Expression of angiotensin II type 1 (AT₁) receptor in the rostral ventrolateral medulla in rats

LIAN HU,1 DA-NIAN ZHU,2 ZHANG YU,1 JOHN Q. WANG,1 ZHONG-JIE SUN,1 AND TAI YAO1,3
1Department of Physiology, 3Laboratory for Electronmicroscopy, and 2National Key Laboratory for Medical Neurobiology, Medical Center of Fudan University (Former Shanghai Medical University), Shanghai 200032, China

Received 23 March 2001; accepted in final form 28 December 2001

THE ROSTRAL VENTROLATERAL MEDULLA (RVLM) has been verified to be the basic area in central cardiovascular regulation. The sympathetic neurons in the RVLM provide descending impulses down to the preganglionic sympathetic neurons in the intermediolateral column (IML) of the spinal cord and function as the major source of sympathetic excitatory activities from the brain. It has been known that administration of angiotensin II (ANG II) into the RVLM increases the mean arterial pressure (MAP) and the sympathetic nerve activity (SNA) (1, 2, 13, 37, 39). ANG type 1 (AT₁) receptor is its major receptor subtype in the brain of adult animals that has been characterized pharmacologically (2, 9, 13, 33, 39) and morphologically (1, 34, 48). The pressor effect of ANG II in the brain mainly results from the activation of AT₁ receptors (25, 44–46). However, electrophysiological studies from different laboratories differ and conflict with each other (4, 5, 41, 42). There are no constant results on ANG II-responsive neurons in the RVLM. On the other hand, there were reports (3, 48) showing that distribution of AT₁ receptors overlapped with tyrosine hydroxylase (TH) in some brain areas, including RVLM. Some investigators (18) observed by in vitro patch-clamp studies that ANG II evoked depolarization in some catecholaminergic spinal projecting neurons. Presympathetic motoneurons were thought to be catecholaminergic neurons in the past decades (8, 49). Those observations lead to an impression that activation of catecholaminergic systems contributes to the pressor response to ANG II application in the RVLM. However, it has been proposed that glutamatergic, other than catecholaminergic, neurons are the most probable candidates for sympathetic vasomotor neurons that project to the spinal cord (23, 24, 27, 28, 33). There is evidence showing that central catecholaminergic pathways mainly contribute to inhibition of renal sympathetic motoneurons caused by endogenous ANG II in the brain (8, 38). Furthermore, only some of the catecholaminergic neurons were overlapped with AT₁ receptor immunoreactivity, and both catecholamine and noncatecholamine neurons were activated by ANG II (44). On the basis of the above reasons, the main aim of this study was to examine the relationship of AT₁ receptors with gluta-

Address for reprint requests and other correspondence: Tai Yao, Dept. of Physiology, Medical Center of Fudan Univ., Shanghai 200032, P. R. of China.
release in the IML during microinjection of ANG II into the RVLM. There is evidence indicating that spontaneously hypertensive rats (SHR) are more sensitive to ANG II than normotensive rats, producing a greater pressor response to ANG II application in the RVLM (4, 5, 29, 49). Microinjection of ANG II receptor agonist into the RVLM has been found to evoke greater depressor response in SHR than in Wistar Kyoto rats (4, 29). In this study, we applied immunohistological and immunogold staining combined with electron microscopy to detect the precise distribution of AT_1 receptors in the RVLM of SHR and Wistar rats; meanwhile, some differences in distributions between the two species were analyzed.

**METHODS**

*General preparation for in vivo experiment.* Male Wistar rats (250–300 g, n = 12) were anesthetized with urethane (800 mg/kg ip) mixed with chloralose (35 mg/kg). The common carotid artery was catheterized to monitor arterial pressure throughout the experiment. Rectal temperature was maintained at 37.5 ± 0.5°C by using an infrared lamp and a warm pad. The animals were air ventilated via trachea intubation. Under dissection microscope, the spinal cord at thoracic T_8 segment was exposed by a dorsal incision.

*Microinjection in RVLM and microdialysis procedure at the T_8 segment.* Anesthetized rats were placed in the prostrate position with their heads fixed in a stereotactic apparatus (Narishige, SN-3) and tilted forward at 45° to make the medulla oblongata horizontal. An ocipital incision was made, and the dorsal surface of the medulla oblongata was exposed. Microinjection (0.1 μl) of L-glutamate (100 pmol) or ANG II (100 pmol) was carried out by a fine stainless steel tube (0.2 mm outer diameter), which was inserted vertically and stereotaxically into the RVLM. In microdialysis experiments, an incision was made on the back to expose the thoracic spinal segment of the level of T_8. The bone and meningeal membrane were carefully removed to expose the surface of the spinal cord. A microdialysis probe (outer diameter 220 μm, length for effective dialysis 0.5 mm, EICOM) was inserted into the spinal cord (T_8) 450 μm lateral to midline and 900 μm below the dorsal surface ipsilateral to the microinjection site. The tip of the probe was in the IML. Perfusion was made through the probe at 2 μl/min with artificial cerebrospinal fluid (in mmol/l: 124 NaCl, 25 NaHCO_3, 3.3 KCl, 0.4 KH_2PO_4, 1.2 MgSO_4, 2.0 CaCl_2; pH 7.4). At least a 90-min balanced period was needed before sample collections. Each sample (20 μl) was collected manually within 10 min. In vivo recovery rates of amino acids through the microdialysis membrane were determined as follows: the microdialysis probe was placed in a standard solution (25 pmol each of aspartate, glutamate, glutamine, glycine, and taurine) and perfused at a rate of 2 μl/min for 10 min, then the actual concentration was measured by HPLC-fluorescence detection. The recovery rates of aspartate, glutamate, glutamine, glycine, and taurine were 12.20 ± 1.50, 15.71 ± 1.30, 11.31 ± 1.81, 10.70 ± 1.62, and 19.20 ± 0.41% (n = 4), respectively. All the amino acids were purchased from Sigma Chemical. At the end of the experiment, the rat was killed with overdose anesthesia and transcardially perfused with 10% formaldehyde solution. The brain and thoracic spinal cord were rapidly removed, and 30-μm-thick transverse sections of brain stem and spinal cord were cut on a freezing microtome to verify the locations of the microinjection tube and microdialysis probe according to the atlas of Paxinos and Watson (31).

*Amino acid analysis by HPLC.* Amino acids in each sample were separated by reverse-phase HPLC and fluorescence detection (Gold System, Beckman) with C18 ultrasphere octadecyl silane (ODS) column (average particle size 5 μm, Beckman). The samples were derivatized by orthophthaldehyde (Sigma Chemical). The derivatized reagent was made up in the proportions by dissolving 27 mg of orthophthalaldehyde in 5 ml absolute ethanol, adding 5 ml 0.1 M sodium tetraborate (pH 9.5–9.6) followed by 40 μl of β-mercaptoethanol (Sigma Chemical). Twenty microliters of the sample was mixed with 9 μl of derivatized reagent for exactly 120 s before being injected into the HPLC system. The mobile phase was comprised of 62% 0.1 M KH_2PO_4 (pH 6.0), 30% methanol, and 3% tetrahydrofuran at a gradient flow rate from 1.0 up to 1.98 ml/min during 10 min. The excitation and emission wavelengths were 280 and 340 nm, respectively. The relative fluorescence unit was set at 0.01. For each sample, the analysis time was not more than 21 min.

*General preparation for morphological experiments.* Male Wistar rats (250–290 g) and SHR (280–310 g) were anesthetized with chloral hydrate (300 mg/kg). The animals were then transcardially perfused with 150 ml saline followed by 250 ml 4% paraformaldehyde in 0.1 M sodium phosphate buffer (0.1 M PB; pH 7.4). The brain was rapidly dissected and postfixed in the same fixative solution at 4°C.

*Frozen section preparation.* After 6 h of postfixation at 4°C, the brains (n = 4 in Wistar rats and n = 3 in SHR) were washed several times in 0.1 M PB (pH 7.4), then transferred sequentially into 20 and 30% sucrose in 0.1 M PB to be cryoprotected overnight until sinking. Transverse serial medullary sections (30 μm thick) were cut with a microtome (Reichert-Jung) at 1.5–1.7 mm rostral to the obex according to Paxinos and Watson’s atlas (31).

*Paraffin sections preparation.* Thirty-six hours after fixation at 4°C, paraffin-embedded brains (n = 5 in each group) were prepared as usual and cut into 5-μm-thick serial horizontal sections at 1.5–1.7 mm rostral to the obex according to Paxinos and Watson’s atlas (31).

*Double staining of AT_1 receptor with glutamate, GABA, and TH, respectively.* Sections were immersed in 4% paraformaldehyde for 10 min followed by 6 × 5-min washing in 0.01 M phosphate-buffered saline (0.01 M PBS, pH 7.4). Free floating sections were firstly incubated in 2% BSA and 0.2% Triton X-100 in 0.01 M PBS for 30 min at 37°C to eliminate nonspecific binding, then sequentially in the following antibodies for 1 h at 37°C and additional 24 h at 4°C for the first primary antibody (Table 1).

After incubation, all sections were detected with a confocal microscope (Leica, TCS-NT). Those in which the cell body showed obvious nuclei were identified as positive cells. The number of positive glutamatergic, TH-immunoreactive (TH-IR), and GABAergic cells and the percentage of those cells double labeled with AT_1 receptors was calculated. Negative counterstaining was performed by replacing either one or both primary antibodies with nonimmune serum from the same species. All negative controls displayed no detectable staining.

*Immunocytochemical staining and image analysis.* One section from every five serial sections was picked up, with a total of six sections in each animal. The paraffin sections were dewaxed with xylene, then immersed into gradient diluted alcohols and distilled water. To block endogenous peroxidase activity, sections were incubated in 3% H_2O_2 in 0.01 M PBS for 15 min. Nonspecific staining was blocked by 2% normal goat serum (Vector, diluted in PBS) for 30 min at
37°C. Sections were incubated in rabbit AT1 antibody (polyclonal anti-AT1, Santa Cruz, 1:50 diluted in 0.01 M PBS containing 1% goat serum) for 45 min at 37°C followed by 24 h at 4°C. These sections were further washed and incubated with biotinylated goat anti-rabbit IgG (Vector, 1:200) for 30 min at 37°C, rinsed in 0.01 M PBS, and incubated with avidin-biotin complex for 30 min at 37°C according to the manufacturer’s instructions. Diaminobenzidine 0.05% (Sigma Chemical, diluted in Tris-buffered saline containing 0.01% H2O2) was applied for chromogen. Finally, all sections were prepared for image analysis (Q500IW). There were 30 sections to be analyzed in each group (n=5). Optical density (OD) is used to calculate the pixels per area. The OD values gradually ascend from white to black. The mean OD (MOD) of each feature (e.g., a positive substance) was calculated from the value of OD of every pixel within an area the feature occupied, representing mean expression level for a positively-stained substance. To analyze the intracellular part and the edge of the cell separately, 0.226 mm of thickness as one pixel was eroded from the border of the cell to be identified as “cell surface,” which includes the cytoplasmic membrane and the area below. Cells that showed an obvious outline of the nuclei were picked up for image analysis. With the use of a similar staining method as described above in the staining of the AT1 receptor, polyclonal rabbit AT2 receptor antibody (presented by Prof. Robert M. Carey, University of Virginia Health Sciences Center, 1:50) was also applied to detect AT2 receptor expression. Nonimmune rabbit IgG (1:5 dilution) replacing the primary antibody gave no detectable staining.

Immunogold staining and electronmicroscopy. Two sections from each animal, with a total of 10 sections in each group (n=5 animals) at similar levels, were chosen for immunogold staining by preembedded method. Frozen sections were first incubated with rabbit polyclonal anti-AT1 antibody (Santa Cruz, 1:50 diluted in 0.01M PBS containing 1% BSA) for 45 min at 37°C followed by 24 h at 4°C. Second, they were incubated with colloidal gold (10 nm)-conjugated protein A (preparation by Ms. Dan Gong, 1:10 diluted in the same solution as the primary antibody) for 2 h at 37°C. Before incubation, the sections were immersed in 0.01 M PBS with 0.5% Triton X-100 for 30 h to enhance the penetration of proteins through the cell membrane. After incubation, sections were fixed in 2.5% glutaraldehyde for 30 min, dehydrated, and embedded. Ultrathin sections were observed under a JEOL 1200EX transmission electron microscope at 80 KV.

Statistical analysis. Data were expressed as means ± SE for the microinjection and microdialysis experiments, statistically analyzed by paired t-test. The MOD was expressed as means ± SE, statistically analyzed by two-way ANOVA. Statistical significance was set at P < 0.05.

RESULTS

Microinjection of ANG II in RVLM immediately increased the release of aspartate and glutamate in the IML with an elevated MAP. Observations were made in Wistar rats (n=12) with basal MAP and HR levels of 96.4 ± 13.3 mmHg and 315.9 ± 12.6 beats/min, respectively. Microinjection of either l-glutamate (100 pmol) or ANG II (100 pmol) into the RVLM (n=12) resulted in a significant increase in MAP (ΔMAP was 15.45 ± 4.22 for L-glutamate and 15.29 ± 5.20 for ANG II; P<0.05) but no significant change in HR (P>0.05).

The basal levels of various amino acids released in the IML of T8 segment (n=11) were: aspartate 4.8 ± 1.0 pmol/20 μl; glutamate 19.0 ± 8.6 pmol/20 μl; glutamine 22.8 ± 15.2 pmol/20 μl; glycine 4.5 ± 2.3 pmol/20 μl; taurine 88.0 ± 20.7 pmol/20 μl.

Microinjection of ANG II (100 pmol, n=11) into the RVLM significantly (P<0.01) increased the release of aspartate (8.9 ± 2.3 pmol/20 μl) and glutamate (73.9 ± 29.3 pmol/20 μl) in the IML. There was no significant change (P>0.05) in taurine release (85.1 ± 20.6 pmol/20 μl). All changes returned to basal levels within the third 10-min period after treatment. Glutamine (32.2 ± 17.8 pmol/20 μl) and glycine (5.2 ± 2.6 pmol/20 μl) release did not change after ANG II injection (P>0.05). The chromatographic recordings are shown in Fig. 1.

Localization of AT1 receptor in glutamate immunoreactive (glutamatergic), TH-IR, and GABA immunoreactive (GABAergic) neurons. Most of the glutamatergic neurons (62–91% in Wistar rats; 73–92% in SHR), GABAergic neurons (56–78% in Wistar rats; 53–84% in SHR), and TH-IR neurons (74–93% in Wistar rats; 67–91% in SHR) in the RVLM were double labeled with AT1 receptor (Fig. 2).

Differences in distribution of AT1 receptor on the rostral medulla oblongata neurons between SHR and Wistar rats. In the rostral medulla, AT1 receptor immunoreactive cells were present in the inferior olive, nucleus ambiguus, reticular formation including the nucleus paragigantocellularis lateralis and C1 area, nucleus tractus solitarius, and the areas surrounding the fourth ventricle. High immunointensity was observed in the nucleus ambiguus and areas surrounding the fourth ventricle, whereas the inferior olivary and reticular formation showed a moderate intensity. MOD

Table 1. Immunofluorescence double staining of AT1 receptor with glutamate, GABA, or TH

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<th>Glutamate and AT1</th>
<th>GABA and AT1</th>
<th>TH and AT1</th>
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<tr>
<td><strong>First primary antibody</strong></td>
<td>Mouse monoclonal anti-AT1 (6313G2, presented by Prof. Gavin P. Vinson, 1:50)</td>
<td>Mouse monoclonal anti-GABA (Sigma Chemical, 1:1,000)</td>
<td>Rabbit polyclonal anti-AT1 (Santa Cruz, 1:80)</td>
</tr>
<tr>
<td><strong>First secondary antibody</strong></td>
<td>Rhodamine-conjugated anti-mouse IgG (Dako, 1:40)</td>
<td>Rhodamine-conjugated anti-mouse IgG (Dako, 1:40)</td>
<td>Rhodamine-conjugated anti-mouse IgG (Dako, 1:40)</td>
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<tr>
<td><strong>Second primary antibody</strong></td>
<td>Rabbit polyclonal antiglutamate (Sigma Chemical, 1:3,000)</td>
<td>Rabbit polyclonal anti-AT1 (Santa Cruz, 1:50)</td>
<td>Mouse monoclonal anti-TH (Sigma Chemical, 1:4,000)</td>
</tr>
<tr>
<td><strong>Second secondary antibody</strong></td>
<td>FITC-conjugated anti-rabbit IgG (Dako, 1:40)</td>
<td>FITC-conjugated anti-rabbit IgG (Dako, 1:40)</td>
<td>FITC-conjugated anti-rabbit IgG (Dako, 1:40)</td>
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TH, tyrosine hydroxylase.
for the whole cell or cell surface in the RVLM was significantly higher ($P < 0.05$) in SHR ($0.36 \pm 0.03$ and $0.35 \pm 0.03$, respectively, $n = 5$) than in Wistar rats ($0.23 \pm 0.03$ and $0.21 \pm 0.03$, respectively, $n = 5$). MOD for the intracellular part also appears to be higher in SHR ($0.36 \pm 0.04$) than in Wistar rats ($0.26 \pm 0.04$), although it is not statistically significant ($P > 0.05$; see Fig. 3).

**Negative immunoreactivity of AT$_2$ receptor in the RVLM.** AT$_2$ receptor immunoreactivity was also examined in SHR and Wistar rats. There was no detectable staining of AT$_2$ receptor in the rostral medulla, although a few positive cells were observed in the inferior olive nucleus (Fig. 4).

**Subcellular localization of AT$_1$ receptor in RVLM by immunoelectronmicroscopy.** In both groups of rats, many immunogold particles (10 nm) were observed on rough endoplasmic reticulum (RER) as well as the location on the membrane of cells. The gold particles were also observed in some neuronal processes (Fig. 5).

**DISCUSSION**

Generally, consistent pressor effects are observed when ANG II is applied into the RVLM. Regarding ANG II-evoked increases in MAP, SNA, and neural

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**Fig. 1.** Chromatography of amino acids release in the intermediolateral column of the spinal cord IML induced by microinjection of ANG II (100 pmol) into the rostroventrolateral medulla (RVLM). A: after saline application. B: first 10-min period after microinjection of ANG II (100 pmol). C: recovery of amino acids released after microinjection of ANG II.

**Fig. 2.** Confocal microscopic demonstration of angiotensin AT$_1$ receptor expressed in glutamatergic cells (A1, A2), tyrosine hydroxylase-immunoreactive (TH-IR) cells (B1, B2) and GABAAergic cells (C1, C2). Top (green) and bottom (red) part of the left of each photo show staining of a single substance, whereas yellow dye on the right presents the colocalization of the former 2 substances. A2, B2, and C2 are a magnifications of A1, B1, and C1, respectively.
activity, previous investigators paid greater attention to the role of catecholaminergic pathways than to amino acid pathways (16, 18, 27, 48). However, according to the hypothesis that glutamatergic neurons in the RVLM projecting to the spinal cord ranks as the top possibility to be vasomotor neurons (23, 24, 27, 28), it is very important to investigate the relations of AT₃ receptors with glutamatergic neurons in the RVLM. Our observation of AT₁ receptors overlapped with large population of glutamatergic neurons indicates, for the first time, that the ANG II-evoked pressor effect via AT₁ receptor in the RVLM is closely related to glutamatergic pathways. Although glutamatergic neurons in the RVLM have various projections to other central areas, the IML of the spinal cord is one of the major projecting destinations (6). In the present study, a high percentage of glutamatergic neurons colocalized with AT₁ receptor-positive neurons was found, up to 92% in some cases. Thus at least part of them are probably sympathoexcitatory vasomotor neurons and directly

Fig. 3. A: expression of AT₁ receptor in the RVLM as well as in inferior olive nucleus (infer. oliv) and nucleus ambiguous (Amb) in a Wistar rat and a spontaneously hypertensive rat (SHR) (lower magnification). B: cells in RVLM showed stronger staining in SHR than that shown in Wistar rats, especially on the cell surface (higher magnification).
projecting to the IML of the spinal cord. The prompt responses of glutamate release in the spinal cord to ANG II or/and losartan treated in the RVLM suggest that exogenous ANG II in the RVLM elevates MAP by increasing the release of excitatory amino acids, like glutamate and aspartate in the IML, and in turn exciting the preganglionic sympathetic neurons. Taking the results together, one of the important possibilities for mechanisms of ANG II-evoked pressor effects is that ANG II activates AT1 receptors on the glutamatergic neurons, which then further elevates directly or indirectly the sympathoexcitatory outflow to the IML. Our results provide indirect evidence that supports the previous hypothesis (2, 5, 11, 15, 19, 44) that ANG II activates sympathetic vasomotor neurons in the RVLM. The release of glutamate in the IML responding to ANG II also indirectly supports the hypothesis that spinally projecting neurons from RVLM to IML are glutamatergic (17, 24, 28).

The second interesting finding in the present study is the expression of AT1 receptors in GABAergic neurons in the medulla oblongata. The results suggest that ANG II in the RVLM not only acts on excitatory neurons but also on inhibitory neurons. The unspecific targeting property of the AT1 receptor in the RVLM implies the complex roles of ANG II in the central nervous system. Under natural conditions, a conversion from ANG I to ANG II probably takes place extracellularly, as angiotensin-converting enzyme is an ectoenzyme. Therefore, neurons and glia may behave in a paracrine fashion in producing ANG II (22, 33). The diffusing ANG II would act on unspecific target cells, unlike the classical synaptic transmission pattern (33).

With regard to our present results, activation of AT1 receptor on GABAergic cells, which have connections with glutamatergic cells, may cause desensitization of the latter neurons to ANG II stimulation (45). The inhibitory GABAergic neurons may functionally counterbalance the pressor effect of ANG II because it has been confirmed that GABA and L-glutamate are not colocalized in the same neuron and nerve terminal (23). Therefore, the result of ANG II administration in the brain may vary in different conditions, depending on the balance of sympathoexcitatory and sympathoinhibitory effects. Although the main effect of ANG II in the brain is sympathoexcitatory, the role of ANG II may switch to sympathoinhibitory while animals are set under some specific conditions, such as for conscious animals (10, 12) or in hypoxia (8). However, which and why one of the two opposing effects will predominate in specific conditions have not been at all well clarified. The above reports suggest that cat-
echolaminergic pathways contribute to the inhibition of SNA caused by ANG II administration (8, 10, 12, 16). Our present results obviously suggest the importance of the GABAergic system in regulating the role of brain ANG II. Nevertheless, the activation of the excitatory neurons predominated over that of the inhibitory GABAergic neurons in response to ANG II with the dose used in our present experiment. Whether the GABAergic neurons activated by ANG II play a role in the subsequent ANG II-produced baroreflex inhibition remains to be elucidated. The expression of AT1 receptor in TH-IR neurons (catecholaminergic neurons) in the RVLM is consistent with some other reports (8, 48). Our results differ from the previous double-labeling studies, which did not investigate the distribution of AT1 receptor in glutamatergic and GABAergic cells in the RVLM. ANG II was supposed to selectively act on catecholaminergic neurons within the brain (16, 18, 27, 48). Our results indicate that ANG II acts as an important modulator or transmitter with the extensive distributions of its receptors. Some researchers reported that 17–27% of glutamatergic neurons in the RVLM contained phenylethanolamine-N-methyltransferase immunoreactivity (27). It is therefore interesting to further study whether the AT1 receptor is located in glutamatergic neurons containing TH immunoreactivity.

It has been reported that the number of ANG II receptors in some brain areas, such as circumventricular organs, is greater in SHR than that in normotensive rats (25, 36, 40). It might account for some types of hypertension that are related to brain ANG. Similarly, the present results showed that the average level of AT1 receptors in the cells of RVLM was significantly greater in SHR than that in normotensive Wistar rats. Furthermore, it was also highlighted that the expression in cell surface of the SHR was higher. AT1 receptor is characterized as a seven-transmembrane domain G protein-coupled receptor, which needs to translocate to the cell membrane to bind to its extracellular ligand (ANG II). Thus cell surface expression level should make sense representing the prompt responsiveness of the cell to stimuli from extracellular ANG II. Thus our results were consistent with the other reports that SHR is more sensitive to acute ANG II application to the RVLM (4, 29, 48). Although the MOD value in SHR was bigger than in Wistar rats, there was no statistical significance on intracellular expression between the two species in the present experiment. The importance of the intracellular AT1 receptors remains to be studied.

Immunogold staining and electronmicroscopic studies verified that the extensive intracellular staining of AT1 receptors under light microscopy was distributed in RER, which reflects the receptor biosynthesis and further translocation to the cell membrane. Cytoplasmic ANG II binding sites have been reported in the brain (32, 48). Researchers hypothesized that the cytoplasmic receptors might form endocytic vesicles, suggesting receptor-ligand internalization (14, 32, 45). However, AT1 receptors on the RER demonstrated in the present experiment do not appear to represent a receptor desensitization or resensitization process related to internalization. The expression of AT1 receptor in the nerve process indicates a different function from that in the cell body. It has been known that AT1 receptors act on presynapse as well as postsynapse (35). Activation of the presynaptic AT1 receptors on the nerve terminals facilitates the transmitter release from the terminals. There are reports that projections from paraventricular nuclei (PVN) to RVLM are ANG II immunoreactive (20, 21). A recent report from Tagawa and Dampney (43) suggests that there exist functional AT1 receptors on the projections to the RVLM. The authors observed that the increase of MAP and SNA evoked by injection of bicucullin into the PVN could be attenuated by injection of losartan into the RVLM. This is an example that AT1 receptors in the RVLM may mediate ANG II release from PVN-RVLM terminals. A report from Zhu et al. (49) demonstrated that microinjection of ANG II into the RVLM increased glutamate release in the same area. This is an example that AT1 receptors may regulate the release of glutamate input to the RVLM and in turn affect the sympathetic outflow. In another experiment (data not published), we observed by patch clamp on rostral medulla slices that both frequency and amplitude of miniature excitatory postsynaptic current were regulated by application of ANG II in the bath solution. Combined together, the processes in the RVLM expressing AT1 receptors are morphological clues to presynaptic mechanisms of the AT1 receptors in the RVLM, no matter that they are projected from either inside or outside of the RVLM.

It has been demonstrated that AT2 receptor subtype predominates in all tissues in fetuses but persists only in very restricted tissues in adults, including such brain areas as septum, thalamus, colliculi, locus ceruleus, and inferior olive (7, 26, 32, 46). The AT2 receptor tends to be reexpressed or upregulated in some pathological disorders (30, 47). There were no AT2 receptors detected in the RVLM either in SHR or Wistar rats in the present experiment, indicating that AT2 receptor is not involved in the RVLM ANG II-related pressor effect.

In summary, AT1 receptors in the RVLM are not only located in catecholaminergic neurons, more specifically also in glutamatergic and GABAergic neurons. In combination with the finding that ANG II applied to the RVLM resulted in an prompt increase in the release of glutamate and aspartate in the IML of the spinal cord and a concomitant pressor response, glutamatergic pathways in the RVLM may act as important mechanisms contributing to ANG II-produced pressor effects. The results indirectly support the hypothesis that exogenous ANG II in the RVLM activate vasomotor neurons in this area, leading to an increase in sympathetic nerve activity and arterial blood pressure. The existence of AT1 receptors on the GABAergic neurons in the RVLM suggests a speculation that the intensity of ANG II-induced pressor effect may depend on a balance between activation of the excitatory glutamatergic neurons and the inhibitory GABAergic neurons.

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The higher density of AT1 receptors in the RVLM of SHR than that in the normotensive controls suggests the possible role of ANG II in the pathogenesis of hypertension. Its further mechanisms remain to be elucidated.

We are grateful to Prof. Gavin P. Vinson, Department of Biochemistry, Queen Mary and Westfield College, University of London, United Kingdom, for kindly presenting mouse monoclonal anti-AT1 (6313/G2) antibody. We also thank Prof. Robert M. Carey, Department of Medicine, University of Virginia Health Sciences Center, for kindly providing polyclonal rabbit AT2 receptor antibody. Dan Gong and Jun Zhou from China Gene Tech Biotechnology kindly helped us prepare the colloidal gold-conjugated protein A.

This work was supported by a grant from National Natural Science Foundation (39970267) of China and the Chinese Medical Board of New York.

Present addresses: J. Q. Wang, Division of Pharmacology, School of Pharmacy, Univ. of Missouri-Kansas City, MO 64108; Z.-J. Sun, Dept. of Physiology, College of Medicine, University of Florida, Gainesville, FL 32610-0274.

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