Effect of Natriuretic Peptide Receptor Antagonist on Lipopolysaccharide-Induced Fever in Rats: Is Natriuretic Peptide an Endogenous Antipyretic?

Michio Miyoshi, Yoshinori Kitagawa, Toshiaki Imoto, and Tatsuo Watanabe
Division of Integrative Physiology, Department of Functional, Morphological, and Regulatory Science, Tottori University Faculty of Medicine, Tottori, Japan

Received February 15, 2006; accepted June 1, 2006

ABSTRACT
We investigated whether natriuretic peptide (NP) acts as an endogenous antipyretic inside and/or outside the blood-brain barrier in rats made febrile by systemic administration of bacterial endotoxin (lipopolysaccharide; LPS). Intravenous (i.v.) injection of LPS induced a triphasic fever, the second phase of which was significantly enhanced by an i.v. injection of the NP receptor (A-type and B-type) antagonist HS-142-1, a glucose-caproic acid polymer. In contrast, the same antagonist (i.v.) had no effect on the fever induced by i.v. injection of interleukin (IL)-1β. An i.v. administration of HS-142-1 enhanced the LPS (i.v.)-induced IL-1β response in the rat spleen. An i.v. treatment with atrial NP (ANP) significantly attenuated the second phase of the LPS-induced fever. On the other hand, i.c.v. injection of the above-mentioned NP receptor antagonist resulted in an augmentation of the third phase of the fever induced by i.v. administration of LPS, the same phase that was attenuated by ANP given i.c.v. When given intracerebroventricularly (i.c.v.), the antagonist had no effect on the fever induced by i.v. IL-1β. Finally, the fever induced by i.c.v. injection of LPS was not affected even by an i.c.v. administration of the antagonist. These results suggest that the production of pyrogenic cytokines (such as IL-1β) that follows i.v. LPS injection may be inhibited by NP acting outside the blood-brain barrier, leading to an inhibition of the fever. In contrast, inside the blood-brain barrier NP may inhibit cytokine-independent mechanisms present within the rat brain that mediate LPS (i.v.)-induced fever.

Natriuretic peptides (NPs), such as atrial NP (ANP), brain NP, and C-type NP, are bioactive peptides that decrease blood pressure and increase natriuresis (Pandey, 2005). In contrast, angiotensin II (ANG II), another bioactive peptide, has the opposite effects, namely, an increase in blood pressure and a retention of sodium within the body (Ganong, 2005). In other words, NP and ANG II participate in blood pressure and body fluid regulation through physiological mechanisms that act counter to each other. Recently, we found that ANG II and its type 1 receptor are involved in the bacterial endotoxin (lipopolysaccharide; LPS)-induced fever and production of pyrogenic cytokines such as interleukin (IL)-1β (a step involved in fever induction) (Watanabe et al., 2000; Miyoshi et al., 2003). This finding is in good agreement with the fact that ANG II plays an important role in the induction of inflammatory responses (Suzuki et al., 2003). Because NP reportedly contributes to the inhibition of such inflammation (Vollmar, 2005), it is likely that the effect of NP on the fever is opposite (i.e., inhibitory) to that of ANG II.

It has been shown that NPs and their receptors are present both inside and outside the blood-brain barrier and that those in each location contribute to blood pressure and body fluid regulation (Imura et al., 1992; Pandey, 2005). To determine whether peripheral and/or brain NP plays important roles in antipyresis (i.e., exerts an inhibitory action against fever), we investigated the effects of the NP receptor antagonist HS-142-1, a glucose-caproic acid polymer (Morishita et al., 1991) and those of ANP, when administered either i.v. or i.c.v., on the fever induced by an i.v. injection of LPS or IL-1β. In addition, we examined the effect of i.v. injection of HS-142-1 on the splenic concentration of IL-1β in rats given an i.v. injection of LPS. HS-142-1 has been reported to interact with guanylyl-cyclase-linked NP receptors (A-type and B-...
type receptors), which are presumed to be the biological receptors, but not with the guanylyl-cyclase-free NP receptor (C-type receptor), which is thought to play a role in storage or clearance of NP (Matsuda, 1997).

Romanovsky et al. (2005) in their recent review noted that i.v. injection of LPS actually induces a triphasic fever in rats, although the small first phase has sometimes been considered to be the latent period and has therefore been overlooked. That being so, the present results showed that the second phase of this LPS (i.v.)-induced triphasic fever was enhanced by i.v. administration of the NP receptor antagonist, whereas the third phase was enhanced by its i.c.v. administration. These results suggest that in rats, separate mechanisms for the NP-mediated inhibition of LPS-induced fever exist inside and outside the blood-brain barrier.

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 close to the lower limit of 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 at 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 was made up of four experiments (experiments 1–4), all on freely moving rats. Each rat took part in only one experiment. Details of the experimental protocols are given below.

Surgery

When needed for i.c.v. injections (experiments 3 and 4; see below), a stainless steel cannula (0.8 mm o.d.) was implanted in each rat under general anesthesia (50 mg/kg i.p. sodium pentobarbitone), so that its tip lay in the lateral cerebral ventricle (coordinates anteroposterior, −0.8; lateral, 1.5; dorsoventral, 3.5 mm; atlas of Paxinos and Watson, 1998) using standard stereotaxic technique. The implantation was performed under strictly sterile conditions at least 10 days before implantation of the transmitter used to measure body temperature. Body temperature was measured using a biotelemetry system (Data Science, Inc., St. Paul, MN) (Lange et al., 1991). When required (experiments 1, 3, and 4; see below), rats with or without an i.c.v. cannula (see above) were anesthetized with 50 mg/kg i.p. sodium pentobarbitone, and a battery-operated transmitter (model TA10TA-F40) was implanted i.p. 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 an IBM 6587-JC3 computer. This implantation was performed at least 10 days before the measurement of body temperature.

When required for i.v. injections (experiments 1, 2, and 3; see below), in animals (with or without transmitters) anesthetized with 50 mg/kg i.p. sodium pentobarbitone, a polyvinyl tube was inserted into the jugular vein, so that its tip lay in the superior caval vein near the right atrium (Harms and Ojeda, 1974). The free end of the catheter was passed subcutaneously to the mid-scapular region, where it was exteriorized dorsally behind the neck. It was kept patent by flushing it every day with heparinized 0.9% saline (50 U/ml). This implantation was performed at least 1 week after the implantation of the transmitter and at least 3 days before any measurements of body temperature (experiments 1 and 3) or at least 3 days before the sampling of the rat spleen (experiment 2). A body weight of the rat usually decreases a little (−5 g) in the 1st day after such surgery. However, within 3 days, the weight starts to increase and reaches a level higher than that seen before the surgery. We excluded rats from the present data whose body weight did not recover in that way after the surgery. At 3 days after the surgery for i.v. cannula implantation, there is no increase in the resting plasma adrenocorticotropic level of rats (which is approximately 25–50 pg/ml) (Watanabe et al., 1991). Because proinflammatory stimuli such as LPS (Watanabe et al., 1994) and IL-1 (Watanabe et al., 1991) induce marked increases in plasma adrenocorticotropic, we believe that no significant inflammation was present at 3 days after jugular catheter implantation surgery.

In this study, two or three surgeries were performed in a given rat, and any animals exhibiting signs of infection (such as fever, reduced appetite and drinking, and/or piloerection) and/or other indications of poor body condition (such as loss of body weight or reduced motility) were excluded from the study. All rats were handled for 5 min each day for at least 5 days to acclimatize 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. An antagonist of A-type and B-type NP receptors, HS-142-1, dissolved in sterile saline for injections, was a kind gift from Kyowa Hakko Co., Inc. (Shizuoka, Japan). 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 × 10^6 units/µg (thymocyte coproferration assay). The IL-1β preparation was free (<0.05 pg/µg protein) of significant endotoxin contamination (Limulus amoebocyte assay). IL-1β was dissolved in sterile saline, as was ANP (Sigma-Aldrich). The doses injected in each experimental group are given below.

Experimental Protocols

Experiment 1. We investigated the effect of an i.v. injection either of the NP receptor antagonist HS-142-1 (50 µg/kg) or of ANP (3 µg/kg) on the fever induced by LPS (2 µg/kg i.v.) or IL-1β (2 µg/kg i.v.). Also examined was the effect of i.v. injection of saline, HS-142-1 alone, or ANP alone on resting body temperature. We believe that the dose of HS-142-1 used was sufficient to inhibit the binding of endogenous NP to the receptors because at a dose above 0.1 µg/ml, HS-142-1 inhibits 15%-iANP binding to the receptors on adrenocortical membranes (Matsuda, 1997). Although we did not establish a dosing rationale for HS-142-1 and ANP in intact animals, we can say the following. If a 300-g rat has approximately 60 ml of extracellular fluid (ECF), given that the ECF volume is approximately 20% of the body weight (Ganong, 2005), then the ECF concentration achieved when HS-142-1 is given at a dose of 50 µg/kg (=15 µg/300 g) would be 0.25 µg/µl (=15 µg/60 ml), which is more than 0.1 µg/ml. Furthermore, it has been reported that 300 ng/100 g (=3 µg/kg, a dose less than the 50 µg/kg used in the present study) of HS-142-1 given i.v. to rats abolished the increase in urinary sodium excretion induced by IL-1β (which induces an increase in the plasma level of ANP), suggesting that the increase in urinary sodium excretion induced by IL-1β involves the action of endogenous ANP (Ohta and Ito, 1999). Here, we used a 3-µg/kg dose of ANP to obtain an effect because an i.v. injection of 1 µg of ANP per rat decreases blood pressure and increases natriuresis in rats weighing 280 to 310 g (Caron et al., 1995), that dose being equivalent to approximately 3 µg/kg. Because a high dose of ANP alone (given i.v.) induces hyperthermia in rats (Pataki et al., 1999), three doses of ANP alone (0, 30, and 300 µg/kg) were given i.v. to determine whether a similar response profile occurs with i.v. dosing. 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. Rats were divided into three groups.

**Group 1.** LPS was administered simultaneously with the HS-142-1. The injectate (LPS solution or HS-142-1 mixed with LPS solution) was given i.v. to each animal in a volume of 1 ml/kg over a period of 30 s. Other rats were given HS-142-1 or saline (0.5 ml/kg i.v.) 220 min after the LPS injection. The total injection volume was set at 1 ml/kg in each case.

**Group 2.** IL-1β and HS-142-1 were administered simultaneously. The injectate (IL-1 solution or HS-142-1 mixed with IL-1 solution) was given i.v. to each animal.

**Group 3.** Rats were given ANP or saline 90 min after the LPS injection. LPS or IL-1β was always given around 11:30 AM. We measured changes in body temperature as a result of LPS or IL-1β for 7 h after the injection.

**Experiment 2.** We examined the effect of HS-142-1 (50 μg/kg i.v.) on the LPS (2 μg/kg i.v.)-induced changes in the splenic concentration of IL-1β. An i.v. injection of LPS was given simultaneously with or without HS-142-1 (HS-142-1 + LPS group or LPS group). The control rats received an i.v. injection either of saline (saline group) or of HS-142-1 alone (HS-142-1 group).

Animals were sacrificed by CO2 stunning followed by decapitation 150 min after the injection. The spleen was quickly removed, frozen, and powdered in liquid nitrogen.

The splenic concentration of IL-1β was measured by enzyme-linked immunosorbent assay. In brief, each powdered tissue sample was added to isovolemic culture medium containing a cocktail protease inhibitor (Sigma-Aldrich), was mechanically homogenized on ice using a post-mounted laboratory homogenizer (Omni International, Gainesville, VA). Homogenized samples were centrifuged at 10,000 rpm for 10 min at 4°C. Supernatants were then transferred to a fresh test tube and stored at −85°C until needed for measurement of IL-1β and total protein content. The IL-1β content was measured using a commercial enzyme-linked immunosorbent assay 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 (Bio-Rad, Hercules, CA). The tissue concentration of IL-1β is expressed as the cytokine content per 100 μg of protein.

**Experiment 3.** The effect of an i.c.v. injection of HS-142-1 (2.5 μg; 625 pm) or ANP (40 ng) was investigated on the LPS (2 μg/kg i.v.)- or IL-1 (2 μg/kg i.v.)-induced fever in rats. Also examined was the effect of i.c.v. injection of saline, HS-142-1 alone, or ANP alone on resting body temperature.

To our knowledge, there is no accepted value for the rat’s cerebrospinal fluid (CSF) volume. However, the approximate concentration of the drug in the CSF achieved in this study can be calculated as follows. Consiglio and Lucion (2000), who developed a technique for collecting CSF from the cisterna magna of conscious rats, found that the volume of the CSF collected was 120 μl. On this basis, the concentration of HS-142-1 (2.5-μg dose) in the CSF in the present study would have been approximately 20.1 μg/ml CSF (2.5 μg/0.12 ml = 20.1 μg/ml = 50.25 μM/l). As mentioned above, at a dose above 0.1 μg/ml, HS-142-1 reportedly inhibits 125I-ANP binding to its receptors. Therefore, the dose of this antagonist used in the present study seems appropriate. However, the above-mentioned estimation may be oversimplified since it presumes a simple nondynamic one-compartment model. Therefore, the actual HS-142-1 concentrations could be somewhat different from our estimation. Some in vivo dose-ranging data would be useful. In our preliminary study, i.c.v. injection of ANP was performed to examine whether the peptide itself affected resting body temperature. We found that 400 ng of ANP induced hyperthermia, as reported previously by Pataki et al. (1999), whereas 40 ng had no effect. Therefore, we selected 40 ng of ANP to observe its effect on fever, although data obtained using 40 and 400 ng of ANP alone are included in the present results.

In experiment 3, HS-142-1 or saline was given i.c.v. to each animal in a volume of 5 μl over a period of 30 s, immediately before an i.v. injection of LPS (group 1) or IL-1β (group 2). An i.v. injection of ANP (40 ng in 5 μl) was given 220 min after the LPS administration in group 3. The i.c.v. injections were made via a stainless steel needle (0.4 mm o.d.) inserted through the implanted cannula and attached to a microsyringe via polyethylene tubing. The other procedures were essentially the same as those described for experiment 1, except that the recording period was 10 h after the injection of LPS or IL-1β.

After its involvement in experiment 3, each animal was subjected to CO2 stunning. Fast Green FCF solution (5 μl) was then injected i.e.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.

**Experiment 4.** To observe the effect of HS-142-1 (2.5 μg i.c.v.) on the LPS (2 μg i.c.v.)-induced fever, each injectate (LPS solution or HS-142-1 mixed with LPS solution) was given i.c.v. The other procedures were essentially the same as those described for experiment 3.

**Statistical Analysis**

All results are expressed as mean ± S.E.M. Body temperature data were analyzed for statistical significance by means of a repeated measures analysis of variance, followed by Fisher’s protected least significant difference test (post hoc test) (Macintosh, StatView 4.0; SAS Institute, Cary, NC). A one-way analysis of variance, followed by Fisher’s protected least significant difference test (Macintosh, StatView 4.0) was carried out to analyze IL-1β data. Differences were considered significant at p < 0.05.

**Results**

**Effect of Intravenous Treatment with an NP Receptor Antagonist, HS-142-1, on the Fever Induced in Rats by Intravenous Injection of LPS or IL-1 (Experiment 1).** As shown in Fig. 1A, i.v. injection of 2 μg/kg LPS induced a triphasic fever with peaks at around 45, 150, and 360 min after the injection, although the first phase was small in amplitude (see Romanovsky et al., 2005). When an NP receptor antagonist, HS-142-1 (50 μg/kg), was given i.v. with the LPS, the second phase of the triphasic fever was significantly enhanced (65–200 min), but the first and the third phases were not. In contrast, i.v. injection of HS-142-1 at 220 min after LPS (the starting point of the third phase) had no effect on the LPS-induced fever (Fig. 1B), indicating that the drug was actually effective in increasing only the second phase of the LPS-induced fever.

The maximum temperature reached during the second phase in the group injected with LPS alone in Fig. 1A (around 38°C) seemed lower than the corresponding values in the two experimental groups in Fig. 1B (around 38.4°C). When the data in Fig. 1B were added to Fig. 1A and a comparison was made between the second phases in the LPS and HS-142-1 + LPS groups, a statistically significant difference was detected between them (p < 0.01; 65–200 min).

Figure 2 shows that i.v. injection of 2 μg/kg IL-1β produced an immediate biphasic fever. It should be noted that i.v. treatment with HS-142-1 had no effect on the IL-1β-induced fever in our rats.

**Effect of Intravenous Treatment with ANP on the Fever Induced in Rats by Intravenous Injection of LPS (Experiment 1).** An i.v. injection of 3 μg/kg ANP was performed at 90 min after an i.v. injection of LPS, because, as shown in Fig. 1, only the second phase of the LPS fever (starting at around 90 min) was enhanced by the NP receptor.
antagonist. Moreover, the peptide ANP is known to be rapidly inactivated in the circulation. Figure 3 shows that ANP significantly attenuated the second phase of the LPS fever (between 90 and 155 min), thus supporting the results obtained in the HS-142-1 experiment.

Effect of Intravenous Treatment with Saline, HS-142-1 Alone, or ANP Alone on Resting Body Temperature in Rats (Experiment 1). An i.v. injection of either HS-142-1 (50 μg/kg) alone or ANP (3, 30, or 300 μg/kg) alone had no effect on resting body temperature, compared with that in the saline-injected control group (Table 1).

Effect of Intravenous Treatment with an NP Receptor Antagonist, HS-142-1, on the Concentration of IL-1β in the Spleen in Rats Given an Intravenous Injection of LPS (Experiment 2). As shown in Table 2, the splenic concentration of IL-1β was significantly increased at 150 min after an i.v. injection of LPS (2 μg/kg; LPS group versus saline group). In rats treated with HS-142-1 (50 μg/kg; HS-142-1 + LPS group), this LPS-induced IL-1β response was significantly enhanced. There was no difference in the splenic concentration of IL-1β between the saline group and the HS-142-1 group.

Effect of Intracerebroventricular Treatment with an NP Receptor Antagonist, HS-142-1, on the Fever Induced in Rats by Intravenous Injection of LPS or IL-1 (Experiment 3). As illustrated in Fig. 4, in saline (5 μl; i.c.v.)-treated control rats, an i.v. injection of LPS resulted in a triphasic increase in body temperature. Interestingly, 2.5 μg of HS-142-1 administered into the cerebral ventricle enhanced the third phase of the fever induced by an i.v. injection of 2 μg/kg LPS, but it did not affect either the first or the second phases. In contrast, Fig. 5 shows that HS-142-1 (2.5 μg i.c.v.) had no effect on the fever induced by i.v. injection of 2 μg/kg IL-1.

Effect of Intracerebroventricular Treatment with ANP on the Fever Induced in Rats by Intravenous Injection of LPS (Experiment 3). Because the third phase of fever started at around 220 min after an injection of LPS, 40 ng of ANP was given to each animal at this time-point. As depicted in Fig. 6, the third phase of the LPS-induced fever
TABLE 2
Effect of i.v. treatment with an NP receptor antagonist, HS-142-1, on the IL-1β concentration in the spleen of rats given an i.v. injection of LPS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IL-1β (pg/100 g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (n = 6)</td>
<td>19.4 ± 5.59</td>
</tr>
<tr>
<td>LPS (n = 6)</td>
<td>179.8 ± 7.45*</td>
</tr>
<tr>
<td>HS-142-1 + LPS (n = 6)</td>
<td>221.5 ± 18.41*</td>
</tr>
<tr>
<td>HS-142-1 (n = 6)</td>
<td>18.2 ± 1.01†</td>
</tr>
</tbody>
</table>

* P < 0.05 vs. saline.
† P < 0.05 vs. LPS.

Fig. 5. Effect of i.c.v. treatment with an NP receptor antagonist, HS-142-1, on fever induced in rats by i.v. injection of IL-1β. Mean ± S.E.M. values obtained for body temperature (°C) in rats after i.v. injection at time 0 of IL-1β (2 μg/kg). HS-142-1 (2.5 μg) or saline was administered i.c.v. immediately before the IL-1β.

Fig. 6. Effect of i.c.v. treatment with ANP on fever induced in rats by i.v. injection of LPS. Mean ± S.E.M. values obtained for body temperature (°C) in rats after i.v. injection at time 0 of LPS (2 μg/kg). ANP (40 ng) or saline was administered i.c.v. at 220 min after the injection of LPS.

was significantly attenuated by i.v. ANP between 330 and 460 min, supporting the results illustrated in Fig. 4.

Effect of Intracerebroventricular Treatment with Saline, HS-142-1 Alone, or ANP Alone on Resting Body Temperature in Rats (Experiment 3). The i.c.v. injection of either 2.5 μg of HS-142-1 alone or 40 ng of ANP alone had no effect on resting body temperature (over and above any changes seen on saline administration; Table 3). However, ANP given i.c.v. at a dose of 400 ng did induce a significant increase (120–420 min) in body temperature.

Effect of Intracerebroventricular Treatment with an NP Receptor Antagonist, HS-142-1, on the Fever Induced in Rats by Intracerebroventricular Injection of LPS (Experiment 4). An i.c.v. injection of 2 μg of LPS induced a fever that peaked at between 420 and 480 min after the injection, and this fever underwent no change after i.c.v. treatment with 2.5 μg of HS-142-1 (Fig. 7). We wondered whether the febrile response induced by 2 μg of LPS might be too large to be clearly enhanced by the antagonist, so we repeated the experiment using a lower dose (0.2 μg) of LPS. As expected, the lower dose of LPS induced a weaker fever than the higher dose, but treatment with 2.5 μg of HS-142-1 had no effect on this smaller rise in body temperature (n = 4; data not shown).

Table 1
Resting body temperature in rats following i.v. injection of saline, HS-142-1, or ANP

<table>
<thead>
<tr>
<th>Time</th>
<th>Saline (n = 6)</th>
<th>HS-142-1 (n = 4)</th>
<th>ANP (5 μg/kg) (n = 5)</th>
<th>ANP (30 μg/kg) (n = 6)</th>
<th>ANP (300 μg/kg) (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>37.1 ± 0.1</td>
<td>37.0 ± 0.2</td>
<td>37.2 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>36.9 ± 0.1</td>
</tr>
<tr>
<td>60 min</td>
<td>37.2 ± 0.1</td>
<td>37.0 ± 0.2</td>
<td>37.2 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>36.9 ± 0.1</td>
</tr>
<tr>
<td>120 min</td>
<td>37.3 ± 0.2</td>
<td>37.2 ± 0.2</td>
<td>37.3 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>37.1 ± 0.1</td>
</tr>
<tr>
<td>180 min</td>
<td>37.2 ± 0.1</td>
<td>37.0 ± 0.2</td>
<td>37.2 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>37.1 ± 0.1</td>
</tr>
<tr>
<td>240 min</td>
<td>37.2 ± 0.2</td>
<td>37.2 ± 0.1</td>
<td>37.2 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>37.1 ± 0.1</td>
</tr>
<tr>
<td>300 min</td>
<td>37.2 ± 0.1</td>
<td>37.0 ± 0.2</td>
<td>37.2 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>37.1 ± 0.1</td>
</tr>
<tr>
<td>360 min</td>
<td>37.2 ± 0.1</td>
<td>37.0 ± 0.2</td>
<td>37.2 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>37.1 ± 0.1</td>
</tr>
<tr>
<td>420 min</td>
<td>37.2 ± 0.1</td>
<td>37.0 ± 0.2</td>
<td>37.2 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>37.1 ± 0.1</td>
</tr>
</tbody>
</table>

Mean ± S.E.M. values obtained for body temperature (°C) in rats after i.v. injection of saline, HS-142-1 (50 μg/kg), or ANP (3, 30, or 300 μg/kg) at time 0.
The administration of HS-142-1 enhanced LPS (i.v.)-induced IL-1 production of the LPS-induced fever. Indeed, we found that an i.v. administration of HS-142-1, on fever induced in rats by i.c.v. injection of LPS. Mean ± S.E.M. values obtained for body temperature (°C) in rats after i.c.v. injection of saline, HS-142-1 (2.5 μg), or ANP (40 or 400 ng) at time 0.

**Table 3**

Resting body temperature in rats following i.c.v. injection of saline, HS-142-1, or ANP

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
<th>240 min</th>
<th>300 min</th>
<th>360 min</th>
<th>420 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (n = 5)</td>
<td>37.0 ± 0.1</td>
<td>37.0 ± 0.2</td>
<td>37.1 ± 0.1</td>
<td>37.1 ± 0.2</td>
<td>37.2 ± 0.1</td>
<td>37.3 ± 0.1</td>
<td>37.3 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>HS-142-1 (n = 6)</td>
<td>37.0 ± 0.2</td>
<td>37.2 ± 0.2</td>
<td>37.1 ± 0.2</td>
<td>37.2 ± 0.2</td>
<td>37.2 ± 0.1</td>
<td>37.3 ± 0.2</td>
<td>37.5 ± 0.2</td>
<td>37.6 ± 0.2</td>
</tr>
<tr>
<td>ANP (40 ng) (n = 4)</td>
<td>37.1 ± 0.1</td>
<td>37.2 ± 0.1</td>
<td>37.2 ± 0.1</td>
<td>37.2 ± 0.1</td>
<td>37.1 ± 0.1</td>
<td>37.3 ± 0.1</td>
<td>37.3 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>ANP (400 ng) (n = 4)</td>
<td>36.9 ± 0.1</td>
<td>37.3 ± 0.1</td>
<td>37.7 ± 0.2</td>
<td>38.4 ± 0.2</td>
<td>38.8 ± 0.2</td>
<td>38.9 ± 0.1</td>
<td>38.4 ± 0.3</td>
<td>38.3 ± 0.1</td>
</tr>
</tbody>
</table>

**Discussion**

The present results show that i.v. treatment with the NP receptor antagonist HS-142-1 enhanced the second phase of the triphasic fever induced by i.v. LPS, suggesting that endogenous NP may inhibit this second phase by an action exerted outside the blood-brain barrier. This idea was strengthened by the second phase of the LPS-induced fever being significantly attenuated by an i.v. injection of ANP. However, the above-mentioned antagonist had no effect on the fever induced by i.v. injection of IL-1β. Because LPS induces fever at least partly though the production of such pyrogenic cytokines as IL-1 (Blatteis et al., 2005; Romanovsky et al., 2005), induced by peripherally acting IL-1 (and/or those effects exerted by the PGE2 that lead to fever), was not altered by the antagonist via an action inside the blood-brain barrier.

For that reason, we speculate that the production of IL-1β within the brain is inhibited by i.v. NP and that this leads to an inhibition of the fever induced by i.v. injection of LPS. Indeed, IL-1 production within the brain is reportedly induced by i.v. administration of LPS (Nakamori et al., 1994). Therefore, we examined the effect of an i.c.v. injection of HS-142-1 on the fever induced by i.v. LPS (2 μg), because we previously reported an increase in the brain concentration of IL-1β in rats after an i.v. injection of this dose of LPS (Shimizu et al., 2004). To our surprise, the drug had no effect on the fever in this experiment. This indicates that the mechanisms underlying the development of the fever induced by brain LPS, including the production of brain cytokines (Tsushima and Mori, 2000), are not controlled by NP acting within the brain. How then can we explain the facilitatory effect of HS-142-1 given i.c.v. on the fever induced by i.v. LPS? First, it has been reported that systemic administration of LPS activates the complement system and that this stimulates macrophages to release PGE2, which in turn stimulates vagal afferents (Blatteis et al., 2004, 2005). These signals are relayed in the medulla to noradrenergic neurons projecting to the preoptic area, where the released norepinephrine stimulates PGE2 secretion, causing fever (Blatteis et al., 2005). If this mechanism is operating during the third phase of LPS-induced fever, brain NP might conceivably be interfering with this system within the brain. However, two problems arise. 1) Why, when injected i.c.v., did the NP receptor antagonist have no effect on the IL-1β-induced fever, even though peripheral IL-1, like peripheral LPS, reportedly stimulates the vagus to induce fever...

![Graph showing body temperature changes](image-url)
(Watkins et al., 1995)? 2) It has been inferred that the vagal afferent pathway is important only in the mediation of the monophasic fever induced by low doses of LPS (Romanovsky, 2000). Another possible cytokine-independent mechanism might entail the LPS-activated complement in the blood reaching the organum vasculosum laminae terminalis and passing through the fenestrated capillaries to stimulate macrophages within the perivascular space to synthesize PGE₂ (Hansch et al., 1984). The released PGE₂ might then either diffuse to the preoptic area or activate organum vasculosum laminae terminalis neurons projecting to the preoptic area (Ota et al., 1997), and thereby evoke fever. We cannot exclude the possibility that brain NP acts as an inhibitor within this pathway. Alternatively, other unknown pathways for the production of LPS-induced fever might exist in rats, and brain NP might interfere with these. To uncover the true explanation will require characterization of the mechanisms underlying the inhibitory effect of brain NP on the LPS (i.v.)-induced fever.

In the present study, a weak attenuation of the LPS-induced fever was observed when ANP was given either i.v. or i.c.v. This may reflect rapid inactivation of ANP when given as a bolus injection, because high neutral endopeptidase activity exists inside and outside the blood-brain barrier (Kubota et al., 2003). We will need to give ANP by continuous infusion to see clearer effects, and this should be done soon. On the other hand, Pataki et al. (1999) observed that i.c.v. injection of ANP at a dose of 40 to 400 ng elicits hyperthermia in rats. In our hands, 400 ng of ANP i.c.v. did indeed induce hyperthermia, but 40 ng did not induce hyperthermia (Table 3). This discrepancy may reflect the different experimental procedures used. We used a telemetry system, enabling us to make measurements in freely moving rats without imposing undue stress. The resting body temperature in our study was approximately 37°C, considerably less than the 38.1°C recorded by Pataki et al. (1999), who used a conventional method of measurement employing a thermistor probe inserted into the colon. Therefore, their rats were probably already under a degree of stress before any injection of ANP. Then, why did i.c.v. injection of 400 ng of ANP induce hyperthermia, whereas 40 ng had no effect? One possibility is that the higher (but not the lower) dose of ANP exceeded the threshold for “unknown” pharmacological effects on the neuronal networks associated with thermoregulation. Indeed, in our hands, i.c.v. HS-142-1 alone had no effect on resting body temperature, suggesting no role for endogenous brain NP (presumably secreted in small amounts) in the control of resting body temperature. However, 40 ng of ANP might interfere with the mechanism of fever induction (normal thermoregulation and fever being different phenomena) since this dose (i.c.v.) attenuated LPS-induced fever. This implies that endogenous ANP might actually inhibit the fever via an action exerted inside the blood-brain barrier in rats, because endogenous ANP is presumed to be released in small amounts.

In summary, the present results represent the first in vivo evidence that separate mechanisms for NP-mediated inhibition of LPS-induced fever exist inside and outside the blood-brain barrier in rats. These observations also imply that the different mechanisms present inside and outside the blood-brain barrier operate so as to induce a multiple-phase LPS-induced fever, supporting the idea put forward by Morimoto et al. (1987) and Romanovsky et al. (2005). However, as described above, the details of the mechanisms mediating NP-dependent antipyresis remain to be established. Furthermore, because HS-142-1 interacts with both A-type and B-type receptors (Matsuda, 1997), we need to clarify which guanylyl-cyclase-linked NP receptor(s) is responsible for this antipyresis. Because ANP is an effective activator of A-type receptors, they are the more likely candidates. Indeed, A-type receptors are reportedly involved in the ANP-mediated inhibition of inflammation (Vollmar, 2005). The data we obtained using HS-142-1 would be strengthened if, in NP-deficient (John et al., 1995) or NP receptor-deficient mice (Lopez et al., 1995), LPS is found to induce febrile responses that exceed those seen in control animals. Finally, we must keep in mind the possibility that the effects of ANP on fever could be mediated partly by changes in peripheral vascular function. Because ANP dilates blood vessels, the blood flow to the tail (a major regulator of body temperature in rats) might increase, leading to increased heat loss responses.

Acknowledgments

We are grateful to Dr. Robert J. Timms (Birmingham, UK) for critical reading of the English in the manuscript. We thank Otsuka Pharmaceutical and Kyowa Hakko Co., Ltd. for the kind supply of human recombinant IL-1β and HS-142-1, respectively. We express our gratitude to Masanari Kuwabara (Tottori, Japan) for valuable assistance.

References

antagonist for ANP receptor, from Aureobasidium sp.


Address correspondence to: Dr. Tatsuo Watanabe, Division of Integrative Physiology, Department of Functional, Morphological, and Regulatory Science, Tottori University, Faculty of Medicine, Yonago, Tottori 683, Japan. E-mail: watanabe@grape.med.tottori-u.ac.jp