The rostral ventrolateral medulla (RVLM) is known to be a major center regulating sympathetic and cardiovascular activities. A possible association between neurovascular compression of the RVLM and essential hypertension has been indicated. The present study was performed to determine the role of angiotensin II (AngII) in the pressor and sympathetic responses to pulsatile compression of the RVLM. To determine the role of glutamate and AngII in the RVLM, L-glutamate (Glu) 2 nmol or AngII 100 pmol was injected into the RVLM with or without RVLM pretreatment of kynurenate (Glu receptor antagonist) 3 nmol, candesartan (AngII type 1 (AT1) receptor antagonist) 2 nmol, or PD123319 (AngII type 2 (AT2) receptor antagonist) 1 nmol in anesthetized Wistar rats. In addition, to determine the role of glutamate and AngII in the pressor and sympathetic effects to the RVLM compression, kynurenate, candesartan, or PD123319 was locally injected before pulsatile compression of the RVLM. Finally, to determine the effects of peripherally administered AngII antagonists in these pressor and sympathetic excitatory responses, candesartan 0.25 μmol or PD123319 0.05 μmol was intravenously injected before pulsatile compression of the RVLM. Glu injected into the RVLM significantly increased mean arterial pressure (MAP) and splanchnic sympathetic nerve activity (SNA), and these effects were reduced by RVLM pretreatment with kynurenate, but were unaffected by candesartan or PD123319. AngII injected into the RVLM and pulsatile compression of the RVLM also increased MAP and SNA. However, in contrast with Glu injections, these effects were reduced by RVLM pretreatment with candesartan or kynurenate, but were unaffected by PD123319. Pressor and sympathetic excitatory responses to RVLM compression were reduced by intravenous pretreatment with candesartan but not with PD123319. These results indicate that, upon pulsatile compression of the RVLM, AngII may activate RVLM neurons via AT1 receptors and stimulate Glu release to thereby elicit sympathetic activation and pressor effects. Candesartan may exert its hypotensive effect at least in part by affecting the RVLM neurons to reduce sympathetic outflow induced by pulsatile compression of the RVLM. (Hypertens Res 2004; 27: 427–432)

Key Words: angiotensin II, rostral ventrolateral medulla, angiotensin II type1 receptor antagonist, pulsatile compression

Introduction

The rostral ventrolateral medulla (RVLM) is an important center regulating sympathetic and cardiovascular activities (1, 2). Studies have reported that blood pressure is increased by electrical or chemical stimulation of the RVLM and decreased by chemical inhibition in the RVLM (3–6). In 1978, Jannetta and Gendell (7) first suggested an association between essential hypertension and neurovascular compression...
of the RVLM, and many subsequent studies have also suggested such an association. In previous studies using MRI and MRA with a high-resolution matrix, we reported that the incidence of observed neurovascular contact between the RVLM and surrounding arteries was significantly higher in a group of patients with essential hypertension than in a secondary hypertension group or normotensive group (8, 9). In addition, in a refractory hypertensive patient with neurovascular compression, high blood pressure was normalized and sympathetic nerve activity (SNA) was decreased by surgical removal of surrounding arteries from the RVLM (10). We also reported that pulsatile compression of the RVLM increased blood pressure and sympathetic nerve activity in pulse-frequency- and pulse-strength-related manners in rats (9). In pulsatile compression of the RVLM, L-glutamate (Glu) released from RVLM vasomotor neurons stimulated sequential neurons and elicited the increases in blood pressure and SNA via the production of NO, guanylate cyclase, cyclic GMP and Fos, the protein product of c-fos (11).

Although the precise mechanism of Glu release from the vasomotor neurons in the RVLM remains unclear, angiotensin II (AngII) has been suggested to play an important role in mediating SNA in the RVLM (12–16). Furthermore, the role of AngII in releasing Glu from the vasomotor neurons on pulsatile compression of the RVLM has not been investigated. Therefore, the present studies were performed to investigate the role of AngII and to determine the effects of AngII antagonist in the pressor and sympathetic responses to pulsatile compression of the RVLM in rats. In addition, we investigated whether intravenously administered AngII antagonists would affect the responses induced by RVLM compression.

Methods

Experiments were performed on male Wistar rats (Charles River Breeding Laboratories, Kanagawa, Japan) that weighed between 250 g and 300 g. Animal care and use procedures were approved by the Experimental Animal Care Committee of the Kyoto Prefectural University of Medicine, Japan. The rats were anesthetized with urethane (100 mg/100 g, i.p.). Each rat was mounted on a stereotaxic apparatus in the supine position (David Kopf Instruments, Tujunga, USA) (6, 17). The lower trachea was cannulated, and the rat was artificially ventilated at a rate of 60 breaths/min with a respirator (Ealing Co., Ltd. South Natick, USA) and paralyzed with decamethonium bromide (0.2 mg/100 g, i.v.). Whenever spontaneous breathing was recognized during experiments, a minimal dose (10–50% of the initial dose) of decamethonium bromide was added. The catheter inserted into the right femoral artery was connected with a pressure transducer (MPV-290; NEC Sanei Co., Tokyo, Japan). A polygraph (141-16; NEC Sanei Co., Tokyo, Japan) was used for continuous recording of blood pressure. Heart rate (HR) was measured with a calculator (NEC Sanei Co.) by way of phasic pressure. The other catheter was inserted into the right femoral vein for drug injection. To record SNA, the splanchnic nerve was placed over a bipolar stainless steel electrode and spike potentials were amplified with a Grass P15AC Amplifier (Grass Medical Instruments, Quincy, USA) and biophysiocomputer (NEC Sanei Co.) and monitored on a storage oscilloscope (CDR 5541; Kikusui Electric Co., Kanagawa, Japan). To quantify nerve activity, original analogue signals were fed into a spike counter (DSE 332P; Biomedical System, Branford, USA) whose output was recorded as a histogram and printed out digitally. The level of the window discriminator was routinely set to filter out any background noise (18). The upper trachea, esophagus, and surrounding musculature were excised. The bilateral longus capitis muscles were removed to expose the occipital foramen and the basal aspect of the occipital bone. The ventral surface of the medulla oblongata was then exposed. We used glass micropipettes with tip diameters of 50 µm for microinjections. The left RVLM was identified by a pressor response of more than 25 mmHg of mean arterial pressure by microinjection of Glu (2 nmol, 100 nl) (17, 19). A polyurethane cannula (outer diameter: 1.5 mm) was connected to a computer-controlled pneumatic pump, and a rubber membrane was attached to the opposite end (11). By pumping air set at 200 mmHg of the pressure inside the cannula and 300 cycle/min, the cannula was placed in contact with the ventral surface of the ipsilateral RVLM 1 mm dorsally.

Changes of blood pressure, HR and SNA were monitored under several conditions. 1) AngII (100 pmol) or Glu (2 nmol) was injected into the left RVLM 5 min after RVLM pretreatment with vehicle, candesartan (AngII type 1 (AT1) receptor antagonist, 2 nmol), PD123319 (AngII type 2 (AT2) receptor antagonist, 1 nmol), or kynurenic (Glu receptor antagonist, 3 nmol) (n = 6 each) (20). 2) The effects of these antagonists on sympathetic excitatory and pressor responses to pulsatile compression of the RVLM by pneumatic pump (300 cycles/min) were examined (n = 6 each). 3) In addition, to determine the effects of peripherally administered AngII antagonists on these pressor and sympathetic responses, candesartan (0.25 µmol) or PD123319 (0.05 µmol) was intravenously injected 5 min before pulsatile compression of the RVLM (n = 5 each) (21, 22). The doses of candesartan and PD123319, which were injected into the RVLM or intravenously, were decided according to the previous papers (20–22).

Statistical Analysis

The data were expressed as the mean ± SD. Results from two groups were compared using a paired Student’s t-test. P values less than 0.05 were considered statistically significant. Since the absolute value of SNA could vary from rat to rat, the changes of SNA were expressed as a percentage, and were compared using the Wilcoxon’s non-parametric test.
Results

Pressor and Sympathetic Excitatory Effects of Glu and AngII

Glu injected into the RVLM significantly increased MAP (Δchange 30 ± 7 mmHg, \( p < 0.05 \) vs. baseline) and SNA (% Δchange 13 ± 7, \( p < 0.05 \) vs. baseline) with a peak after 10 s of administration (Table 1). These sympathetic excitatory and pressor effects were significantly reduced by RVLM pretreatment with kynurenate (MAP: Δchange 7 ± 7 mmHg, \( p < 0.01 \) vs. vehicle; SNA: % Δchange 4 ± 5, \( p < 0.01 \) vs. vehicle), but were unaffected by candesartan (MAP: Δchange 22 ± 7 mmHg; SNA: % Δchange 11 ± 11), or PD123319 (MAP: Δchange 27 ± 7 mmHg; SNA: % Δchange 9 ± 5) (Fig. 1). AngII injected into the RVLM also significantly increased MAP (Δchange 15 ± 4 mmHg, \( p < 0.05 \) vs. baseline) and SNA (% Δchange 10 ± 3, \( p < 0.05 \) vs. baseline) with a peak after 1 min of administration (Table 1). These effects were significantly reduced by RVLM pretreatment with candesartan (MAP: Δchange 2 ± 2 mmHg, \( p < 0.01 \) vs. vehicle; SNA: % Δchange 1 ± 1, \( p < 0.01 \) vs. vehicle) or kynurenate (MAP: Δchange 6 ± 6 mmHg, \( p < 0.01 \) vs. vehicle; SNA: % Δchange 10 ± 10, \( p < 0.01 \) vs. vehicle) (Fig. 2).

Table 1. Cardiovascular and Sympathetic Responses to RVLM Injection of Glu and AngII, and Pulsatile Compression of the RVLM

<table>
<thead>
<tr>
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<th>Glu (n = 6)</th>
<th>AngII (n = 6)</th>
<th>Compression (n = 6)</th>
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<tbody>
<tr>
<td>Mean arterial pressure (mmHg)</td>
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<td></td>
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<tr>
<td>Baseline</td>
<td>76 ± 13</td>
<td>84 ± 14</td>
<td>66 ± 12</td>
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<tr>
<td>Δchange</td>
<td>30 ± 7*</td>
<td>15 ± 4*</td>
<td>8 ± 3*</td>
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<tr>
<td>Heart rate (bpm)</td>
<td></td>
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<tr>
<td>Baseline</td>
<td>304 ± 10</td>
<td>295 ± 17</td>
<td>280 ± 11</td>
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<tr>
<td>Δchange</td>
<td>3 ± 4</td>
<td>3 ± 3</td>
<td>2 ± 3</td>
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<tr>
<td>Sympathetic nerve activity (%)</td>
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<td></td>
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<tr>
<td>% Δchange</td>
<td>13 ± 7*</td>
<td>10 ± 3*</td>
<td>9 ± 5*</td>
</tr>
</tbody>
</table>

RVLM, rostral ventrolateral medulla; Glu, l-glutamate; AngII, angiotensin II. * \( p < 0.05 \) vs. baseline.

Fig. 1. Pressor and sympathetic responses to RVLM injection of L-glutamate. Open, closed, striped, and cross-hatched bars indicate the RVLM pretreatments with vehicle, kynurenate, candesartan, and PD123319, respectively. MAP, mean arterial pressure; SNA, sympathetic nerve activity. ** \( p < 0.01 \) vs. vehicle group.

Fig. 2. Pressor and sympathetic responses to RVLM injection of angiotensin II. Open, closed, striped, and cross-hatched bars indicate the RVLM pretreatments with vehicle, kynurenate, candesartan, and PD123319, respectively. Abbreviations are as in Fig. 1. ** \( p < 0.01 \) vs. vehicle group.
Pressor and Sympathetic Excitatory Effects of RVLM Compression

Pulsatile compression of the RVLM immediately increased MAP (Δchange 8 ± 3 mmHg, p < 0.05 vs. baseline) and SNA (% Δchange 9 ± 5, p < 0.05 vs. baseline), and these effects continued during RVLM compression and normalized soon after cessation of compression (Table 1, Figs. 3, 4). These sympathetic excitatory and pressor effects were significantly reduced by RVLM pretreatment with candesartan (MAP: Δchange 1 ± 5 mmHg, p < 0.05 vs. vehicle; SNA: % Δchange 1 ± 2, p < 0.05 vs. vehicle), or kynureinate (MAP: Δchange 0 ± 2 mmHg, p < 0.01 vs. vehicle; SNA: % Δchange 1 ± 2, p < 0.05 vs. vehicle), but were unaltered by PD123319 (MAP: Δchange 9 ± 11 mmHg; SNA: % Δchange 8 ± 6) (Fig. 3). HR was not significantly changed by pulsatile compression of the RVLM (Δchange 2 ± 3 bpm) (Table 1).

Effects of Intravenous Administration of Angiotensin Receptor Antagonists

To determine whether intravenously administered AngII antagonists would affect the responses induced by RVLM compression, candesartan and PD123319 were intravenously injected 5 min before pulsatile compression. Pressor and sympathetic excitatory responses to pulsatile compression of the RVLM were significantly reduced by intravenous pretreatment with candesartan (Fig. 5) but were unaltered with
The present study has demonstrated that pressor and sympathetic excitatory effects are evoked by microinjection of AngII and Glu in the RVLM, as previously reported (13–15, 23). These responses were attenuated by RVLM pretreatment with kynurenate. The response of AngII was reduced by RVLM pretreatment with candesartan, but the response of Glu was not affected. These responses were unaltered by RVLM pretreatment with PD123319. Therefore, it seems possible that AngII activates RVLM neurons via AT1 receptors and stimulates Glu release to thereby elicit sympathetic activation and pressor effects. As we reported previously, Glu released from RVLM neurons stimulated sequential neurons through NMDA receptors and induced a pressor response and sympathetic nerve activation via the production of NO, guanylate cyclase, cyclic GMP, and Fos, the protein product of c-fos (11). Supporting our results that AngII stimulates Glu release in the RVLM, Dampney et al. (23) and Zhu et al. (24) showed Glu release from RVLM vasomotor neurons by AngII stimulation. In contrast, another AT1 blocker, losartan, was reported to reduce the pressor effect of Glu in the RVLM (15), which indicates that losartan had an inhibitory effect on sympathetic-excitatory responses evoked by Glu. This conflict in results may be due to the different nature of the two drugs. Supporting this assumption, microinjection of losartan into the RVLM evoked pressor responses (unpublished data), whereas candesartan did not affect the basal sympathetic and cardiovascular activities.

Since Jannetta and Gendell first reported that neurovascular compression of the RVLM by surrounding vertebral artery, or anterior- or posterior-inferior cerebellar arteries was more common in essential hypertensives than normotensives (7), numerous studies using angiography or MRI have shown that neurovascular compression of the RVLM is a cause of essential hypertension (8, 9, 25–28). As stated earlier, on pulsatile compression of the RVLM, Glu was released from RVLM neurons and elicited a pressor response and sympathetic nerve activation. But the underlying mechanism of Glu release from RVLM neuron has remained unclear. In the present studies, pulsatile compression of the RVLM induced the pressor response and sympathetic activation, and these responses were reduced by RVLM pretreatment with candesartan and kynurenate, but were unaltered by RVLM pretreatment with PD123319. It therefore appears that, upon pulsatile compression of the RVLM, endogenous AngII stimulated vasomotor neurons through AT1 receptor and released Glu, thereby stimulating sequential neurons and evoking the sympathetic nerve activation and pressor response. It has been reported that endogenous AngII was increased in the RVLM under certain conditions, such as salt deprivation or heart failure, or in SHR (23), and AT1 blocker injected into the RVLM decreased blood pressure in these rats but not in normal rats. Therefore, local AngII might be involved in sympathoexcitatory and pressor responses to pulsatile compression of the RVLM, since an AT1 blocker, candesartan, injected into the RVLM reduced the sympathetic activation and pressor responses in the present study. It remains unclear how endogenous AngII was produced in the RVLM. However, because some glia cells or neurons contain whole components of the renin-angiotensin system, it seems possible that endogenous AngII could be produced in the astrocytes of the RVLM under certain conditions, such as pulsatile compression (29, 30).

Pressor and sympathetic excitatory responses to pulsatile compression of the RVLM were reduced by intravenous injections with AT1 receptor antagonist but not with AT2 receptor antagonist. It has been reported that orally administered AT1 receptor antagonist can pass through the brain blood barrier (31). Therefore, it is possible that orally administered AT1 antagonists have at least partial effects on the central nervous system, and especially on the RVLM, that lead to lowering of elevated blood pressure in essential hypertensives. Alternatively, intravenously injected AT1 recep-

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**Fig. 5.** The changes of mean arterial pressure and sympathetic nerve activity upon pulsatile compression of the RVLM. Open and striped bars indicate the intravenous pretreatments with vehicle and candesartan, respectively. Abbreviations are as in Fig. 1. **p < 0.01 vs. vehicle group.**

**Fig. 6.** The changes of mean arterial pressure and sympathetic nerve activity upon pulsatile compression of the RVLM. Open and striped bars indicate the intravenous pretreatments with vehicle and PD123319, respectively. Abbreviations are as in Fig. 1.
tor antagonists may affect peripheral sympathetic nerve endings to reduce norepinephrine release, which also contributes to blood pressure lowering. In conclusion, AngII may activate RVL M neurons via AT1 receptors and stimulate Glu release to thereby elicit sympathetic activation and pressor effects. Upon pulsatile compression of the RVL M, local AngII may activate RVL M vasomotor neurons via AT1 receptors and evoke sympathetic activation and pressor effects.

References