported in patients with essential hypertension^{4,5} and experimental models⁶⁻⁸. It would be very interesting if these two mechanisms are

connected to each other. The possibility of this connection may be supported by the fact that hypertonic NaCl injected intracisternally elicits a pressor response accompanied by a corresponding increases in sympathetic nerve activity (SNA).⁹ However, its mechanism, how centrally applied sodium can act, is not yet clear. Angiotensin II (Ang II) can elicit similar response as hypertonic NaCl when it is injected into cerebroventricles,¹⁰ and Andersson et al.¹¹ reported that Ang II could enhance the effect of sodium injected into the ventricles in sheep.

These findings suggest that Ang II might be involved in the pressor effect of centrally administered sodium. In the present study, to elucidate the roles of Ang II and the Ang I converting

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Fig.1. Basal blood pressure after intracisternal injections of 1-Sar-8-Ile-Ang II ($n = 5$) and saline ($n = 5$) in urethane anesthetized SHR. Values are shown as mean $\pm$ SEM.

Fig.2. Pressor responses to intracisternal injections of 5% NaCl solutions in 1-Sar-8-Ile-Ang II and saline treated SHR anesthetized with urethane. Values are shown as mean $\pm$ SEM.

enzyme system in the brain in the pressor responses to intracisternal (i.c.) injection of NaCl, hypertonic saline was injected intracisternally in urethane anesthetized rats pretreated with 1-Sar-8-Ile-Ang II, captopril, or Ang II.

METHODS

Male albino Wistar rats and spontaneously hypertensive rats (SHR) weighing 200–250 g were used in this experiment. All rats were anesthetized with urethane (1.2 g/kg, i.p.). Catheters were inserted into the right common carotid artery and the left jugular vein. Blood pressure was recorded using an inkless writing recorder (Sanei Sokki) by connecting a cannula to a strain gauge transducer (MPV-290, Sanei Sokki). Heart rate was determined from the phasic pressure signals by a heart rate meter (Sanei Sokki). After cannulations, all rats were placed on the stereotaxic apparatus and then the atlanto occipital membranes were exposed by dissecting the dorsal neck muscles and the cisterna magna was punctured with 27 gauge needles connected through silastic tubing to a 50 μ l microsyringe (Terumo). Three drugs were used in this study. 1-Sar-8-Ile-angiotensin II, an angiotensin II analog, was dissolved in 0.9% NaCl solution just before use, and 100 μ g/10 μ l of it was injected intracisternally as a pretreatment. Captopril, angiotensin I converting enzyme inhibitor, dissolved in saline was injected into vein (2 mg/kg) through cannula and into the cisterna magna (200 μ g/10 μ l). Angiotensin II (Hypertensin, CIBA) was administered intracisternally (50 ng/10 μ l).

Data (mean $\pm$ SEM) obtained from all groups were analyzed using Student's t test and p values less than 0.05 were considered significant.

RESULTS

Angiotensin II Analog Abolished the Pressor Responses to Intracisternal Injections of Hypertonic NaCl in Urethane Anesthetized SHR.

1-Sar-8-Ile-Ang II (100 μ g/10 μ l) was administered intracisternally in urethane anesthetized SHR 10 min prior to i.c. injections of 5% NaCl. In the control group, only physiological saline was administered intracisternally. Base line blood pressure was not affected by physiological saline. Although 1-Sar-8-Ile-Ang II reduced blood pressure somewhat, there were no significant differences between control and treated groups (Fig. 1). Pressor responses to i.c. injections of 5% NaCl solution were completely abolished in Ang II analog treated SHR, while marked pressor responses were produced in vehicle treated SHR (Fig. 2). These findings indicate that Ang II analog could abolish the

Fig.3. Pressor responses to intracisternal injections of 10% NaCl in intravenously treated captopril ($n = 4$) and control ($n = 6$) rats. The solid lines show captopril treated rats and the dotted lines the controls.

Fig.4. Basal blood pressure after intracisternal injections of captopril ($n = 8$) and saline ($n = 7$) in urethane anesthetized normotensive Wistar rats.

pressor responses to i.c. injection of 5% NaCl in urethane anesthetized SHR.

Captopril did not Reduce the Pressor Responses

Fig.5. Pressor responses to intracisternal injections of 5% NaCl normotensive Wistar rats treated intracisternally with captopril and saline.

to Intracisternal Injection of Hypertonic NaCl in Urethane Anesthetized Normotensive Wistar Rats.

After intravenous injection of captopril, blood pressure was lowered significantly (from 93 ± 3 to 65 ± 8 mmHg, $p < 0.025$, $n = 4$). However, pressor responses to i.c. injections of 10% NaCl

Fig.6. Basal blood pressure after intracisternal injections of Ang II ($n = 5$) and saline ($n = 7$) in urethane anesthetized normotensive Wistar rats.

Fig.7. Pressor responses to intracisternal injections of 5% NaCl solution in normotensive Wistar rats treated intracisternally with Ang II and saline. Values are shown as mean $\pm$ SEM.

Fig.8. Responses to additional intracisternal injection of 1-Sar-8-Ile-Ang II in the enhanced pressor responses to intracisternal injections of 5% NaCl in Ang II i.c. treated rats. Tracings represent mean blood pressure (mmHg), phasic blood pressure (mmHg) and heart rate (per min) from top to bottom. Arrow mark indicates an additional injection of Ang II analog.

were not reduced as compared with those in the control group treated with the vehicle (Fig. 3). Moreover, in the rats treated intracisternally with captopril ($200 \mu\text{g}/10 \mu\text{l}$), blood pressure was reduced but not significantly (from 93 ± 6 to 81 ± 3 mmHg, $n = 8$) (Fig. 4), and also pressor responses to i.c. injections of 5% NaCl were not inhibited, when compared with those in the control group treated centrally with vehicle (Fig. 5).

These results indicate that the angiotensin I converting enzyme system may not be involved in these pressor mechanisms.

Angiotensin II Enhanced the Pressor Responses to Intracisternal Injections of 5% NaCl Solution in Urethane Anesthetized Normotensive Wistar Rats.

To confirm whether or not Ang II can really

be involved in the pressor responses to i.c. injections of hypertonic NaCl, Ang II (50 ng/10 μ l) was administered intracisternally as a pretreatment in 5 normotensive Wistar rats. Basal blood pressure was not changed after i.c. treatments of Ang II (86 ± 6 vs 85 ± 6 mmHg) (Fig. 6). Pressor responses to i.c. injections of 5% NaCl were compared with those in saline pretreated control rats. Pressor responses were significantly enhanced in Ang II treated rats (Fig. 7). Furthermore, these pressor responses were rapidly abolished after an additional i.c. injection of Ang II analog (100 μ g/10 μ l) and blood pressure fell to the basal line (83 ± 10 mmHg, 2 min after the injection of Ang II analog, $n = 4$) (Fig. 8).

DISCUSSION

The pressor responses elicited by intracisternal injection of hypertonic NaCl were inhibited by an Ang II antagonist. This inhibition indicates that brain Ang II is involved in the pressor responses of hypertonic NaCl. It is also confirmed by the fact that Ang II itself enhanced the pressor responses to i.c. injection of hypertonic NaCl. However, hypertonic NaCl did not stimulate Ang I converting enzyme system, since converting enzyme inhibitor did not alter the pressor responses of hypertonic NaCl. These findings suggest that Ang II is involved in the pressor responses of hypertonic NaCl without mediation by a converting enzyme system. Our results might be supported by the study demonstrating that saralasin attenuated the pressor responses to intraventricular injection of hypertonic saline in awake normotensive rats.¹² Whether the stimulus to centrally administered hypertonic sodium is osmolality or sodium itself remain controversial,^{13,15,16} but stimulus of intracisternal injections of hypertonic NaCl is not likely osmotic, because i.c. injection of other fluid with same osmotic pressure as hypertonic saline could not elicit any pressor response.¹⁴

Several mechanisms of Ang II may be involved in the pressor responses of hypertonic NaCl, i.e., Ang II may 1) modulate sodium sensitive receptors, 2) activate neurons stimulated by increased sodium concentration in cerebral spinal fluid (CSF) and 3) transmit neural (or humoral) systems mediating biologic responses. The first mechanism is supported by the study showing that Ang II augments the effect of hypertonic NaCl administered centrally by lowering the threshold of the sodium sensitive site.^{15,16}

Enzymatically these receptors have been regarded as Na⁺-K⁺-ATPase.^{15,16} Therefore, evidence that Ang II increases Na⁺-K⁺-ATPase in several tissues¹⁷ might also support this theory. Our data show that sodium sensitive site are located around brain stem, which may be independent from paraventricular system.⁹ The second mechanism, i.e., activation of neurons stimulated by an increase of sodium concentration in CSF, is supported by the interaction of Ang II and norepinephrine. Ang II can accelerate norepinephrine release¹⁸ and inhibit its reuptake,¹⁹ and also stimulate norepinephrine synthesis,²⁰ because neurons may be partly adrenergic. However, this possibility is remote, since clonidine and propranolol did not affect the pressor responses to i.c. injection of hypertonic saline (unpublished data). Since opiates inhibit central actions of angiotensin²¹ and opioid peptides inhibit the pressor responses to centrally injected hypertonic NaCl,²² interaction of the Ang II and the opioid systems may be possible. As the third mechanism, Ang II could act like a neurotransmitter at a synapse from sodium sensitive receptors. Evidence that Ang II could be a neurotransmitter in CNS has been accumulated²³ and the renin angiotensin system which includes renin, Ang I, Ang II and converting enzymes are localized in the brain.²³ However, the present study indicates that the action of Ang II is not mediated by a converting enzyme system. Another possibility is that Ang II may increase the binding affinity of the receptors to sodium or increase receptor numbers. However, since enhanced pressor responses to intracisternal injection of hypertonic NaCl in Ang II pretreated rats were abolished rapidly by an intracisternal addition of an Ang II analog, these possibilities may be unlikely.

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