Involvement of central angiotensin II type 1 receptors in LPS-induced systemic vasopressin release and blood pressure regulation in rats

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The purpose of this study was to evaluate the involvement of central angiotensin II (ANG II) and ANG II type 1 (AT1) receptors in systemic release of arginine vasopressin (AVP) and blood pressure regulation during endotoxemia. LPS (150 μg/kg) was injected intravenously 30 min after intracerebroventricular (icv) losartan (50 μg), an AT1 receptor antagonist, or subcutaneous (sc) captopril (50 mg/kg), an angiotensin-converting enzyme inhibitor. Rats with icv and sc saline injections served as control. LPS administration increased plasma AVP concentration from 2.1 ± 0.2 to 15.2 ± 2.5 pg/ml (60 min after LPS injection) without significant changes in plasma osmolality or hematocrit. LPS-induced AVP secretion was significantly attenuated by pretreatment with icv losartan (2.3 ± 0.5 to 3.7 ± 0.5 pg/ml) but was not attenuated after peripheral captopril treatment (2.2 ± 0.6 to 17.6 ± 4.2 pg/ml). LPS administration significantly decreased systolic blood pressure (SBP) by 22.7 ± 5.4 mmHg after intravenous LPS injection in icv losartan-treated rats, while SBP remained unchanged in vehicle-treated or sc captopril-treated rats by intravenous LPS. These results indicate that central AT1 receptors, not responsive to peripheral ANG II, play an important role in systemic AVP secretion and maintenance of blood pressure during endotoxemia.

endotoxemia; lipopolysaccharide; losartan; arginine vasopressin; captopril

ENDOTOXEMIA, produced by lipopolysaccharide (LPS) administration, induces the release of cytokines from immune cells, resulting in a variety of physiological and potentially pathophysiological responses (3, 14). We have demonstrated that intravenous or intraperitoneal injection of LPS significantly increases the number of c-Fos-immunoreactive magnocellular AVP and oxytocin neurons in the supraoptic nucleus (SON) and magnocellular neurons in the paraventricular nucleus (PVN), in addition to increased c-Fos immunoreactivity in parvocellular neurons containing these peptides in the PVN (19), as confirmed by others (37, 38). These findings suggest that LPS could stimulate both the hypothalamic-pituitary-adrenal (HPA) axis and the hypothalamic-noradrenergic system (20). Several investigators have reported that endotoxemia stimulates systemic AVP secretion (2, 4, 10). LPS-induced AVP secretion is unlikely to be a secondary effect of LPS administration with changing body fluid status, as administration of LPS stimulates AVP secretion independent of physiological variables that are known to induce vasopressin secretion (10), such as plasma hyperosmolality, hypovolemia, hypotension, and hypoglycemia (1). However, the central mechanisms underlying systemic AVP secretion induced by endotoxemia have yet to be elucidated.

Endotoxemia also stimulates thirst in rats (35), suggesting that a common neural pathway could be involved in dehydration- and LPS-induced systemic AVP. Osmotically induced AVP secretion and thirst have been demonstrated to be mediated, at least in part, by brain ANG II type 1 (AT1) receptors (7, 12, 18). Kregel et al. (15) reported that heat-stress-induced AVP secretion is mediated by brain AT1 receptors. In addition, intravenous or intracranial injection of ANG II reportedly causes ACTH and AVP release (13, 27, 32), and brain ANG II and its receptors are involved in the development of sympathetic and neuroendocrine responses to stress (8, 36). Lesions of the anteroventral region of the third ventricle (AV3V) attenuate LPS-induced vasopressin secretion (4), again suggesting a possible role of AT1 receptors in LPS-induced AVP secretion, since AT1 receptors are densely located in the AV3V region (21, 29). Taken together, we hypothesized that ANG II AT1 receptors may be involved in AVP secretion and maintenance of blood pressure during endotoxemia.

In the present study, to clarify the involvement of central angiotensin AT1 receptors in LPS-induced AVP secretion and blood pressure regulation, we examined the effects of the central administration of AT1 receptor antagonist, losartan, on vasopressin secretion and blood pressure regulation. In addition, to clarify the contribution of peripheral ANG II, accessing AT1 receptors locating circumventricular organs outside the blood-brain barrier (BBB), to systemic AVP secretion and blood pressure regulation during endotoxemia, we examined effect of peripheral administration of captopril, an angiotensin-converting enzyme inhibitor, on systemic AVP secretion and blood pressure regulation during endotoxemia.

METHODS AND MATERIALS

Experiments were performed using male Sprague-Dawley rats (Shimizu Laboratory Supplies, Kyoto, Japan) weighing 250–400 g. All experiments were conducted in accordance with the institutional guidelines for animal care, and all experimental procedures were approved by the animal care committee of Kyoto Prefectural University of Medicine. Animals were housed under a 12:12-h light-dark cycle and provided with ad libitum access to food and water, in a humidity- and temperature-controlled environment. We examined the effects of intracerebroventricular injection of losartan and peripheral captopril administration (subcutaneous) on responses of plasma AVP concentration and arterial pressure change to LPS challenges.

Surgical procedures. Rats were anesthetized with pentobarbital sodium (50 mg/kg); then an intravenous catheter (3-Fr vinyl tubing; ATOM, Tokyo, Japan) was inserted into the right jugular vein with
the free end of the catheter was passed subcutaneously and exteriorized dorsally behind the neck through the midscapular incision. An intracerebroventricular stainless guide cannula (0.5-mm OD; Eicom, Kyoto, Japan) was stereotaxically implanted into the right lateral ventricle (anteroposterior, −0.8 mm; lateral, 1.5 mm from the bregma; and at a depth of 4 mm) (26), and fixed on the skull with anchor screws and dental resin. Silicone-coated temperature data loggers (SubCue, Calgary, Canada) for measurement of abdominal temperature (T\textsubscript{abdo}) were implanted in the abdominal cavity in some rats. Experiments were conducted ≥5 days after the surgical operation to allow for recovery from surgical stress. Two days after the surgery, we confirmed the placement of the cerebroventricular cannula by injecting ANG II (5 ng), and rats that did not drink within a few minutes after intracerebroventricular ANG II were excluded from the study. Patency of the venous catheter was maintained by flushing every day with heparinized (100 U/ml) isotonic saline. During the recovery period, rats were kept in individual cages. Food and water intake and body weight were measured daily after surgery, and rats with abnormal water or food intake and/or abnormal weight gain were excluded from the experiments.

**Protocol.** Food and water were removed at 1000, and experiments were started at 1100. At the beginning of the experiments, rats were injected (icv) with 50 μg (in 5 μl) of losartan (generously donated by Merck) or vehicle (5-μl isotonic saline). At 30 min after intracerebroventricular injection, a 1-ml blood sample was drawn and either 150 μg/kg LPS (in 3 ml/kg isotonic saline; *Esherichia coli* serotype 055:B5; Sigma-Aldrich) or 3 ml/kg saline was infused through the intravenous catheter over 1 min. A blood sample was taken at 60 min after intravenous injection. Systolic blood pressure (SBP) and heart rate (HR) were measured before intracerebroventricular losartan injection, and before and 60 min after intravenous LPS injection. After the experiments, we injected (icv) 5 ng ANG II (Sigma) and observed the drinking response in order to verify the inhibitory action of losartan.

We also examined the involvement of peripheral ANG II in LPS-induced systemic vasopressin secretion and blood pressure regulation. The protocol in this experiment is similar to the first series of experiment. Rats were injected with intracerebroventricular saline followed by subcutaneous captopril (50 mg/kg; Sigma) or saline 30 min before intravenous LPS injection. Because subcutaneous injections were not performed in the first series of experiments, we performed the subcutaneous injection of saline just before intracerebroventricular losartan or saline in two rats in each experiment. The responses to LPS injection were similar between rats with and without subcutaneous injection. Thus all data from rats received with or without subcutaneous injection of saline were used for further analysis.

**Measurements.** Plasma osmolality was measured by freezing point depression (Fiske One-Ten osmometer; Fiske Associates, Norwood, MA), and hematocrit was determined by conductivity-based method (i-STAT; Abbott Point of Care). Plasma AVP concentration was determined by radioimmunoassay (Mitsubishi Chemical, Tokyo, Japan). Intra- and interassay coefficients of variation for 1.21 pg/ml AVP were 4.4% and 4.5%, respectively. SBP and HR were measured using the nonpreheating tail-cuff method (MK-2000; Muromachi Kikai, Tokyo, Japan). We used this apparatus for blood pressure measurement because preheating is not required and measurement time is very short, so the stress on rats is relatively small. We measured blood pressure daily after surgery to accustom rats to this apparatus and thus minimize stress. T\textsubscript{abdo} was measured with data loggers (SubCue, Canada) every 1 min, and the average of every 5-min period was used for data analyses.

**Data analyses.** Data are shown as means ± SE. The number of rats examined is shown in the text and figures. ANOVA with repeated measures (one within and one between factor) was used to determine the effects of pretreatment and LPS challenge on measured variables (plasma AVP, SBP, HR, hematocrit, P\textsubscript{osmalt} and T\textsubscript{abdo}, responses). Differences between data of specific interest were determined using the Tukey post hoc test. The effect of pretreatment (vehicle, icv losartan, and sc captopril) on blood pressure before LPS administration within the group (before and after pretreatment) was determined by paired t-test. Values of *P* < 0.05 were considered statistically significant.

**RESULTS**

Figure 1 shows the effects of intracerebroventricular losartan and subcutaneous captopril on LPS-induced AVP secretion. LPS administration stimulated AVP secretion, and plasma AVP concentration increased from 2.07 ± 0.18 pg/ml to 15.22 ± 3.22 pg/ml in rats with intracerebroventricular vehicle (*n* = 9). Pretreatment with intracerebroventricular injection of losartan completely inhibited LPS-induced systemic AVP secretion. In rats with intracerebroventricular losartan administration, intravenous injection of LPS did not alter plasma AVP concentrations (from 2.90 ± 0.59 pg/ml to 3.93 ± 0.61 pg/ml; *n* = 9). In contrast, the increase in plasma AVP after pretreatment with subcutaneous captopril was not different from the increase after vehicle pretreatment (2.20 ± 0.55 to 17.63 ± 4.18 pg/ml; *n* = 9). No change in plasma osmolality was seen in either group, nor was any difference seen in changes of hematocrit among groups (Table 1).

Baseline SBP before pretreatment was 119 ± 29 mmHg in the vehicle group (*n* = 7), 110 ± 4 mmHg in the losartan group (*n* = 7), and 114 ± 2 mmHg in the subcutaneous captopril group (*n* = 6), not representing a significant difference among the three groups. Neither intracerebroventricular losartan nor vehicle administration altered SBP, while subcutaneous captopril administration significantly decreased SBP by 13 ± 3 mmHg. Before LPS administration in captopril-pretreated rats was lower than the vehicle-treated rats but was not different from the losartan-pretreated rats (Fig. 2, top). LPS injection significantly decreased SBP in losartan-treated rats (from
107 ± 5 to 84 ± 3 mmHg) but did not alter SBP in captopril-treated (from 100 ± 5 to 98 ± 8 mmHg) or vehicle-treated rats (from 120 ± 6 to 112 ± 6 mmHg). SBP was significantly lower in losartan-treated rats than in vehicle-treated rats (Fig. 2, top).

Figure 2, bottom, shows the effects of intracerebroventricular losartan and subcutaneous captopril pretreatments on HR response to intravenous LPS (right). The pretreatment with subcutaneous captopril significantly increased HR by 39 ± 13 beats/min, while HR in losartan- and vehicle-treated rats were not changed by pretreatment. HR did not change in response to intravenous LPS in all three groups.

DISCUSSION

The present study demonstrated that central ANG II AT1 receptors responding to central ANG II are involved in systemic AVP secretion and blood pressure regulation during endotoxemia. LPS-induced AVP secretion was inhibited almost completely by intracerebroventricular losartan pretreatment but was not inhibited by a large dose of peripheral captopril (50 mg/kg). Blood pressure was maintained after systemic LPS administration in rats with vehicle and systemic captopril pretreatments but decreased in rats with central losartan pretreatment. Therefore, AVP secretion and maintenance of blood pressure during endotoxemia are mediated by central AT1 receptors, and peripheral ANG II does not play a role in AVP secretion and blood pressure regulation during endotoxemia.

To elucidate the role of peripheral ANG II in systemic AVP secretion and blood pressure regulation during endotoxemia,
we conducted peripheral administration of captopril. The reason for this procedure is that peripheral administration of losartan can reportedly inhibit blood pressure response to intracerebroventricular administration of ANG II, indicating that losartan can penetrate the BBB (39). It has been reported that captopril does not penetrate the BBB (9) although several studies reported that this dose of captopril inhibits central angiotensin-converting enzyme (5). In any case, in the present study, a relatively large dose of peripheral captopril pretreatment did not alter the responses of systemic AVP release and blood pressure to systemic LPS challenge. This suggests that central AT1 receptors, responding to ANG II at the brain side of the BBB, are involved in the systemic AVP secretion and blood pressure regulation.

McKinley et al. (22) reported that osmoregulatory fluid intake was intact in mice lacking angiotensin, although many studies reported that central AT1 antagonism significantly inhibits osmoregulation (7, 12, 18). These results suggest the possibility that an unknown ligand for AT1 receptors stimulated by increased osmolality, and possibly LPS, is involved in the AVP secretion and blood pressure regulation, or that another type of receptor blocked by losartan plays a role in these responses (22).

As reported in some earlier studies (10, 35), intravenous LPS administration at this dose (150 μg/kg) did not alter plasma osmolality or hematocrit, and intracerebroventricular losartan inhibited LPS-induced systemic AVP secretion, suggesting that AVP secretion is caused by factors other than changes in body fluid status. A large dose of LPS causes septic shock, and severe hypotension is a factor inducing AVP release (2, 4). In the present study, we confirmed that LPS administration at a dose of 150 μg/kg did not decrease blood pressure in vehicle-treated control (10, 35), while LPS administration caused hypotension in centrally losartan-treated rats. In addition, LPS did not alter blood pressure in rats with systemic captopril pretreatment. These results indicate that LPS at this dose stimulates central angiotensinergic neurons and AVP secretion independently of changes in body fluid status or hypotension.

Hyperthermia induced by endotoxemia could be a direct or indirect factor stimulating AVP secretion (6, 11, 33). Kregel et al. (15) reported that AVP secretion induced by severe hyperthermia is mediated, at least in part, by brain AT1 receptors in passively heated rats. However, heat stress they employed was extremely severe, the core temperature elevated to 41°C after passive body heating. In the present study, Tambo did not change significantly 60 min after the LPS injection, and Tambo was similar among the groups throughout the experiment. Therefore, change in body temperature is not a mechanism for LPS-induced activation of central ANG II and systemic AVP release.

Xu et al. (37) reported that central α1-adrenergic receptor antagonist treatment attenuated activation of magnocellular AVP neurons in the PVN and SON. However, α1-adrenergic receptor antagonism only partially inhibited LPS-induced activation of SON and PVN magnocellular AVP neurons. In contrast, AT1 receptor antagonism almost completely inhibited LPS-induced AVP secretion in the present study. However, there is a possibility that adrenergic and angiotensinergic systems cause AVP secretion in a serial manner. Bathalo et al. (2) reported that glucocorticoid attenuated LPS-induced increases in plasma AVP concentration. LPS dose in that study was higher than in our study and caused hypotension, and glucocorticoid treatment attenuated the decrease in blood pressure induced by LPS administration. Glucocorticoid thus seems to inhibit AVP release by inhibiting hypotension. In contrast, with 150 μg/kg LPS, central AT1 receptor antagonist inhibited AVP secretion and decreased blood pressure. These suggest that glucocorticoid or change in blood pressure is not involved in the inhibition of LPS-induced AVP secretion by central AT1 receptor antagonism.

The present data also demonstrated that central AT1 receptors probably play an important role in maintaining blood pressure during endotoxemia. Blood pressure regulation is mainly mediated by the sympathetic nervous system, and also by circulating AVP, particularly during severe hypotension. Central ANG II reportedly plays a significant role in sympathetic regulation of blood pressure (8, 27, 29). In the present study, blood pressure was well maintained following LPS administration in vehicle- and systemically captopril-treated rats, but decreased in centrally losartan-treated rats. Peripheral captopril treatment before LPS injection decreased blood pressure and increased HR. In centrally losartan-treated rats, LPS injection decreased blood pressure but did not alter HR. These results suggest that baroreceptor-mediated activation of sympathetic nerves, as well as AVP release, is attenuated by central losartan (17, 31). In addition, central AT1 receptor blockade inhibited systemic AVP release. Thus maintenance of blood pressure during endotoxemia at this dose is probably achieved by activation of both sympathetic nervous and hypothalamic-neurohypophyseal systems via central AT1 receptors, although it remains unclear the contributing weight of sympathetic nervous activity and systemic AVP to blood pressure regulation.

Our data indicate that central AT1 receptors responding to ANG II inside the BBB is involved in AVP release and blood pressure regulation during endotoxemia. However, it remains unclear how peripheral LPS stimulates central angiotensinergic neurons. We speculate that LPS-induced activation of central angiotensinergic neurons is mediated by IL-1β (16, 24). Landgraf et al. (16) reported that intracerebroventricular IL-1β administration stimulates both central and systemic AVP and oxytocin release in rats. In addition, it has been reported that intracerebroventricular IL-1β administration exerts hypertension, and that the IL-1β-induced pressor response was inhibited by intracerebroventricular losartan pretreatment (34). Thus we speculate that centrally acting IL-1β plays a role in AVP release and blood pressure regulation during endotoxemia via a central angiotensinergic pathway. It is unlikely that the site of action on IL-1β is in the SON or PVN magnocellular neurons, because local IL-1β application into the PVN or SON failed to increase systemic AVP release (16).

The site of IL-1β production by LPS might be in the circumventricular organs, such as the organum vasculosum of the lamina terminalis (OVLT) (25). However, a large dose of LPS (40 μg/kg) can also induce IL-1β at the hypothalamic parenchyma (25). Thus we speculate that LPS at a dose of 150 μg/kg can induce IL-1β production both inside and outside of the BBB.

ANG II is known to enhance the induction of proinflammatory agents, such as IL-1β, via NF-κB and activator protein-1 (AP1) (23). Central AT1 receptor antagonism with losartan has been reported to attenuate the LPS-induced fever and central
IL-1β production (30), and AT1 receptor blockade attenuates activation of cultured microglial cells by LPS (23). In the present study, central AT1 receptor blockade inhibited LPS-induced AVP secretion and blood pressure regulation, suggesting a possibility that the LPS-induced AVP secretion is partly enhanced by central microglial IL-1β production via central AT1 receptors. However, it is also possible and more plausible that the action of central IL-1β on systemic AVP release and blood pressure regulation is mediated by central ANG II (34).

Our data demonstrate that the site of AT1 receptors, which are involved in LPS-induced vasopressin release and blood pressure regulation, is located at the brain side of the BBB. However, the specific site of AT1 receptor playing a role in the AVP release and blood pressure regulation during endotoxemia remains unknown from the present data. Intracerebroventricular administration of ANG II has been reported to induce c-Fos expression in the AV3V region, including OVLt, subformical organ, and median preoptic nucleus (MnPO), in addition to PVN and SON (28). Therefore, AT1 receptors facing to the brain side of the BBB are located at the AV3V region (28). Further, McKinley et al. (21) hypothesized that an angiotensinergic synapse in the MnPO plays a role in vasopressin release. Thus AT1 receptors inside the BBB in the AV3V region, probably at the MnPO, might play a role in AVP secretion and blood pressure regulation during endotoxemia.

Subcutaneous captopril pretreatment decreased SBP by 13 ± 3 mmHg, which corresponds to 12 ± 3%. It has been accepted in humans that a fall in blood pressure by 8–10% is required to stimulate vasopressin release in humans (1), but the fall in blood pressure in the present study did not stimulate AVP release. It might be because we measured SBP in the present study. The decreased mean arterial pressure by peripheral captopril treatment might be a little smaller than threshold for induction of AVP release, although the decrease in SBP was larger than 10%.

In summary, the present study demonstrated that systemic LPS stimulates AVP release without any changes in plasma osmolality, hematocrit, body temperature, or blood pressure. Central AT1 receptor antagonism with losartan inhibited the LPS-induced AVP secretion and blood pressure regulation, but inhibition of peripheral ANG II production with captopril did not alter AVP and blood pressure responses to systemic LPS. Our results indicate that central AT1 receptors, which are not responsive to peripheral ANG II, are involved in AVP release during endotoxemia via central AT1 receptors. Our data also suggest that central ANG II plays an important role in blood pressure regulation during endotoxemia.

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