Role of AT2 receptor in the brain in regulation of blood pressure and water intake

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Submitted 21 June 2002; accepted in final form 29 August 2002

Li, Zhen, Masaru Iwai, Lan Wu, Tetsuya Shiuchi, Toyohisa Jinno, Tai-Xing Cui, and Masatsugu Horiuchi. Role of AT2 receptor in the brain in regulation of blood pressure and water intake. Am J Physiol Heart Circ Physiol 284: H116–H121, 2003.—The effects of intracerebroventricular (ICV) injection of angiotensin II (ANG II) on blood pressure and water intake were examined with the use of ANG II receptor-deficient mice. ICV injection of ANG II increased systolic blood pressure in a dose-dependent manner in wild-type (WT) mice and ANG type 2 AT2 receptor null (knockout) (AT2KO) mice; however, this increase was significantly greater in AT2KO mice than in WT mice. The pressor response to a central injection of ANG II in WT mice was inhibited by ICV preinjection of the selective AT1 receptor blocker valsartan but exaggerated by the AT2 receptor blocker PD-123319. ICV injection of ANG II also increased water intake. It was partly but significantly suppressed both in AT2KO and AT1aKO mice. Water intake in AT2/AT1aKO mice did not respond to ICV injection of ANG II. Both valsartan and PD-123319 partly inhibited water intake in WT mice. These results indicate an antagonistic action between central AT1a and AT2 receptors in the regulation of blood pressure, but they act synergistically in the regulation of water intake induced by ANG II.

ANGIOTENSIN II (ANG II) is a potent vasoactive substance of the renin-angiotensin system (RAS) and plays a major role in hemodynamic regulation, aldosterone release, and cell growth in peripheral tissue (11). Two distinct ANG II receptor subtypes, type 1 (AT1) and type 2 (AT2), were cloned in 1991 and 1993, respectively (19, 24, 25, 29). The AT2 receptor is abundantly and widely expressed in fetal tissues, but its expression declines rapidly after birth (30) and is retained in certain tissues, such as the brain, adrenal gland, uterus, and ovary (36). Recent studies (17, 34) have demonstrated the existence of all components of RAS, including ANG II receptors, in the central nervous system. The AT1 receptor, a major subtype of ANG II receptor in adult tissue, is dominantly localized in the regions that control sympathetic activity and vasopressin secretion, such as the ventrolateral medulla, nucleus tractus solitarius, and paraventricular nucleus (3, 18, 27). In addition, both the AT1 and AT2 receptors are expressed in the forebrain and brain stem, which are related to the regulation of blood pressure (17). Moreover, the AT2 receptor is also located in the hypothalamus (17, 21) and is predominantly expressed in the ventral part of the brain (12). These findings suggest that the brain RAS plays an important role in hemodynamic regulation.

It has been reported (6, 7) that the intracerebroventricular (ICV) injection of ANG II increased systemic blood pressure in mice. This increase was significantly suppressed in AT1a receptor null (knockout) (AT1aKO) mice but not in AT1bKO mice, suggesting that the AT1a receptor plays a major role in the regulation of blood pressure. Because the AT2 receptor is also present in the brain, this subtype may contribute to blood pressure regulation by the brain RAS. Recent evidence (1, 5, 37) suggests that AT2 receptor stimulation exerts antagonistic effects against the AT1 receptor-mediated functions in peripheral tissues. Thus, in the present study, we explored the effect of ICV microinjection of ANG II and the involvement of the central AT2 receptor in blood pressure regulation in relation to that of the AT1 receptor with the use of receptor gene KO mice.

MATERIALS AND METHODS

Animals. Adult male wild-type (WT), AT2 receptor null (AT2KO) (13) AT1aKO (32), and AT2/AT1aKO mice aged 10–12 wk (25–30 g body wt) were used in this study. AT2/AT1aKO mice were generated by crossing AT2KO and AT1aKO mice. The animals were housed in a room where lighting was controlled (12 h on and 12 h off), and the temperature was maintained at 25°C. They were given a standard diet (MF, Oriental Yeast) and water ad libitum. The genotype of animals was determined by RT-PCR after DNA was extracted from tail samples. All experiments were approved by the Animal Studies Committee of Ehime University.

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Implantation of microcannula into intracerebroventricle and central administration of ANG II. While under anesthesia with ketamine (70 mg/kg) and xylazine (4 mg/kg), the mice were stereotaxically implanted with a chronic double-walled stainless steel cannula in a unilateral cerebral ventricle, as previously reported (31). The stereotaxic coordinates were 0.4 mm posterior and 1.0 mm lateral to the bregma, according to the method of Franklin and Paxinos (9), and 2.5 mm below the surface of the skull. Animals were allowed 7 days of recovery before the experiments took place. The localization of the cannula was confirmed by preparing brain sections following injection of dye after the experiment.

ANG II was diluted in saline and administered in 0.5 μl into the cerebral ventricle. The AT1 receptor blocker valsartan (donated by Novartis Pharma) and the AT2 receptor blocker PD-123319 (Research Biochemicals International) were diluted with saline and administered in 1.0-μl increments into the cerebral ventricle 10 min before ANG II injection. Experiments were started between 9 and 11 AM. Mice were allowed at least 30 min to adapt before ANG II injection to obtain a stable basal level of blood pressure. After ANG II injection with the use of a microsyringe connected to a microinjector, systolic blood pressure was measured in conscious mice for 30 min by the indirect tail-cuff method with a blood pressure monitor (model MK-1030, Muromachi Kikai, Tokyo, Japan) (33).

To measure the water intake of mice, the animals were kept in metabolic cages (Natsume Seisakusyo; Tokyo, Japan) for 7 days before the experiment. Water intake was measured by weighing the water bottle in the metabolic cage for 30 min before and after ICV injection of ANG II.

**Statistical analysis.** Values are expressed as means ± SE in the text and figures. Data were analyzed by one-way analysis of variance. If a statistically significant effect was found, a Newman-Keuls test was performed to detect the difference between the groups. A value of $P < 0.05$ was considered to be statistically significant.

**RESULTS**

**Effect of central ANG II injection on arterial blood pressure.** As shown in Fig. 1, basal systolic blood pressure was not different between WT and AT2KO mice (98.4 ± 0.8 and 101.9 ± 0.9 mmHg, respectively) but was significantly lower in AT1aKO and AT2/AT1aKO mice (79.3 ± 0.9 and 80.1 ± 0.8 mmHg, respectively; $P < 0.05$ vs. WT). AT2/AT1aKO mice did not show any apparent physiological changes, such as body weight gain, and histological features of heart, artery, and brain. ICV injection of ANG II increased systolic blood pressure in WT mice. The pressor response reached a peak ~3–5 min after ANG II injection and recovered to the basal level within 20 min (Fig. 1). The time course of the pressor response in AT2KO, AT1aKO, and AT2/AT1aKO mice was not different from that in control mice. The maximum increase in blood pressure induced by central ANG II injection was dose dependent in WT mice between 50 and 200 ng (Fig. 2). The pressor response after the central injection of ANG II was significantly larger in AT2KO mice within the range of 50–200 ng. In contrast, AT1aKO mice showed a smaller response of blood pressure after central ANG II injection (Fig. 2). In these mice, there was no obvious dose dependence of the response, although a high dose of ANG II (200 ng) slightly increased blood pressure. The response in AT2/AT1aKO mice to a higher dose (200 ng) of ANG II tended to be larger than the response in AT1aKO mice (Fig. 2).

In WT mice, the increase in blood pressure induced by central injection of 100 ng ANG II was inhibited by ICV preinjection of the selective AT1 receptor blocker valsartan in a dose-dependent manner (Fig. 3A). Near-maximum inhibition by valsartan was observed at 0.5 nmol for 30 min by the indirect tail-cuff method with a blood pressure monitor (model MK-1030, Muromachi Kikai, Tokyo, Japan) (33).

![Fig. 1. Change of systolic blood pressure after central injection of angiotensin II (ANG II) in wild-type (WT) mice, ANG type 2 receptor null (knockout) (AT2KO) mice, and ANG type 1a KO (AT1aKO) mice and AT2/AT1a KO mice (AT2/AT1aKO). A microcannula was implanted into the cerebral ventricle and ANG II was injected, as described in MATERIALS AND METHODS. ICV, intracerebroventricular. Values are means ± SE (n = 7–9 mice per group). *P < 0.05 vs. WT.](image1)

![Fig. 2. Dose response of systolic blood pressure after central injection of ANG II in WT, AT2KO, AT1aKO, and AT2/AT1aKO mice. A microcannula was implanted into the cerebral ventricle, and ANG II was injected (see MATERIALS AND METHODS), and blood pressure was determined for 30 min after ANG II injection. Δ, Change in systolic blood pressure. Values are expressed as the maximal response from basal level. Values are means ± SE (n = 7–9 mice per group). *P < 0.01 vs. without ANG II; †P < 0.05 vs. without ANG II; §P < 0.05 vs. WT.](image2)
μg (~70%; Fig. 3A). In contrast, pretreatment of mice with the selective AT<sub>2</sub> receptor blocker PD-123319 enhanced the pressor response induced by central ANG II injection dose dependently in WT mice (Fig. 3A). The maximum effect was caused by 5 μg PD-123319. Central injection of 0.5 μg valsartan or 5 μg PD-123319 without ANG II did not significantly change systolic blood pressure, whereas blood pressure tended to increase after injection of PD-123319 (Fig. 3B). In AT<sub>2</sub>KO mice, the pressor response after central injection of ANG II was inhibited ~45% by valsartan; however, this inhibition was significantly smaller than that in control (Fig. 3B; P < 0.05). PD-123319 did not significantly affect the blood pressure in AT<sub>2</sub>KO mice.

On the other hand, ICV injection of ANG II (100 ng) did not influence systolic blood pressure in AT<sub>1</sub>aKO mice (Fig. 3B). In these mice, PD-123319 did not significantly change the blood pressure after central injection of ANG II (Fig. 3B), suggesting that AT<sub>1</sub>a receptor stimulation is a prerequisite for the AT<sub>2</sub> receptor to exert an antagonistic effect on blood pressure regulation.

**Effect of central ANG II injection on water intake.** As shown in Fig. 4, ICV injection of ANG II at a dose of 100 ng increased water intake fivefold in WT mice. This increase was decreased ~40% in AT<sub>2</sub>KO mice and ~60% in AT<sub>1</sub>aKO mice compared with that in WT mice. In AT<sub>2</sub>/AT<sub>1</sub>aKO mice, a central ANG II injection did not significantly affect water intake. Figure 5 shows that the effect of ANG II on water intake in WT mice was significantly inhibited by valsartan and by PD-123319.}

**Fig. 3.** Effect of valsartan and PD-123319 on pressor response induced by ANG II in WT, AT<sub>2</sub>KO, and AT<sub>1</sub>aKO mice. A microcannula was implanted into the cerebral ventricle, and ANG II (100 ng), valsartan, and PD-123319 were administered, as described in MATERIALS AND METHODS. A: dose-dependent response of valsartan and PD-123319 in WT mice. B: effect of valsartan (0.5 μg) and PD-123319 (5 μg) in WT, AT<sub>2</sub>KO, and AT<sub>1</sub>aKO mice. Values are expressed as the maximal response from basal level. Values are means ± SE (n = 7–9 mice per group). *P < 0.01 vs. control; §P < 0.01 vs. ANG II.

**Fig. 4.** Effect of central injection of ANG II on water intake in WT, AT<sub>2</sub>KO, AT<sub>1</sub>aKO, and AT<sub>2</sub>/AT<sub>1</sub>aKO mice. A microcannula was implanted into the cerebral ventricle and ANG II (100 ng) was injected, as described in MATERIALS AND METHODS. Values are expressed as the water intake during 30 min after central ANG II injection. Values are means ± SE (n = 7–9 mice per group). *P < 0.01 vs. vehicle; §P < 0.05 vs. WT.

**Fig. 5.** Effect of valsartan and PD-123319 on water intake induced by central ANG II in WT, AT<sub>2</sub>KO, and AT<sub>1</sub>aKO mice. A microcannula was implanted into the cerebral ventricle, and ANG II (100 ng), valsartan (0.5 μg), and PD-123319 (5 μg) were administered, as described in MATERIALS AND METHODS. Values are expressed as the water intake during 30 min after central ANG II injection. Values are means ± SE (n = 7–9 mice per group). *P < 0.01 vs. control; §P < 0.01 vs. ANG II.
In contrast, AT2 receptor stimulation increased water intake synergistically with AT1a receptor stimulation. The basal level of water intake was not different among WT, AT2KO, AT1aKO, and AT2/AT1aKO mice. 

**Plasma AVP after ICV injection of ANG II.** ICV injection of ANG II increased plasma AVP level in WT mice (Fig. 6). This increase was significantly larger in AT2KO mice but smaller in AT1aKO and AT2/AT1aKO mice than in WT. The basal level of plasma AVP was not different among these groups.

**DISCUSSION**

In this report, we studied the roles of ANG II receptor subtypes in the brain in the regulation of systemic blood pressure and water intake by using ANG II receptor gene-deficient mice and selective ANG II receptor blockers. Our results using ANG II receptor gene-deficient mice indicated that stimulation of the central AT1a receptor by ICV injection of ANG II increased both systolic blood pressure and water intake. Stimulation of the AT2 receptor, however, antagonized the AT1a receptor function and lowered blood pressure. In contrast, AT2 receptor stimulation increased water intake synergistically with AT1a receptor stimulation. The effects of an ANG II receptor blocker on blood pressure and water intake were consistent with the results in ANG II gene-deficient mice. We used the doses of ANG II and ANG II receptor blockers according to those in previous studies (2, 6, 10, 28). In our experiments, ICV injection of ANG II showed dose dependency in pressor response up to 200 ng (Fig. 2). AT1 and AT2 receptor blockers also induced the dose-dependent action, and 0.5 µg valsartan and 5 µg PD-123319 showed near-maximal effects, similar to the results in previous studies (2, 10). These results suggest that the AT2 receptor in the brain plays an important role in the regulation of peripheral blood pressure and water intake in combination with the AT1a receptor.

In the peripheral RAS, most of the physiological actions induced by ANG II are thought to be mediated by the AT1a receptor, a major isoform of the AT1 receptor. The AT2 receptor counteracts functions mediated by the AT1 receptor, such as cell proliferation, apoptosis, and aldosterone release (8). It has been reported (20) that a local RAS is present in the central nervous system, and previous studies (12, 17, 18, 21, 27, 36) demonstrated the existence of both AT1a and AT2 receptors in the central nervous system. These ANG II receptors in the brain seemed to be involved in the pressor response, drinking, and vasopressin release (6, 14, 35). Li et al. (22) demonstrated that paraventricular AT1 receptors were critical in salt-sensitive hypertension in mRen-2 transgenic rats. Davison et al. (6) also reported that the AT1a receptor in the brain plays a major role in the control of blood pressure. In our results, AT1aKO mice also showed a lower basal level of blood pressure than WT mice. However, basal blood pressure in AT2KO mice was not significantly different from that in WT mice. Moreover, basal blood pressure in AT2/AT1aKO mice was similar to that in AT1aKO mice. These results indicate that the central AT2 receptor is not essential to maintain the basal level of blood pressure. However, the AT2 receptor-mediated signal in the brain antagonizes the central AT1 receptor-mediated action on blood pressure when the brain AT1 receptor is stimulated. The pressor response to ICV injection of ANG II was greater in AT2KO mice than in WT mice. In addition, ICV preinjection of PD-123319 enhanced the pressor response after central injection of ANG II in WT mice, and the inhibitory action of valsartan on the blood pressure increase induced by central ANG II was weaker in AT2KO mice than in WT mice. Consistent with these observations, Hein et al. (13) reported that AT2KO mice had increased sensitivity to the pressor effect of peripheral injection of ANG II. Nishioka et al. (26) also indicated that AT2 receptors play a depressor role in sodium-induced pressor response. The effect of central ANG II on peripheral blood pressure may be mediated by the peripheral neuroendocrine system. Previous studies (16, 27) reported that RAS in the brain modulates sympathetic nerve activity through brain AT1 and AT2 receptors. Thus it is possible that the AT2 receptor in the brain induces the antagonistic action against the AT1 receptor-mediated pressor response by regulating the sympathetic nervous system, which remains to be explored.

In contrast to the regulation of systemic blood pressure, the central AT2 and AT1a receptors seemed to act additively or synergistically to increase water intake. Previous studies demonstrated the importance of the brain AT1 receptor in the fluid/electrolyte balance.
Morris et al. (23) reported that the AT₁α receptor-deficient mice showed a differential response to dehydration. Davisson et al. (6) indicated that the AT₁b receptor but not the AT₁α receptor in the brain was responsible for a major portion of drinking motivation. The reason for such a discrepancy has not yet been clarified. However, it may be due to the different markers of drinking in these studies because other studies measured drinking behavior and we measured the volume of water intake. Moreover, the present study indicated that not only the AT₁ but also the AT₂ receptor was involved in the regulation of water intake by the central nervous system. Consistent with our results, Hein et al. (13) observed that AT₂KO mice had an impaired drinking response to water deprivation. Other reports using receptor antagonists also supported our results. Alova et al. (2) reported that not only AT₁ receptor blocker but also AT₂ receptor antagonist inhibited water intake induced by ICV injection of ANG II in rats. Moreover, Camara et al. (4) indicated that ICV ANG II stimulated both central AT₁ and AT₂ receptors to induce drinking in high-sodium chloride fed rats. These findings indicate that the regulatory mechanism of water intake mediated by central ANG II differs from that of the pressor response and that AT₁ and AT₂ receptors in the brain seemed to act synergistically in the control of drinking. The mechanism of action of the AT₁ and AT₂ receptors on drinking is not yet fully clarified. Previous reports (14, 15, 17) indicated that ANG II in the brain affects vasopressin secretion. Because vasopressin regulates water retention, we measured the change of AVP in plasma after central injection of ANG II. From the results using ANG II receptor-deficient mice and ANG II receptor blockers, it was concluded that the AT₁ receptor in the brain stimulated AVP release, whereas a central AT₂ receptor antagonized such action of the AT₁ receptor, when the AT₁ receptor signal was activated. Stimulation of the central AT₂ receptor alone did not seem to affect plasma AVP level, because the effect of ICV injection of ANG II did not differ between AT₁αKO and AT₂/AT₁αKO mice. Because such a response pattern of AVP in ANG II receptor-deficient mice was different from that of water intake, the regulation of drinking via the central AT₁ and AT₂ receptors may involve not only vasopressin but also other factors.

Although the mechanism of the regulation of hemodynamic changes by the central RAS has not yet been fully clarified, it is of interest that the pressor response and water intake are regulated by AT₁ and AT₂ receptors in a different manner. This may be at least partly caused by the different distribution of AT₁ and AT₂ receptors in the brain, which remains to be clarified. Our results also suggest the possibility that the functional balance of central AT₁α and AT₂ receptors plays an important role in the mechanism of hypertension.

The authors gratefully acknowledge Novartis Pharma for donating valsartan and Tanabe Pharmaceutical for donating AT₁αKO mice. This study was supported by grants from the Ministry of Education, Science, Sports, and Culture of Japan, Japan Research Foundation for Clinical Pharmacology, Tokyo Biochemical Research Foundation, and a grant from the Smoking Research Foundation.

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