Distribution and Role of Angiotensin II Receptor in the Brain of Spontaneously Hypertensive Rat

KENJI MIZUNO, SYUICHI SHIGETOMI, JUN-ICHIROH MATSUI, SOITSU FUKUCHI

ALTHOUGH angiotensin II plays an important role in maintenance of extracellular fluid volume and blood pressure, the action of which are considered to be peripheral. On the other hand, it is now known that this peptide also acts upon central nervous system to regulate blood pressure. When administered centrally, angiotensin II causes a potent pressor effect exerted by release of antidiuretic hormone and increase sympathetic discharge, and it induces a drinking response in rat. The central loci of this well-known dipsogenic action have been identified by localized administration of angiotensin II into brain. The most sensitive region of this drinking response has been reported to be the septum, the hypothalamus or the preoptic area.

However, to date central site of pressor action of angiotensin II has been scarcely elucidated. Furthermore, a role of angiotensin II receptor in brain with regard to maintenance or development of hypertension has remained to be obscure.

In this report, regional distribution and extent of specific angiotensin II receptor in brain of spontaneously hypertensive rat (SHR) is described by means of a radioligand receptor assay of angiotensin II, and, relationship between changes in binding activity of receptor for angiotensin II and development of hypertension is further reported.

MATERIALS AND METHODS

Rats and brain dissection
Twenty-three female SHR were used for receptor studies. They were classified into two groups by their ages; group I (10 SHR), aged 8–10 weeks, and group II (13 SHR), aged 20–21 weeks. The rats were decapitated after measurement of blood pressure with the method of tail-cuff, and the brains were supplied for receptor assay.

The brains were initially dissected into regions of the cortex, midbrain, thalamus, hypothalamus and striatum according to the procedure of Glowinski and Iversen.

Radioligand receptor assay
Angiotensin II binding activity of the receptor freshly prepared from brain tissues was assayed by modification of the method by Glossman et al. The brain tissue from each region was weighed and homogenized in 20 volumes of saline at 4°C in a Potter-Elvehjem glass homogenizer fitted with a teflon pestle at 1500 rev./min with 15 up and down strokes. The homogenate was then centrifuged at 50,000 × g for 30 min at 4°C and the pellet was resuspended in 10 volumes of 150 mM NaCl, 5 mM ethylenediamine tetraacetic acid (EDTA), 50 mM Tris-HCl (pH 7.4). Portions of the particulate fraction containing 300 µg were incubated for 15 min at 22°C in 300 µl assay buffer containing 150 mM NaCl, 5 mM dithiothreitol, 5 mM EDTA, 50 mM Tris-HCl (pH 7.4), 0.2 % bovine serum albumin and 3H-angiotensin II (about 120,000 cpm).

Key Words:
Brain tissue
Angiotensin II receptor
Spontaneously hypertensive rat
Radioreceptor assay

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After incubation, the samples were placed on ice, diluted with 1.0 ml of cold buffer (150 mM NaCl, 50 mM Tris-HCl) and filtered through Whatman GF/C glass fiber discs (2.5 cm in diameter). The filters were then washed by 6.0 ml of cold buffer and radioactivity remained on filters were determined. Non-specific binding was determined in a separate incubation to which an excess of unlabelled angiotensin II (1 Asp-5 Ile-angiotensin II) was added to saturate the receptor.

All incubations were performed in triplicate. The specific binding was calculated from differences of radioactivity between total binding and non-specific binding. Radioactivity on the glass filters was determined by counting in a liquid scintillation spectrometer. For liquid scintillator counting the filters were placed in a vial to which was added 1.0 ml of distilled water and 10.0 ml of scintillation solution (Aquasol II). Protein determination was performed according to the method of Lowry et al.9

RESULTS

Blood pressure

In the group I the systolic blood pressure was 144.3 ± 7.5 (mean ± S.D.) mmHg. On the other hand, the systolic blood pressure in the group II was significantly high of 189.5 ± 11.5 mmHg.

Properties of angiotensin II binding

Figure 1 shows relationship between protein concentration of angiotensin II receptor in each of the brain particles and specific binding of labelled angiotensin II. Bindings were linear over the protein concentration range from 100 to 900 µg of incubation mixture. In range from 200 to 800 µg protein, receptor fraction from the thalamic region showed the highest binding activity compared with other fractions.

The binding reaction of receptor fraction to labelled angiotensin II was remarkably rapid during first 3 min of incubation, and then proceeded gradually during 30 min over which observations were made, as shown in figure 2.

The binding activity of labelled angiotensin II to each of the brain particles reached a zenith at 22°C of incubation temperature, but it was significantly reduced at 0°C and 52°C.

The inhibition of labelled angiotensin II binding was studied with various concentrations.
Fig. 2. Time course of specific $^3$H-angiotensin II binding to receptor fraction from the thalamic region. The result shows mean ± S.D. of 3 experiments. Incubation condition, 22°C in 5 mM EDTA buffer.

of unlabelled angiotensin II ranging from $10^{-10}$ to $10^{-4}$ M. As shown in table I, unlabelled angiotensin II reduced the binding of receptor fractions to labelled angiotensin II, by about 89% in the thalamic region, about 85% in the hypothalamic region, about 77% in the cortex, about 84% in the striatum and about 80% in the midbrain at $10^{-4}$ M of unlabelled angiotensin II concentration (Table I).

**TABLE I INHIBITORY EFFECT OF UNLABELLED ANGIOTENSIN II ON BINDING OF $^3$H-ANGIOTENSIN II TO VARIOUS FRACTIONS OF SHR BRAIN**

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Molar concentration of unlabelled angiotensin II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$10^{-4}$</td>
</tr>
<tr>
<td>Thalamus</td>
<td>89 ± 4</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>85 ± 7</td>
</tr>
<tr>
<td>Cortex</td>
<td>77 ± 10</td>
</tr>
<tr>
<td>Striatum</td>
<td>84 ± 6</td>
</tr>
<tr>
<td>Midbrain</td>
<td>80 ± 2</td>
</tr>
</tbody>
</table>

Comparison of specific angiotensin II binding between two groups of SHR

Figure 3 shows the result obtained from comparison of labelled angiotensin II binding activity to brain particles between two groups of SHR. In group II, the receptor fraction from the hypothalamic region bound to labelled angiotensin II at the highest degree of 40.3 ± 0.6%, while
in group I the receptor fraction from the thalamic region bound to the labelled angiotensin II at the highest degree of 32.0 ± 1.1%. The binding activity of labelled angiotensin II to the hypothalamic receptor fraction was greater in group II than that of group I; the difference was about 10%. Also the angiotensin II binding was greater to the thalamic receptor fraction in group II than that of group I by about 6%, while binding activity of labelled angiotensin II was almost equally in other receptor fractions between two groups of SHR.

Binding to the particles prepared from each of the brain tissues was studied over a range of labelled angiotensin II concentration from 0.1 to 1.5 mM in two groups of SHR, and the results were analyzed by Scatchard plot (Fig. 4). In group I, the maximal specific binding (Bmax) of angiotensin II was the greatest of 2.9 fmol/mg protein in the thalamic receptor fraction, and Bmax in the hypothalamic receptor fraction was about 2.1 fmol/mg protein. On the contrary, in group II Bmax was the greatest of 2.2 fmol/mg protein in receptor fraction from the hypo-

**TABLE II  MAXIMAL BINDING ACTIVITY OF ANGIOTENSIN II IN SHR BRAIN**

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Number of experiments</th>
<th>Maximal binding (Bmax) (mean ± S.D., fmole/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>group I</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>3</td>
<td>2.20 ± 0.04</td>
</tr>
<tr>
<td>Thalamus</td>
<td>3</td>
<td>2.90 ± 0.14</td>
</tr>
<tr>
<td>Cortex</td>
<td>3</td>
<td>0.78 ± 0.07</td>
</tr>
<tr>
<td>Midbrain</td>
<td>3</td>
<td>2.01 ± 0.14</td>
</tr>
<tr>
<td>Striatum</td>
<td>3</td>
<td>1.21 ± 0.14</td>
</tr>
</tbody>
</table>

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lamic region, and Bmax was about 2.0 fmol/mg protein in the thalamic receptor fraction.

Table II depicts the results obtained from Scatchard plot method between two groups. The maximal binding activity of angiotensin II was greater in receptor fraction of group I thalamic region than that of group II, while it was greater in the fraction from group II hypothalamic region than that in group I. Bmax of angiotensin II to receptor fractions of the cortex, midbrain and striatum was similar in two groups.

DISCUSSION

The present studies have demonstrated characteristics of binding activity for angiotensin II in brain particles prepared from SHR. Although previous study by Glossman et al.9 had indicated very little binding activity of angiotensin II to brain tissues despite well-known central action of angiotensin II, our study has shown a significant specific binding activity of angiotensin II confined to each of brain regions, in particular to the thalamus and the hypothalamus.

The central pressor action of angiotensin II was first demonstrated by Buckley et al.10 who found that injection of large dose of angiotensin II into the carotid artery of vascularly isolated dog’s head with intact neural connection to the rest of the body caused a remarkable elevation of systemic arterial blood pressure. This central site of pressor action of angiotensin II has been thought to be the area of postrema11 in rat. However, since our study demonstrated that in SHR a highly specific binding activity of angiotensin II was found in the regions of the hypothalamus and the thalamus, this difference should be further examined including problem whether the difference is caused simply by species of rat.

The depressed maximal binding of angiotensin II to the thalamic region of SHR with severe development of hypertension may give one possibility that this abnormality contributes to development of hypertension. Hallbäck et al.12 have suggested that in both the genetically hypertensive rat from New Zealand strain and SHR the genetic predisposition to hypertension is neurogenic in nature, which is supported by study showing an increase of sympathetic activity in the genetically hypertensive rat.13

In SHR with development of hypertension, a depression of angiotensin II binding activity was found in the thalamus, which has been implicated in the central control of blood pressure.14 This reduced binding activity of angiotensin II to the receptor fraction would explain the results of the ventricular perfusion studies of Ganten et al.15 who reported increased angiotensin II in cerebrospinal fluid of genetically hypertensive rats; a reduced population of brain angiotensin II receptors would be a natural consequence of increased brain angiotensin II.

Another possibility to explain development of hypertension in SHR may be given by the findings that, in our study, the maximal binding activity was increased in the hypothalamic region, rather than in the thalamic fraction, in SHR with severe hypertension. The mechanism is not clearly elucidated, but the increase of binding activity in the hypothalamic region may play some significant role in development of hypertension, though there is not direct evidence to support the suggestion.

CONCLUSION

1. Specific binding activity of angiotensin II to brain tissues of the cortex, the thalamus, the hypothalamus, the midbrain and the striatum was detected in SHR.

2. Angiotensin II bound with the highest activity to the thalamic region in the brain tissue of SHR in non-advanced stage.

3. Maximal binding activity of angiotensin II of SHR was the highest in the hypothalamus in advanced stage, while the greatest in the thalamus in non-advanced stage.

4. From these observations, it is postulated that the binding activity of angiotensin II to the receptor in thalamus-hypothalamus has increased with some significant role in occurrence or development of hypertension in SHR.

Acknowledgements

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REFERENCES


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