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Short Communication

Decreased Expression of Angiotensin II Type 1 and Type 2 Receptors in the Brain After Long-Term Administration of Antihypertensive Drugs in Stroke-Prone Spontaneously Hypertensive Rat

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Abstract. The present study examined the levels of Angiotensin II type 1 receptor (AT_1) and type 2 receptor (AT_2) in the brain stem and cerebral cortex of the stroke-prone spontaneously hypertensive rat (SHR-sp) after long-term treatment with three types of antihypertensive drugs: valsartan, enalapril, and amlodipine. In both tissues, expression of the AT_1 was decreased by administration of each drug. Expression of the AT_2 was decreased in the cerebral cortex by drug administration, but did not change in the brain stem. This study may contribute to elucidating the relationship between AT_1 and AT_2 expressions and the effect of antihypertensive drugs in SHR-sp brain.

Keywords: angiotensin II receptor, antihypertensive drug, brain

Angiotensin II (Ang II) is the principal vasoactive peptide of the renin–angiotensin system, having a variety of physiological actions, including vasoconstriction, aldosterone release, and cell growth (1) Ang II binds two major receptors, Ang II type 1 receptor (AT_1) and type 2 receptor (AT_2). The AT_1 is mainly expressed in blood vessels, heart, kidney, and adrenal and is also expressed in the brain (2, 3). In the brain, the AT_1 is also expressed in microvessels (4), and the AT_2 is also expressed in neurons (5). The majority of Ang II actions are mediated via the AT_1; AT_1 blockers (ARBs) have been widely used as antihypertensive drugs. The AT_2 is reported to be expressed in fetal tissues but is present at low levels in adult tissues and is re-expressed in certain pathological conditions, such as neuronal injury and vascular injury. The AT_2 has also been reported to be expressed in the adult rat brain stem, in the inferior olivary nuclei (6) that contain sympathetic premotor neurons for the maintenance of vasomotor tone and blood pressure. Because AT_2 activation is believed to oppose AT_1-mediated effects (7), it would be of interest to know whether AT_2 expression in the brain stem is associated with blood pressure through an interaction with AT_1 signaling. However, AT_1 and AT_2 expressions and their association in the brain stem are not well defined.

The present study examined the levels of AT_1 and AT_2 gene expression in the brain stem and cerebral cortex of the stroke-prone spontaneously hypertensive rat (SHR-sp) after long-term treatment with three types of antihypertensive drugs: ARBs, ACE inhibitors, and a calcium channel blocker (CCB); and it clarified the effect of these antihypertensive drugs and blood pressure on AT_1 and AT_2 expressions. We hope to elucidate the possible role of the AT_1 or AT_2 in the brain underlying the regulation of the blood pressure.

Forty male SHR-sp/Izm rats were used for this experiment. The experimental protocol was approved by the Ethical Committee of Nihon University, School of Medicine. These animals were allowed standard rat chow (CRF-1; Oriental Yeast, Tokyo) and tap water ad libitum for 1 week. Then they were divided into four groups (n = 10): 1, vehicle; 2, valsartan (ARB) (Diovan; Novartis Pharma, Basel, Switzerland) at 30 mg/kg per day; 3, enalapril maleate salt (ACEI) (Sigma-Aldrich, St. Louis, MO, USA) at 10 mg/kg per day; and 4, amlodipine (CCB) (Norvasc; Pfizer, New York, NY, USA) at 6 mg/kg per day. Treatment groups were administered each drug [suspended in carboxy-methyl-

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cellulose (CMC)] orally once a day for 4 weeks from 8 weeks of age. The vehicle group was administered CMC orally once a day for 4 weeks from 8 weeks of age. Systolic blood pressure (SBP) was measured once a week by the tail cuff method, using a Softron BP-98 (Softron, Tokyo). After 4 weeks of treatment (at 12 weeks of age), the brain was dissected into the brain stem and cerebral cortex, which were kept at −80°C until analyzed (8). Figure 1 shows the systolic blood pressure in the treatment groups. SBP increased by about 40 mmHg in the vehicle group during 4 weeks. During 4 weeks, SBP slightly increased in the amlodipine group and slightly decreased in the valsartan and enalapril groups. Although the SBP in the valsartan group and enalapril group was significantly decreased in comparison with that in the amlodipine group from 2 to 4 weeks (P<0.01), SBP in all three treatment groups was significantly decreased in comparison with that in the vehicle group from 1 to 4 weeks (P<0.01).

To validate AT_1 and AT_2 gene expression, TaqMan RT-PCR assay was performed using an ABI Prism 7700 Sequence Analyzer (PE Applied Biosystems, Foster City, CA, USA) as described previously (9). For the primers and the TaqMan probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), we used TaqMan rodent GAPDH control reagents (Roche, Basel, Switzerland). Primers and the TaqMan probe for AT_1 were as follows (5' to 3'): forward primer (TTACCGG CCTTCGGATAAC), reverse primer (ACTCCACCT CAAAACAAGACG), and probe (TGAGCTCATCG GCCAAAAAGCC). Primers and the TaqMan probe for AT_2 were as follows (5' to 3'): forward primer (CCC TAAAAAGGTGTCCAGCA), reverse primer (TTACA TCTTCAATCTGGCTGTGGCTGA), and probe (AAG GGTTGCCAAAAGGAGTAA). Normalized mRNA levels of the AT_1 and AT_2 were determined by dividing the levels of each gene by the level of GAPDH. Statistical analysis of TaqMan RT-PCR data was performed with SAS 9.1.3 (SAS Institute, Cary, NC, USA) statistical software (10). One-way analysis of variance (ANOVA) was used to compare AT_1 or AT_2 expression among treatment groups of valsartan, enalapril, amlodipine, and vehicle, with multiple-comparison

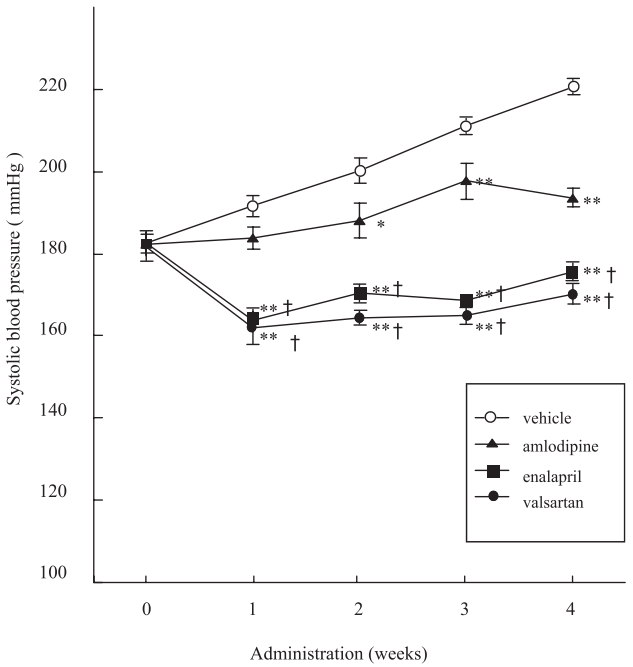

Fig. 1. Effect of antihypertensive drugs on systolic blood pressure in SHR-sp. SHR-sp were administered vehicle (open circle), amlodipine at 6 mg/kg per day (closed triangle), enalapril at 10 mg /kg per day (closed square), or valsartan at 30 mg/kg per day (closed circle) daily. Data are each a mean ± S.E.M. (n = 10). Significantly different from vehicle, *P<0.05, **P<0.01 and from amlodipine, $^{\dagger}P$<0.01 (Tukey post hoc analysis).

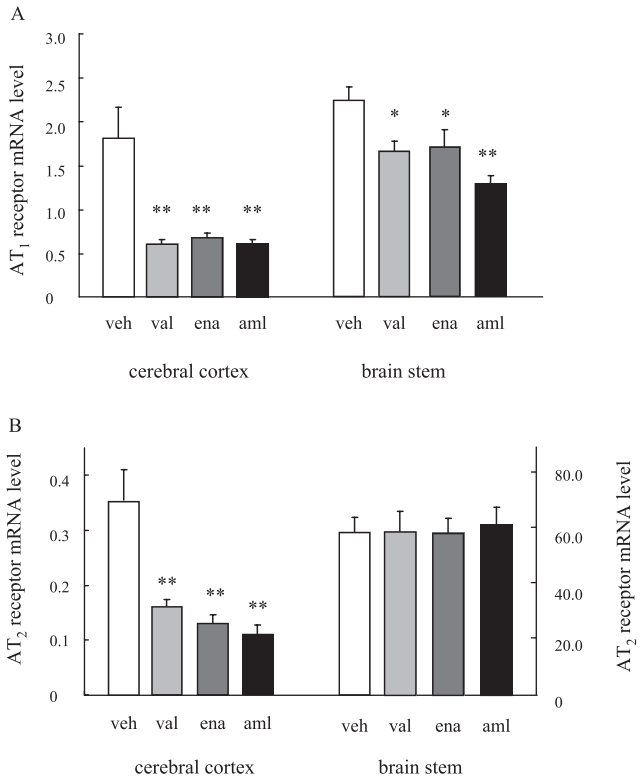

Fig. 2. Effect of antihypertensive drugs on expression of AT_1 and AT_2 in SHR-sp brain. A: AT_1 expression in SHR-sp brain after 4 weeks of administration of antihypertensive drugs (veh: vehicle, val: valsartan, ena: enalapril, aml: amlodipine). Data are each a mean ± S.E.M. (n = 10). Significantly different from vehicle, *P<0.05, **P<0.01 (Tukey post hoc analysis). B: AT_2 expression in SHR-sp brain after 4 weeks of administration of antihypertensive drugs. Data are each a mean ± S.E.M. (n = 10). Significantly different from vehicle, **P<0.01 (Tukey post hoc analysis). The left scale shows AT_2 expression in the cortex and the right scale shows AT_2 expression in the brain stem.

testing (Tukey–Kramer HSD post hoc analysis) set at a significance level of 0.05.

Figure 2 shows AT_1 and AT_2 expression in the brain stem and cerebral cortex of SHR-sp rats treated with valsartan, enalapril, amlodipine, and vehicle. In the cerebral cortex and brain stem, AT_1 expression in all three drug treatment groups was significantly decreased in comparison with that of vehicle group ($P<0.05$), as shown in Fig. 2A. AT_1 expression was not significantly different among the drug treatment groups, despite their different mechanisms of antihypertensive effect. These results suggest that AT_1 expression in the brain stem and cerebral cortex was decreased by a blood pressure-lowering action of antihypertensive drugs but not by a direct action of antihypertensive agents with different mechanisms of action. Although the blood pressure-lowering action of amlodipine was weaker than those of valsartan and enalapril, AT_1 expression was tended to be most inhibited by amlodipine. This discrepancy suggests the possibility that the expression of AT_1 in the brain stem was influenced not only by a blood pressure lowering action but also by a direct action of amlodipine, such as the blockage of calcium channels and the alteration of nitric oxide production (11). Our results are different from those of the previous studies on the effects of candesartan, enalapril, or amlodipine administration on AT_1 expression in the kidney (12, 13). The difference may mainly come from the difference in tissues examined, brain or kidney, between the studies, although differences in age and strain of rats used would also be possible causes of the difference. As the blood brain barrier obstructed the penetration of these agents into the brain, it is considered that expression of the AT_1 decreased through a reduction of blood pressure by administration of antihypertensive agents. Our present study suggested that expression of the AT_1, which mediates the majority of Ang II actions in the brain stem and cerebral cortex, may be influenced by the change of blood pressure, at least in SHR-sp.

The antihypertensive agents affected expression of the AT_2 in the cerebral cortex and brain stem in different manners. In the cerebral cortex, AT_2 expression in all three drug treatment groups was significantly decreased in comparison with that in the vehicle group ($P<0.05$), as shown in Fig. 2B. Similar to the case of AT_1 expression in the cerebral cortex, this result suggests that expression of the AT_2 in the cerebral cortex is decreased by the reduction of blood pressure but not by a direct action of antihypertensive agents. In the brain stem, the difference in AT_2 expression was not significant not only between each of the drug treatment groups and the vehicle group, but also among drug treatment groups, as shown in Fig. 2B. These findings suggest that AT_2 expression in the brain stem is not directly affected by antihypertensive drugs and is not affected by blood pressure. Therefore, regulation of AT_2 expression may be unrelated to blood pressure control and independent of the AT_1 signaling system, suggesting an important role of the AT_2 in the brain stem. Supporting this possibility, evidence showing effects of AT_2 signaling in cerebral protection and neural differentiation has accumulated (14, 15). Although expression of the AT_2 in the adult brain is limited, these receptors are reported to be up-regulated in the brain after ischemic damage, suggesting an important role of AT_2 activation in neural protection. In this study, we found that AT_2 expression was significantly increased in the brain stem in comparison with that in the cerebral cortex, as shown in Fig. 2. Our findings, in combination with previous reports (15), suggest that the brain stem is more resistant to brain damage including ischemic injury than the cerebral cortex because AT_2-mediated neuroprotective activity is stronger in the former than in the latter.

In situ hybridization was not carried out in the present study because mRNA expression of the AT_1 and AT_2 was low and hard to detect. In the brain, however, it has been reported that these receptors are expressed in microvessels (4) and neurons (5). Therefore, it is important to determine the expression site of AT_1 and AT_2, that is, neurons or microvessels; and we would like to clarify this next using a microdissection method.

The present study demonstrated that antihypertensive agents decreased expression of the AT_1 in the brain stem and cerebral cortex of SHR-sp. In addition, we found that expression of the AT_2 was markedly higher in the brain stem than in the cerebral cortex and that antihypertensive agents themselves and reduction of blood pressure did not affect expression of the AT_2 in the brain stem. This study may contribute to elucidating the relationship between AT_1 and AT_2 expressions and the effect of antihypertensive drugs in SHR-sp brain.

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