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The Effect of Central Injection of Angiotensin-Converting Enzyme Inhibitor and the Angiotensin Type 1 Receptor Antagonist on the Induction by Lipopolysaccharide of Fever and Brain Interleukin-1β Response in Rats

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ABSTRACT

We recently reported an involvement of peripheral angiotensin II (ANG II) in the development of both the fever and the peripheral interleukin (IL)-1β production induced in rats by a systemic injection of lipopolysaccharide (LPS). The present study was performed to investigate whether brain ANG II contributes to the fever and IL-1β production in the rat brain induced by i.c.v. injection of LPS. LPS (0.2 and 2 μg i.c.v.) induced dose-related fevers and increases in the brain (hypothalamus, hippocampus, and cerebellum) concentrations of IL-1β. These effects were significantly inhibited by i.c.v. administration of either an angiotensin-converting-enzyme (ACE) inhibitor or an angiotensin type 1 (AT_1) receptor antagonist. By contrast, the ACE inhibitor had no effect on the IL-1β (i.c.v.)-induced fever, whereas the AT_1 receptor antagonist enhanced (rather than reduced) it. The AT_1 receptor antagonist had no effect on the brain levels of prostaglandin E_2 in rats given an i.c.v. injection of IL-1β. These results suggest that in rats, brain ANG II and AT_1 receptors are involved in the LPS-induced production of brain IL-1β, thus contributing to the fever induced by the presence of LPS within the brain.

Angiotensin II (ANG II), a bioactive peptide well known to play an important role in blood pressure and body fluid regulation, seems to participate in inflammatory responses, too. For example, an angiotensin-converting-enzyme (ACE) inhibitor has been shown to have an anti-inflammatory effect (Godsel et al., 2003). Furthermore, ANG II and ANG II type 1 (AT_1) receptors are involved in cardiovascular inflammation, such as monocyte infiltration (Usui et al., 2000). Recently, we reported results suggesting that ANG II is involved in the development of the fever (an inflammation-related response) induced by i.v. injection of lipopolysaccharide (LPS, 2 μg/kg) in euhydrated rats as well as in dehydrated rats (in which the secretion of ANG II is elevated) (Watanabe et al., 2000). In fact, the LPS-induced fever seen in that study was significantly attenuated by an ACE inhibitor, injected i.v. Because, as the first step in fever induction the pyrogenic/proinflammatory cytokine interleukin (IL)-1 is released from macrophages after their stimulation by LPS (Kluger, 1991; Dinarello, 1999), we speculated that ANG II might contribute to the LPS-induced peripheral production of IL-1. Indeed, i.v. injection of LPS increases the liver concentration of IL-1β in dehydrated rats, and this effect can be significantly attenuated by an ACE inhibitor or by an AT_1 receptor antagonist, in each case given i.v. (Miyoshi et al., 2003). However, we found that an i.v. injection of LPS (2 μg/kg) did not induce any detectable changes in the brain level of IL-1β (our unpublished observations). It seemed likely from these results that peripheral ANG II is involved in the development of the peripheral production of IL-1β (induced by LPS).

The brain has its own renin-angiotensin system and its own AT_1 receptors that play important roles in blood pressure and body fluid regulation (Tsutsumi and Saavedra, 1991; Rowe et al., 1992; Wright and Harding, 1992), alongside the regulation mediated via peripheral ANG II. On the other hand, an i.c.v. injection of LPS reportedly leads to

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ABBREVIATIONS: ANG II, angiotensin II; ACE, angiotensin-converting-enzyme; AT_1 receptor, angiotensin type 1 receptor; LPS, lipopolysaccharide; IL, interleukin; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; PG, prostaglandin; ANOVA, analysis of variance; NF-κB, nuclear factor-κB.

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marked fever and IL-1β production in the brain (De Simoni et al.,1997; Tsushima and Mori, 2000). The i.c.v. injection of LPS provides a model of bacterial meningitis, in which bacteria (or LPS) enter the brain and induce pathological changes such as fever and cerebral inflammation (Korytko and Boje, 1996; Tsushima and Mori, 2000). However, it is at present, unknown whether brain ANG II contributes to the production of IL-1β within the brain, just as peripheral ANG II probably promotes the peripheral production of IL-1β (see above).

In this study, we investigated the effects of an i.c.v. injection of an ACE inhibitor or an AT_1 receptor antagonist on the fever and the brain IL-1β response induced in rats by i.c.v. LPS. We also examined the effect of i.c.v. treatment with an ACE inhibitor or an AT_1 receptor antagonist on the fever due to i.c.v. injection of IL-1β.

Materials and Methods

Animals

The animals used in this study were male Wistar rats, weighing 270 to 350 g. They were housed in individual plastic cages (40 × 25 × 20 cm; length × width × depth) with wood-chip bedding in a room maintained at 26 ± 1°C, a temperature within the thermoneutral zone for rats. They experienced a photoperiod of 12-h light:12-h dark, with lights coming on at 7:00 AM. All animals had ad libitum access to drink and standard laboratory rat chow. The protocols were reviewed by the Committee on the Ethics of Animal Experiments in Tottori University Faculty of Medicine, and the experiments were carried out in accordance with the Guidelines for Animal Experiments at Tottori University Faculty of Medicine and the Federal Law (221) and Notification (6) of the Japanese Government.

This study comprised seven experiments (experiments 1–7), all on freely moving euhydrated rats. Each rat took part in only one experiment. Details of the experimental protocols are given below.

Surgery

To permit i.c.v. injections, a stainless steel cannula (0.8 mm o.d.) was implanted in each rat under general anesthesia (sodium pentobarbitone, 50 mg/kg i.p.) so that its tip lay in the lateral cerebral ventricle (coordinates AP, −0.8; L, 1.5; V, 3.5 mm; Paxinos and Watson, 1998) using standard stereotaxic technique. The implantation was performed under strictly sterile conditions.

For experiments 1, 2, 5, and 6 (see below), each rat was again anesthetized with sodium pentobarbitone (50 mg/kg i.p.), and a battery-operated transmitter (model TA10TA-F40) for the measurement of body temperature was implanted i.p. This was done at least 10 days after the implantation of the i.c.v. cannula and 7 days before the start of the experiments to enable us to measure body temperature using a biotelemetry system (Data Science, Inc., St. Paul, MN). The output of the transmitter was monitored by antennae mounted in a receiver board (model CTR86) placed under each animal's cage. The data were fed into a peripheral processor (matrix model BCM100) connected to a Sanyo MBC-17J AX computer (IBM compatible). All rats were handled for 5 min each day for at least 5 days to accustom them to the experimenters.

Drugs

The LPS used in this study, which was derived from *Salmonella typhosa* endotoxin (Sigma-Aldrich, St. Louis, MO), was dissolved in sterile saline. Lisinopril (Sigma-Aldrich) was also dissolved in sterile saline. Losartan, dissolved in sterile saline for injections, was a kind gift from Merck & Co., Inc. (Rahway, NJ). Human recombinant IL-1β supplied by Otsuka Pharmaceutical (Tokushima, Japan), was produced from recombinant strains of *Escherichia coli*. The activity of the IL-1β was found to be 2×10^4 units/μg by means of a thymocyte coproliferation assay. The IL-1β preparation was shown to be free of significant endotoxin contamination by a *Limulus* amoebocyte assay (<0.05 pg/μg protein). IL-1β was dissolved in sterile saline. The doses injected in each experimental group are given below.

Experimental Protocols

Experiment 1. The i.c.v. injection of one of two doses of LPS (0.2 and 2 μg) or saline (5 μl) was performed to observe the dose-related changes in body temperature induced in conscious rats by LPS. We chose these doses of LPS because Tsushima and Mori (2000), who examined changes in body temperature induced by a similar dose of LPS (3 μg i.c.v.), reported a significant fever.

Each rat was gently picked up and its transmitter switched on with a magnet at 18 h before the start of the experiment. The body temperature was then allowed to stabilize at an ambient temperature of 26 ± 1°C before any injections. The injectate (LPS solution or saline) was given i.c.v. to each animal in a volume of 5 μl over a period of 30 s. The i.c.v. injections were made via a stainless steel needle (0.4 mm o.d.) inserted through the cannula and attached to a microsyringe via polyethylene tubing. To minimize the influence of the rat's own circadian rhythm, LPS was always given between 11:00 and 12:00. We measured changes in body temperature due to LPS for 6 h after the injection. After its involvement in experiment 1, each animal was subjected to CO_2 stunning. Fast Green FCF solution (5 μl) was then injected i.c.v. to mark the ventricular space, followed by decapitation. Only data from animals in which the tip of the cannula could be seen to have been located within the cerebral ventricle were included under *Results*. Furthermore, in this and all other experiments, any rats exhibiting signs of infection (such as fever, reduced appetite and drinking, and/or piloerection) were excluded from the study.

Experiment 2. The effect of an i.c.v. injection of an ACE inhibitor, lisinopril (50 μg), or an AT_1 receptor antagonist, losartan (50 μg), was investigated on the fever induced by LPS (2 μg i.c.v.). The injectate (LPS solution, lisinopril mixed with LPS solution, or losartan mixed with LPS solution) was given i.c.v. to each animal in a volume of 5 μl over a period of 30 s. Lisinopril or losartan alone was given to other animals. The remaining procedures were essentially the same as those described for experiment 1.

We believe that the doses of lisinopril and losartan used in this study were successful at inhibiting ACE and ANG II because previous studies have demonstrated 1) that an even smaller dose of lisinopril (2 μg i.c.v.) is effective at inhibiting ACE (namely, the drug suppressed water-deprivation-induced drinking) (Saad et al., 1992); and 2) that less than 10 μg of losartan is sufficient to inhibit ANG II-induced responses such as increases in water intake (Rowland et al., 1992) and arterial blood pressure in rats (Hogarty et al., 1992). In addition, Mathai et al. (2000) reported that 10 μg of losartan (i.c.v.) impaired the thermolytic response to heat exposure in rats. On the other hand, we believe that the doses used in this study are not too high. In many studies, lisinopril and losartan have been given to rats systemically at doses within the range 10 to 30 mg/kg (Watanabe et al., 2000; Carey et al., 2001; Ongali et al., 2003), which is equivalent to about 156 to 468 mg/l blood, because it is known that the blood volume of rats is about 64.1 (57.5–69.9) ml/kg. To our knowledge, there is no report available on the rat's cerebrospinal fluid (CSF) volume. However, Consiglio and Lucion (2000) developed a technique for collecting a conscious rat's CSF from the cisterna magna, and the maximum volume of CSF successfully collected was 120 μl. Therefore, it is reasonable to think that the total volume of the rat's CSF is more than 120 μl. On this basis, the concentration of drugs (after injection of 50 μg) reached in the CSF in the present study would have been less than 417 mg/l CSF (0.05 mg/0.00012 l = 417 mg/l).

Experiment 3. Dose-related changes in the regional IL-1β content in the brain (hypothalamus, hippocampus, and cerebellum) were examined in rats after an i.c.v. injection of LPS (0.2 or 2 μg) or saline (5 μl). The injectate (LPS solution or saline) was given i.c.v. to

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TABLE 1

Effect of i.c.v. treatment with losartan on the concentration of PGE_2 in the hypothalamus, hippocampus, and cerebellum in rats given an i.c.v. injection of IL-1β

Mean values (±S.E.M.) obtained for brain levels of PGE_2 (pg/100 μg of protein) in rats 90 min after i.c.v. injection of IL-1β (200 ng). Losartan (50 μg) was administered simultaneously with the IL-1β. There was no significant difference between the IL-1β and losartan + IL-1β groups in the PGE_2 levels in the hypothalamus, hippocampus, or cerebellum.

	Hypothalamus	Hippocampus	Cerebellum
IL-1β (n = 10)	1.20 ± 0.18	9.22 ± 0.83	1.13 ± 0.16
Losartan + IL-1β (n = 9)	1.19 ± 0.13	8.65 ± 1.77	1.10 ± 0.12

each animal in a volume of 5 μl over a period of 30 s. Animals were subjected to CO_2 stunning either 2 or 4 h after their injection. Fast Green FCF solution (5 μl) was then injected i.c.v. to mark the ventricular space, followed by decapitation. The brain was quickly removed and the hypothalamus, hippocampus, and cerebellum were dissected out, and then frozen and powdered in liquid nitrogen. During the brain dissection, we verified the position of the cannula tip (see above).

The brain regional concentrations of IL-1β were measured by ELISA. In brief, each powdered tissue, immersed in Iscove's culture medium containing a cocktail protease inhibitor (Sigma-Aldrich), was mechanically homogenized on ice, using a postmounted laboratory homogenizer (Omni International, Warrenton, VA). Homogenized samples were centrifuged at 10,000 rpm for 10 min at 4°C. Each supernatant was then transferred into a fresh test tube and stored at −85°C until needed for measurements of IL-1β and total protein content. The IL-1β content was measured using a commercial ELISA kit (TFB Inc., Tokyo, Japan) with a lower detection limit of 3 pg/ml. The total protein content was determined using a Bio-Rad protein-assay kit. The tissue concentration of IL-1β is expressed as the cytokine content per 100 μg of protein.

Experiment 4. The effect of an i.c.v. injection of lisinopril (50 μg) or losartan (50 μg) was investigated on the changes in the brain concentration of IL-1β at 4 h after an administration of LPS (2 μg i.c.v.). The injectate (saline, LPS solution, lisinopril mixed with LPS solution, or losartan mixed with LPS solution) was given i.c.v. to each animal in a volume of 5 μl over a period of 30 s. Lisinopril or losartan alone was given to other animals. The other procedures were essentially the same as those described for experiment 3.

Experiment 5. The i.c.v. injection of one of two doses of IL-1β (20 and 200 ng) or saline (5 μl) was performed to examine the dose-related changes in body temperature in rats. We chose these doses of IL-1β because Nakamori et al. (1993) showed that a moderate fever could be induced by IL-1β at 20 ng i.c.v. The other procedures were essentially the same as those described for experiment 1.

Experiment 6. The effect of an i.c.v. injection of lisinopril (50 μg) or losartan (50 μg) was investigated on the IL-1β (200 ng i.c.v.)-induced fever in rats. The procedures were essentially the same as those described for experiment 1.

Experiment 7. We examined the effect of losartan (50 μg) given i.c.v. on the brain levels of the final fever mediator prostaglandin (PG) E_2 (Blatteis and Sehic, 1997; Ushikubi et al., 2000) in rats given an i.c.v. injection of IL-1β (200 ng). The injectate (IL-1β solution or losartan mixed with IL-1β solution) was given i.c.v. to each animal in a volume of 5 μl over a period of 30 s. After decapitation at 90 min after the injection, the hypothalamus, hippocampus, and cerebellum were dissected out and then frozen and powdered in liquid nitrogen.

The brain concentrations of PGE_2 were measured by ELISA. In brief, each powdered tissue was homogenized in 0.1 M sodium phosphate buffer (pH 7.5) containing 15% methanol. Homogenized samples were centrifuged at 10,000 rpm for 10 min at 4°C. Each supernatant was then transferred into a fresh test tube and stored at −85°C until needed for measurement of PGE_2 and total protein content. The PGE_2 was extracted from the samples using a C18 Sep-Pak column (Waters Milford, MA) and the PGE_2 content measured using a commercial ELISA kit (Neogen Corp., Tokyo, Japan) with a lower detection limit of 0.1 ng/ml. The total protein content was determined using a Bio-Rad protein assay kit. The tissue concentration of PGE_2 is expressed as the PGE_2 content per 100 μg of protein.

Statistical Analysis

All results are expressed as mean ± S.E.M. Body temperature data were analyzed for statistical significance by means of a repeat-

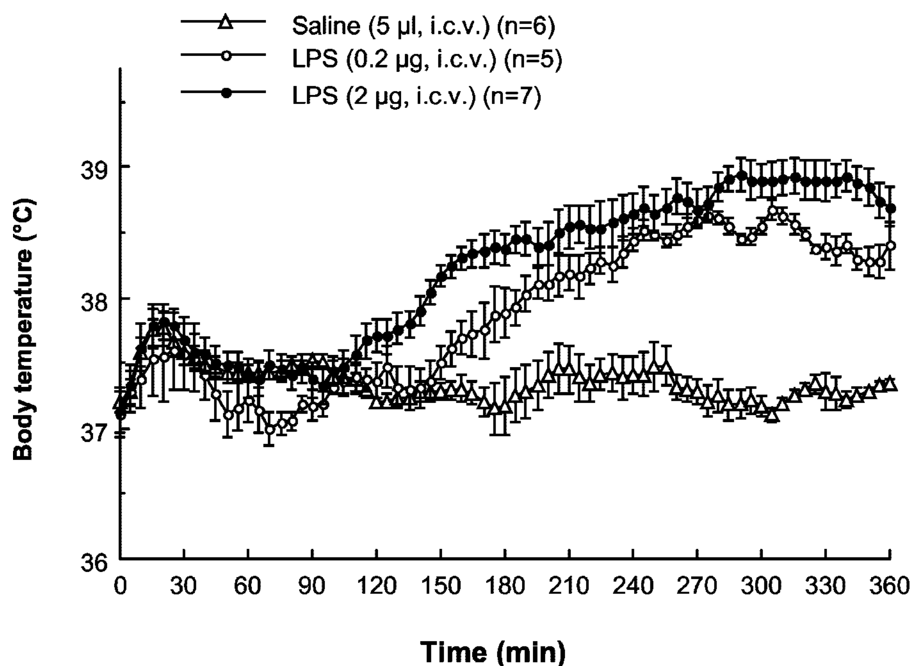

Fig. 1. Febrile responses induced in rats by i.c.v. injection of LPS. Mean values (±S.E.M.) obtained for body temperature (°C) in rats after i.c.v. injection at time 0 of one of two doses of LPS (0.2 or 2 μg) or saline (5 μl). LPS was dissolved in sterile saline. There was a significant ($p < 0.05$) difference in the temperature responses among the three groups. From repeated-measures ANOVA: for treatment effect, $p < 0.0001$; for time effect, $p < 0.0001$; for interaction, $p < 0.0001$.

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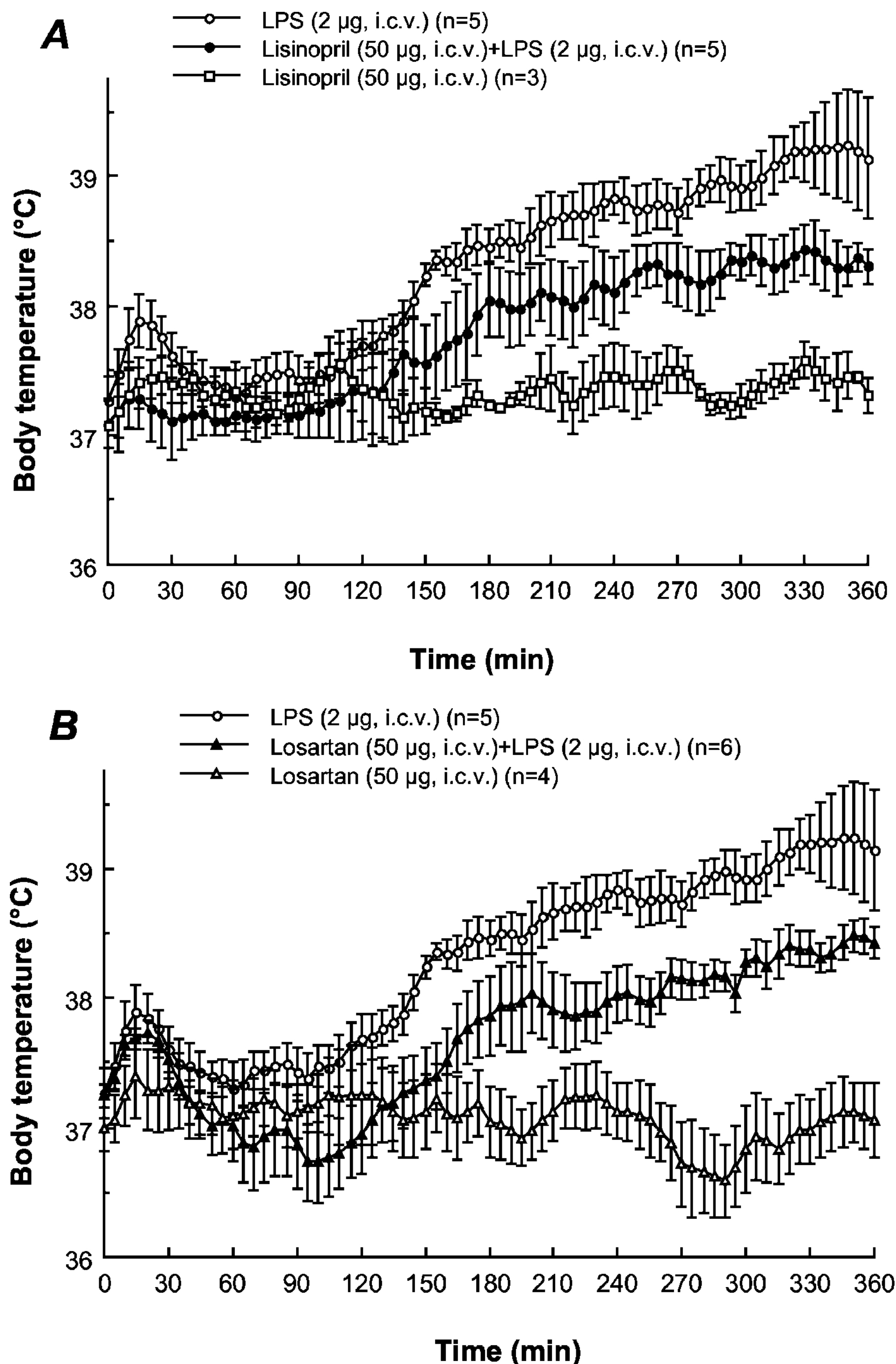

Fig. 2. Effect of i.c.v. treatment with an ACE inhibitor, lisinopril, or an AT_1 receptor antagonist, losartan, on LPS-induced fever in rats. Mean values (±S.E.M.) obtained for body temperature (°C) in rats after i.c.v. injection at time 0 of LPS (2 μg). Lisinopril (A, 50 μg) or losartan (B, 50 μg) was administered simultaneously with the LPS. The effect of lisinopril (A) or losartan (B) (each 50 μg i.c.v.) given alone at time 0 on resting body temperature is also shown. Lisinopril and losartan were each dissolved in sterile saline. There were significant ($p < 0.05$) differences in the temperature responses between the LPS-injected group and the lisinopril + LPS group and between the LPS-injected group and the losartan + LPS group. From repeated-measures ANOVA: for treatment effect, $p < 0.01$ in A and B; for time effect, $p < 0.0001$ in A and B; for interaction, $p < 0.0001$ in A and B.

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ed-measures ANOVA, followed by Fisher's protected least significant difference test (post hoc test) to assess the overall effect (Macintosh, StatView 4.0). This analysis was performed on data collected from the time of drug injection onward (i.e., from time 0 to 360 min). Details of the results of this analysis are given in the legends for Figs. 1 and 2 and 5 and 6. When there was no treatment effect, but a significant interaction, further statistical comparisons between the changes in the groups over given time periods were made by a repeated-measures ANOVA followed by Fisher's protected least significant difference test (post hoc test) (Fig. 6; Macintosh, StatView 4.0). IL-1β data were analyzed for statistical significance by means of a one-way ANOVA, followed by Fisher's protected least significant difference test (post hoc test, Figs. 3–4) (Macintosh, StatView 4.0). PGE_2 data were analyzed for statistical significance using a Student's t test with Bonferroni's correction (Table 1). Differences were considered significant at $p < 0.05$.

Results

Febrile Responses Induced in Rats by i.c.v. Injection of LPS. Injections of LPS (0.2 or 2 μg i.c.v.) produced dose-related ($p < 0.05$) increases in body temperature (fever); they had a latency of about 90 min and reached peak 270 to 330 min after the injection (Fig. 1). Sterile saline induced no change in body temperature after its i.c.v. injection, except for a small increase immediately after the injection (this represents an injection stress-induced hyperthermia), indi-

cating that saline per se has no effect on body core temperature.

Effect of an ACE Inhibitor, Lisinopril, or an AT_1 Receptor Antagonist, Losartan, on LPS-Induced Febrile Responses in Rats. As shown in Fig. 2, the LPS (2 μg i.c.v.)-induced febrile response was significantly ($p < 0.05$) attenuated by i.c.v. treatment with either lisinopril (50 μg; Fig. 2A) or losartan (50 μg; Fig. 2B). The i.c.v. injection of lisinopril or losartan alone had no effect on resting body temperature (over and above any changes seen on saline administration) (Figs. 1 and 2; $p > 0.05$). Furthermore, intravenous injection of either lisinopril (50 μg) or losartan (50 μg) had no effect on the febrile response induced by i.c.v. LPS [2 μg; data not shown ($n = 3$ for each)], indicating that brain ANG II and AT_1 receptors are involved in the febrile response induced by i.c.v. injection of LPS.

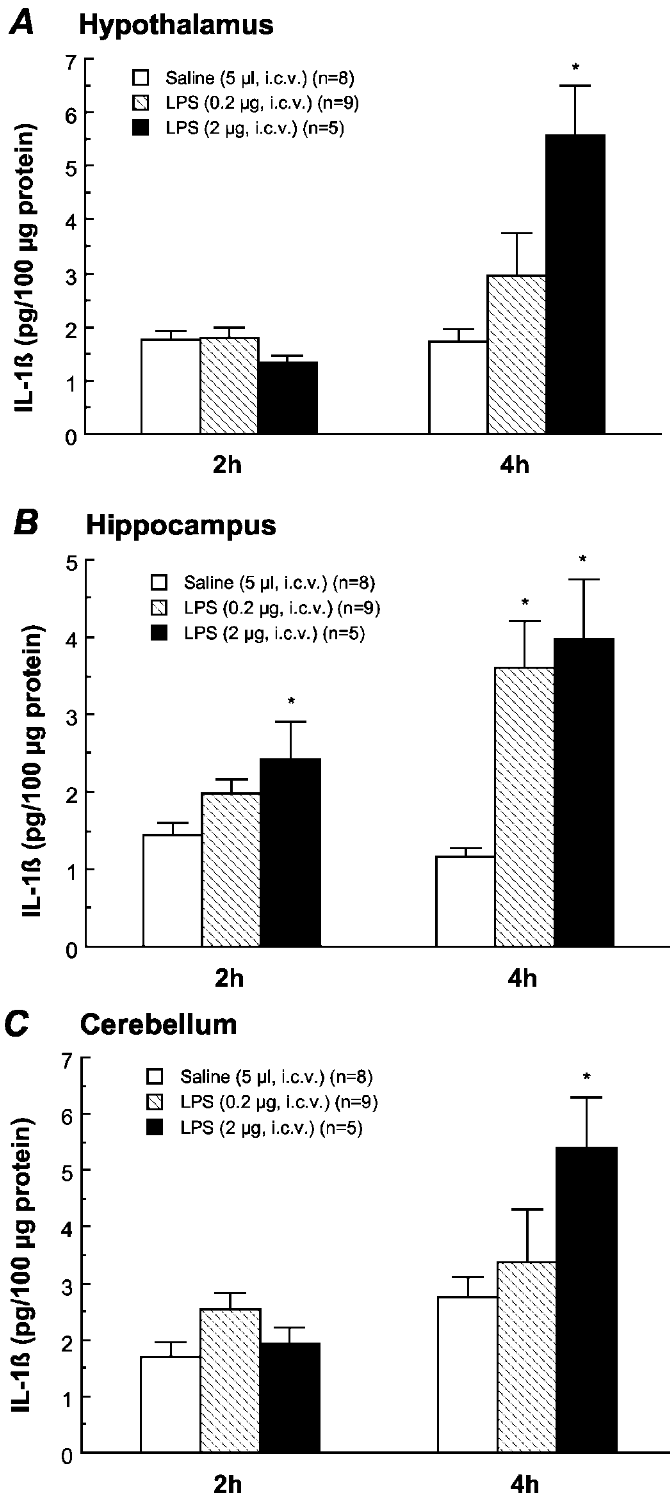

Fig. 3. Changes in IL-1β levels induced in rats by i.c.v. injection of LPS. Mean values (±S.E.M.) obtained for brain [hypothalamus (A), hippocampus (B), and cerebellum (C)] concentrations of IL-1β in rats at 2 and 4 h after i.c.v. injection at time 0 of one of two doses of LPS (0.2 or 2 μg) or saline (5 μl). *, $p < 0.05$ versus saline.

IL-1β Responses Induced in Rats by i.c.v. Injection of LPS. At 2 h after an i.c.v. injection of one of two doses of LPS (0.2 or 2 μg) or saline, there were no differences in the IL-1β levels among the three groups, except that 2 μg of LPS produced a significant increase in the hippocampal concentration of IL-1β (Fig. 3). By contrast, at 4 h after the injection of LPS there were dose-related increases in the concentrations of IL-1β in the hypothalamus, hippocampus, and cerebellum. It is important to note in Fig. 3 that LPS at a dose of 2 μg elicited IL-1β responses at 4 h that were statistically significant in all three brain areas examined.

Furthermore, in rats ($n = 3$) fitted with an i.c.v. cannula but not given any i.c.v. injections, the IL-1β levels in the hypothalamus (1.73 ± 0.34 pg/100 μg protein), hippocampus (1.02 ± 0.03 pg/100 μg protein), and cerebellum (2.85 ± 0.23 pg/100 μg protein) were similar to those in the saline-injected control group (1.62 ± 0.08, 1.06 ± 0.05, and 2.90 ± 0.31 pg/100 μg protein, respectively).

Effect of Lisinopril or Losartan on the LPS-Induced IL-1β Responses in Rats. The brain concentrations of IL-1β were increased significantly at 4 h after an injection of LPS (2 μg i.c.v.) (versus those in the saline-injected group) (Fig. 4). When lisinopril (50 μg; Fig. 4A) or losartan (50 μg; Fig. 4B) was given i.c.v. along with the LPS (2 μg i.c.v.), the LPS-induced IL-1β responses were significantly smaller in the hypothalamus and hippocampus. Furthermore, the i.c.v. injection of lisinopril or losartan alone had no effect on the resting levels of IL-1β in the hypothalamus and hippocampus. On the other hand, in the cerebellum only lisinopril (not losartan) exerted a significant inhibitory effect on the LPS-induced IL-1β response, whereas lisinopril alone resulted in an increase in the concentration of IL-1β (versus the saline group). Although losartan tended to attenuate the LPS-induced IL-1β response in the cerebellum, its effect did not reach significance.

Febrile Responses Induced in Rats by i.c.v. Injection of IL-1β. The i.c.v. injection of IL-1β (20 or 200 ng) resulted in dose-related ($p < 0.05$) fevers (Fig. 5). Saline had no effect on the resting body temperature, except for the injection stress-induced hyperthermia.

Effect of Lisinopril or Losartan on IL-1β-Induced Febrile Responses in Rats. When lisinopril was administered i.c.v. along with IL-1β (200 ng; Fig. 6A), there was no change in the IL-1β-induced fever. On the other hand, the IL-1β-induced fever was enhanced by losartan ($p < 0.05$) in the period 85 to 180 min (Fig. 6B).

Effect of Losartan on IL-1β-Induced PGE_2 Responses in Rats. Table 1 shows that i.c.v. administration of losartan (50 μg) had no effect on the levels of PGE_2 in the hypothalamus, hippocampus, or cerebellum at 90 min after the i.c.v. injection of IL-1β (200 ng).

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Discussion

The present results show that i.c.v. injection of LPS (0.2 and 2 μg) induces a dose-related fever in rats. Furthermore, the fever induced by the larger dose (2 μg) was significantly attenuated by i.c.v. treatment with an ACE inhibitor or an AT_1 receptor antagonist, indicating the involvement of brain ANG II and AT_1 receptors. In addition, dose-related increases in IL-1β concentration were induced in the hypothalamus, hippocampus, and cerebellum by i.c.v. LPS. Furthermore, the ACE inhibitor and the AT_1 receptor antagonist significantly inhibited the LPS (2 μg)-induced IL-1β responses in the hypothalamus and hippocampus. The first step in fever induction reportedly involves an LPS-induced production of IL-1 (Kluger, 1991; Dinarello, 1999), and it is apparently brain IL-1 that actually mediates the rat's febrile response to i.c.v. LPS (Tsushima and Mori, 2000). Hence, brain ANG II, via an action on its AT_1 receptor, may well contribute to the IL-1β production and ultimately to the fever induced in rats by i.c.v. LPS.

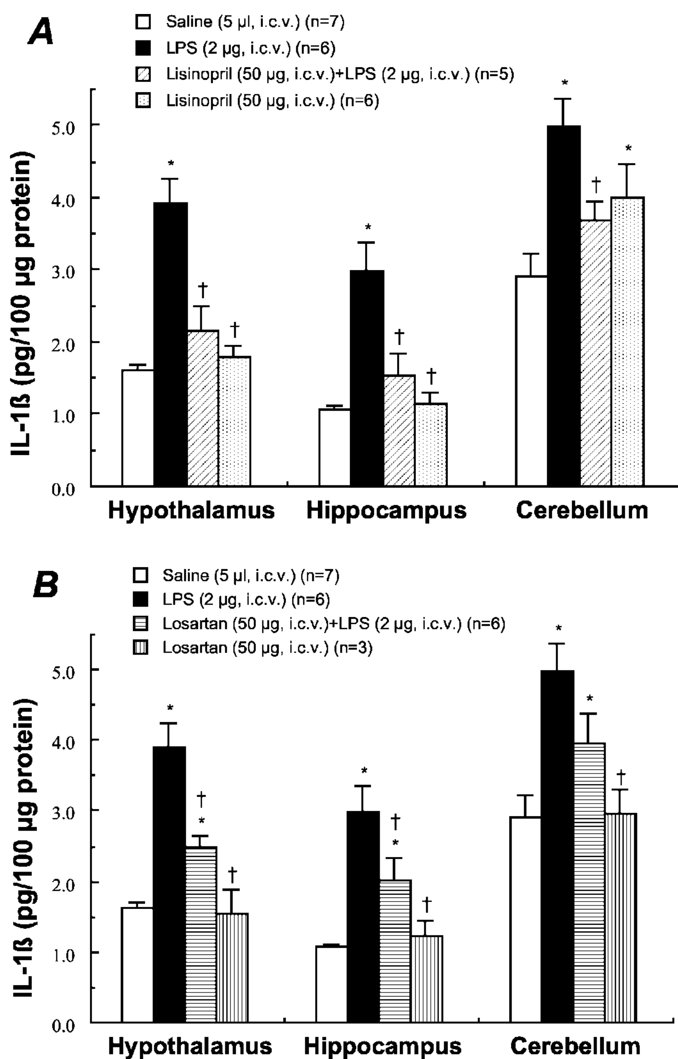

Fig. 4. Effect of i.c.v. treatment with lisinopril or losartan on LPS-induced IL-1β responses in rats. Mean values (±S.E.M.) obtained for brain (hypothalamus, hippocampus, and cerebellum) concentrations of IL-1β in rats at 4 h after i.c.v. injection at time 0 of LPS (2 μg i.c.v.) or saline (5 μl i.c.v.). Lisinopril (A, 50 μg) or losartan (B, 50 μg) was administered simultaneously with the LPS. The effect of lisinopril (A) or losartan (B) (each 50 μg i.c.v.) given alone at time 0 on the resting brain concentrations of IL-1 β is also shown. *, $p < 0.05$ versus saline; †, $p < 0.05$ versus LPS.

The present results are supported by several previous findings. For example, in vitro studies have suggested that ANG II is involved in the production of proinflammatory cytokines from LPS-stimulated leukocytes (Schindler et al., 1995; Peeters et al., 1998). Moreover, application of ANG II onto cultured mesangial cells results in the production of another cytokine, IL-6 (Moriyama et al., 1995), and systemic administration of LPS increases renal IL-6 production, an effect that can be inhibited by an ACE inhibitor or an AT_1 receptor antagonist (Niimi et al., 2002). Finally, we recently demonstrated contributions of ANG II and its AT_1 receptor to LPS-induced hepatic IL-1β production in vivo (Miyoshi et al., 2003). However, the precise mechanism by which ANG II contributes to the induction of cytokine production remains unknown. One possibility is that it activates some proinflammatory transcription factor(s) such as nuclear factor-κB (NF-κB), leading to the production of cytokines. Indeed, 1) LPS activates NF-κB in monocytes (Baeuerle and Henkel, 1994); 2) cytokine expressions are controlled at the transcriptional level through NF-κB (Baeuerle and Henkel, 1994); and 3) ANG II, too, activates NF-κB in monocytes (Kranzhofer et al., 1999). Therefore, it is possible that activation of NF-κB by LPS is mediated or enhanced by ANG II, leading to an increase in cytokine production. This possibility should be examined in the not-too-distant future.

We did not examine whether LPS up-regulates the central renin-angiotensin system in this study. However, systemic injection of LPS up-regulates angiotensinogen mRNA in the liver (Nyui et al., 1997). Because the brain has its own renin-angiotensin system, including angiotensinogen mRNA (Wright and Harding, 1992), it is likely that LPS within the brain up-regulates the central renin-angiotensin system as well. The activation of the peripheral renin-angiotensin system induced by systemic injection of IL-1β is mediated by prostaglandins (PGs; Bataillard et al., 1992), and brain PGs apparently activate the brain renin-angiotensin system (Scholkens et al., 1984). In our recent unpublished microdialysis study, ANG II release in the hypothalamus was increased (on average, by 0.18 pg/2 μl perfusate/min; $n = 5$) during intrahypothalamic perfusion with PGE_2 (2 μg/2 μl perfusate/min). A reasonable inference from the above-mentioned evidence is that brain LPS may up-regulate brain ANG II through an action of PGs. However, to confirm this idea, we need to examine whether brain LPS actually does stimulate the central renin-angiotensin system in the near future.

In the present study, dose-related fevers were evoked by IL-1β (20 and 200 ng i.c.v.). Furthermore, the IL-1β (200 ng)-induced fever was not attenuated by the ACE inhibitor or the AT_1 receptor antagonist (the latter actually enhancing one period of the fever). These results are consistent with our hypothesis that brain ANG II and AT_1 receptors are involved in the LPS-induced production of IL-1β within the brain (and ultimately in the LPS-induced fever). However, they could be interpreted as indicating that 1) the brain AT_1 receptor inhibits the febrile process distal to the action of IL-1β [e.g., by an inhibition either of the production of the final fever-mediator PGE_2 (Blatteis and Sehic, 1997; Ushikubi et al., 2000) or of the subsequent fever-inducing action of such PGE_2 within the brain], whereas 2) another receptor, the AT_2 re-

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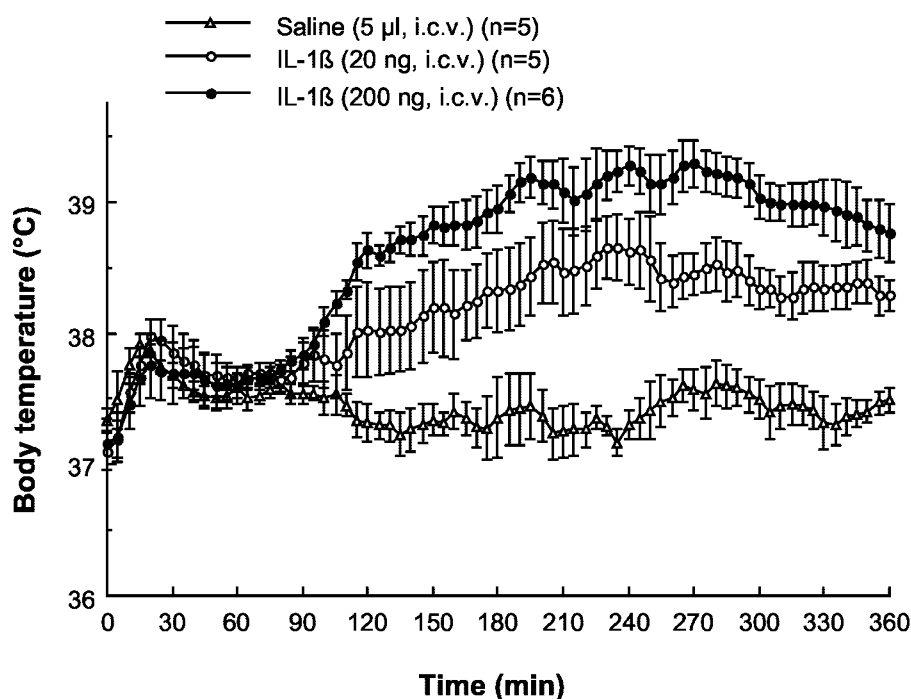

Fig. 5. Febrile responses induced in rats by i.c.v. injection of IL-1β. Mean values (±S.E.M.) obtained for body temperature (°C) in rats after i.c.v. injection at time 0 of one of two doses of IL-1β (20 or 200 ng) or saline (5 μl). IL-1β was dissolved in sterile saline. There were significant ($p < 0.05$) differences in the temperature responses among the three groups. From repeated-measures ANOVA: for treatment effect, $p < 0.0001$; for time effect, $p < 0.0001$; for interaction, $p < 0.0001$.

ceptor, enhances it. Possibility 2 is supported by the finding that although the brain AT_2 receptor contributes to the fever developed when PGE_2 is injected into the brain, the brain AT_1 receptor does not (Watanabe et al., 1997). Thus, the AT_1 receptor could be involved in inhibiting the IL-1β-induced PGE_2 production. However, losartan had no effect on the PGE_2 concentration in the hypothalamus, hippocampus, or cerebellum in rats given IL-1β i.c.v. (Table 1). Collectively, the above-mentioned results do not support the hypothesis that the AT_1 receptor is involved in inhibiting the febrile process downstream of the action of IL-1β. An alternative possibility (which we hope to test soon) is that during an IL-1β-induced fever, the AT_1 receptor is involved in the production of antipyretic substances such as IL-10, vasopressin, α-melanocyte-stimulating hormone, or the arachidonic acid metabolite epoxyeicosatrienoic acid (Kozak et al., 2000; Tatro, 2000; Nakashima et al., 2001).

In this study, we administered LPS i.c.v., which provides a model of bacterial meningitis, a condition in which bacteria (or LPS) within the brain induce pathological changes such as fever and cerebral inflammation (Korytko and Boje, 1996; Tsushima and Mori, 2000). Interestingly, i.c.v. LPS induced a dose-related increase in brain IL-1β levels at 4 h, but not at 2 h (Fig. 3). Likewise, it produced a significant increase in body temperature at 4 h, but not at 2 h (Fig. 1). In other words, the changes in the brain IL-1β concentration paralleled the time course of the LPS-induced fever. Because the fever induced by i.c.v. LPS is reportedly mediated by brain IL-1β (Tsushima and Mori, 2000), it seems likely that IL-1β in the brain, especially in the hypothalamus (which contains the thermoregulatory center), contributes to the development of LPS-induced fever. Of course, a number of factors other than IL-1 could be involved in fever regulation. For example, IL-6, tumor necrosis factor, and interferons are proinflammatory cytokines that may act as pyrogens (Dinarello, 1999), whereas the antipyretic substances mentioned above may modulate the fever.

The hypothalamus plays a well known role in thermoregulation, but this is not the case for the hippocampus and cerebellum. Hence, we chose the latter two regions for the controls in the IL-1β experiments. However, i.c.v. LPS induced increases in IL-1β concentration in all three brain regions. In a previous report, i.c.v. LPS induced IL-1β mRNA expressions in the rat hypothalamus, hippocampus, and striatum (De Simoni et al., 1997). Furthermore, i.v. LPS increases IL-1β mRNA throughout the rabbit brain, with the microglial cells displaying the IL-1β message (Nakamori et al., 1994). If microglial cells (which are widespread) are involved in the LPS-induced production of IL-1β, it is likely that i.c.v. LPS induces IL-1β widely throughout the brain to elicit cerebral inflammation.

The present results suggest that brain ANG II and its AT_1 receptor contribute both to the fever and to the increases in hypothalamic and hippocampal IL-1β levels induced in rats by i.c.v. LPS. Our previous notion (based on our results; Watanabe et al., 2000; Miyoshi et al., 2003) of a peripheral pathway by which ANG II and its AT_1 receptor are involved in the fever and peripheral increase in IL-1β induced by i.v. LPS seems to be paralleled by a similar pathway in the rat brain. In this study, the cerebellum behaved differently from the hypothalamus and hippocampus. For example, although lisinopril significantly attenuated the LPS-induced IL-1β response in the cerebellum, it significantly increased IL-1β in this brain region when given alone. As yet, we have no explanation for this discrepancy. Moreover, losartan tended to reduce the IL-1β response in the cerebellum, although not significantly. Therefore, we cannot exclude the possibility that the AT_2 receptors previously detected in the cerebellum (Reagan et al., 1994; Lenkei et al., 1996) may contribute to the LPS-induced IL-1β response.

In this study, we measured IL-1β in "powdered" brain, so we do not know the source of the IL-1β. Possible candidates are neurons, glial cells, or blood cells within the brain, including bloodborne phagocytes. Soon, we hope to determine

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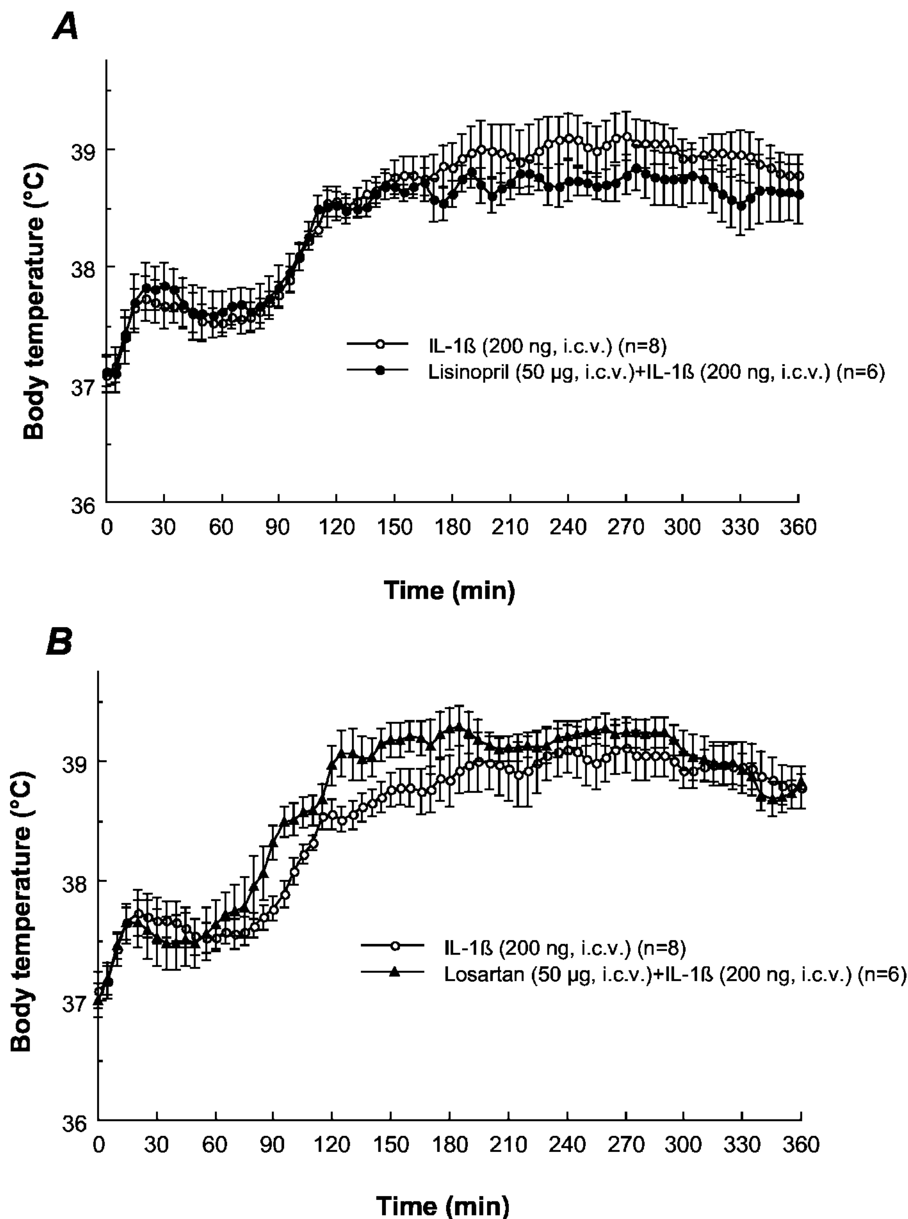

Fig. 6. Effect of i.c.v. treatment with lisinopril or losartan on IL-1β-induced fever in rats. Mean values (±S.E.M.) obtained for body temperature (°C) in rats after i.c.v. injection at time 0 of IL-1β (200 ng). Lisinopril (A, 50 μg) or losartan (B, 50 μg) was administered simultaneously with the IL-1β. There was a significant ($p < 0.05$) difference in the temperature response from 85 to 180 min between the IL-1β-injected group and the losartan + IL-1β-injected group. From repeated-measures ANOVA: for treatment effect, $p > 0.05$ in A and B; for time effect, $p < 0.0001$ in A and B; for interaction, $p > 0.05$ in A, $p < 0.01$ in B.

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which cells in the brain are mainly responsible. However, blood cells are unlikely to be the major responsible cells because they produced no detectable IL-1β in response to i.c.v. LPS (2 μg) (expressed as pg/100 μg protein; our unpublished observation). Finally, the present findings may inform the clinical treatment of bacterial meningitis. Because systemically administered AT_1 receptor antagonists such as losartan can enter the brain (Wang et al., 2003), losartan could potentially be used to treat this disease, to judge from the present findings, which suggest that brain ANG II and AT_1 receptors contribute to the fever and IL-1β response induced by the presence of LPS within the brain.

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