Angiotensin II and Fever in Rodents

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Angiotensin II (ANG II), a bioactive peptide well known to play an important role in blood-pressure and body-fluid regulation, participates in inflammatory responses, too. Recently, we reported results suggesting that ANG II is involved in the development of the fever induced by intravenous (i.v.) injection of lipopolysaccharide (LPS, 2 µg/kg) in dehydrated rats (in which the secretion of ANG II is elevated). Furthermore, we verified the contributions made by ANG II and its type 1 receptor (AT₁ receptor) to the LPS-induced production of interleukin-1β (IL-1β) in those rats. It therefore seems likely that in rats, ANG II and its receptors contribute to the induction of this fever at its 1st step (namely, the LPS-induced production of pyrogenic cytokines such as IL-1β). Moreover, we have revealed that intrahypothalamic (i.h.) ANG II and AT₂ receptors are involved in the development of the fever induced by i.h. injection of prostaglandin E₂ (PGE₂), a final fever mediator. ANG II given i.h., which had no effect on the resting body temperature, facilitated PGE₂-induced fever. These results suggest that in rats, ANG II and its receptors contribute to the induction of fever at its final step, too (namely, the action of PGE₂ to cause fever). Finally, a single i.p. injection of IL-1β results in a weaker IL-1β-induced fever in AT₂-receptor-deficient mice than in wild-type mice, suggesting that the brain AT₂ receptor is involved in the development of fever (possibly at its final step) in mice as well. Collectively, these results suggest that in rodents, ANG II and its receptors contribute to the development of fever, both at the 1st and final steps in the pathway for fever induction.

Key words: AT₁ receptor; AT₂ receptor; interleukin-1; lipopolysaccharide; prostaglandin E₂

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, ANG II and ANG-II-type-1 (AT₁) receptors are involved in certain types of cardiovascular inflammation (Usui et al., 2000). Furthermore, angiotensin-converting-enzyme (ACE) inhibitors have an anti-inflammatory effect (Delfraissy et al., 1984; Martin et al., 1984; Rezkalla et al., 1990). We recently revealed that ANG II and its receptors are involved in the development of fever (another example of an inflammatory response) both in rats (Watanabe et al., 1997, 2000; Miyoshi et al., 2003) and in mice (Watanabe et al., 1999).

This review focuses on the role of ANG II and its receptors in the development of febrile responses in rodents.
**Fig. 1.** Effect of intravenous (i.v.) injection of lisinopril on lipopolysaccharide (LPS)- or interleukin-1β (IL-1β)-induced fever in dehydrated rats. Changes (mean ± SEM) in body temperature (°C) in dehydrated rats after i.v. injection at time 0 of LPS (A: 2 µg/kg) or IL-1β (B: 2 µg/kg). Lisinopril (10 mg/kg) was administered i.v. immediately before the injection of LPS or IL-1β. [ ,] number of subjects. Data from Watanabe et al. (2000).

**ANG II and Fever in Rats**

It is widely accepted that once in the body, bacterial endotoxin [or lipopolysaccharide (LPS)] stimulates leukocytes to produce pyrogenic cytokines, such as interleukin-1 (IL-1) or IL-6, leading to fever induction (Kluger, 1991; Dinarello, 1999). Furthermore, the febrile response to pyrogenic cytokines is believed to be mediated by prostaglandin E (PGE), acting within the brain as a final fever mediator (Blatteis and Sehic, 1997; Ushikubi et al., 2000). We have reported that in rats, ANG II and its receptors contribute to the induction of fever not only at its 1st step [namely, the LPS-induced production of pyrogenic cytokines (Watanabe et al., 2000;
Miyoshi et al., 2003) but also at its final step [namely, the action of PGE₂ to cause fever (Watanabe et al., 1997)]. We should like to present our findings below.

**ANG II is involved in fever production in rats at its 1st step, the LPS-induced production of IL-1β**

It is well known that a host develops a higher fever under dehydrated conditions than under euhydrated conditions. Indeed, in our hands, i.v. injection of a given dose of LPS induced a fever that was significantly greater in dehydrated rats than in euhydrated rats. However, dehydration had no effect on the fever induced by i.v. injection of IL-1β (Watanabe et al., 2000). These results suggested that the LPS-induced production of pyrogenic cytokines such as IL-1β might be enhanced by dehydration. Furthermore, since the secretion of ANG II increases under dehydrated conditions it seemed possible that ANG II might be involved in this enhanced production of pyrogenic cytokines, and ultimately in enhancing the fever seen in response to LPS. To test this possibility, we examined the effect of an angiotensin-converting-enzyme (ACE) inhibitor on febrile responses in dehydrated rats.

Figure 1 illustrates the effect of the ACE inhibitor lisinopril on the fever induced in dehydrated rats by an i.v. injection of LPS (2 µg/kg) (Watanabe et al., 2000). When lisinopril was given i.v. just before the LPS, the LPS-induced fever was significantly attenuated (Fig. 1A), indicating that ANG II is indeed involved in LPS-induced fever in dehydrated rats. In contrast, the same inhibitor had no effect on the fever induced by IL-1β itself (Fig. 1B), suggesting that ANG II may act by contributing to the LPS-induced production of pyrogenic cytokines, such as IL-1β.

To test the above possibility, we then examined the effect of lisinopril on the expression of IL-1β mRNA in the liver of dehydrated rats given an i.v. injection of LPS. Animals were sacrificed, the liver quickly removed and its IL-1β mRNA examined by Northern blot analysis. As shown in Fig. 2, LPS induced a significant increase in the expression of hepatic IL-1β mRNA at both 2 and 4 h after its injection, and this increase was reduced by treatment with lisinopril (Fig. 2A), an effect that, by quantitative analysis, was revealed to be statistically significant (Fig. 2B) (Miyoshi et al., 2003). This result suggested that ANG II contributes to the LPS-
induced induction of IL-1β at the mRNA level in dehydrated rats.

Next we investigated the effect of lisinopril on the production of IL-1β protein in the liver of dehydrated rats given an i.v. injection of LPS, the hepatic concentration of IL-1β being measured by enzyme-linked immunosorbent assay. The results are depicted in Fig. 3. The LPS-induced increase in the IL-1β protein concentration was significantly attenuated by lisinopril at both 2 and 4 h after the injection of LPS (Miyoshi et al., 2003). Furthermore, it should be mentioned that i.v. injection of ANG type 1 (AT₁) receptor antagonist reduced this LPS-induced IL-1β response in the liver as well (Miyoshi et al., 2003).

Taken together, the above results made it seem likely that ANG II and the AT₁ receptor are involved in the peripheral IL-1β production induced in dehydrated rats by LPS.

**ANG II is also involved in fever production in rats at its final step, the action of PGE₂ within the brain to induce fever**

Recently, we suggested that ANG II and its receptors within the brain participate in the development of one particular type of body-temperature increase, namely stress-induced hyperthermia (Saiki et al., 1997). Two types of ANG II receptors are present within the brain, AT₁ and AT₂ receptors (Saavedra, 1994; Wright and Harding, 1994; Aguilera et al., 1995; Watanabe et al., 1998) and we have shown that the brain AT₁ receptor is the one responsible for the induction of restraint-stress-induced hyperthermia in rats (Saiki et al., 1997). We therefore decided to investigate the effect of an intracerebroventricular (i.c.v.) injection of the AT₁-receptor antagonist losartan (60 µg) on the febrile responses induced in rats by an i.c.v. injection of PGE₂ (200 ng), a final fever mediator (Blatteis and Sehic, 1997; Ushikubi et al., 2000). We postulated that AT₁ receptors play an important role in this type of fever, too. Surprisingly, however, i.c.v. treatment with losartan (60 µg) had no effect on the febrile response induced by PGE₂. This dose of losartan (60 µg) is actually rather high, because less than 10 µg of the drug is sufficient to inhibit ANG II-induced responses such as increases in water intake (Rowland et al., 1992) and arterial blood pressure (Hogarty et al., 1992) in rats. Thus, since it seemed that AT₁ receptors are not involved in PGE₂-induced fever, even though they participate in stress-induced hyperthermia, we hypothesized that the other ANG receptor, the AT₂ receptor, might contribute to the development of PGE₂-induced fever. Indeed, we found that when an AT₂-receptor antagonist, CGP 42112A (20 µg), was administered i.c.v. just before i.c.v. PGE₂, the fever was significantly reduced (Watanabe et al., 1997). This result suggested that AT₂ receptors located somewhere in the brain contribute to the induction of fever by PGE₂.

To try to determine where in the brain these AT₂ receptors might be situated, we gave intrahypothalamic (i.h.) injections of CGP 42112A and observed their effects on fevers, because the rostral hypothalamus is thought to be the site at which PGE₂ acts to induce fever. For these i.h. injections, a stainless-steel cannula was implanted into the preoptic/anterior hypothalamic (PO/AH) region on one side at AP 1.8 mm, L 1.2 mm, and V 8.5 mm
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[coordinates from the rat brain atlas of Pellegrino et al. (1979)] under sodium pentobarbitone anaesthesia (50 mg/kg, i.p.).

Figure 4 shows the effects of i.h. injections of CGP 42112A on the fever induced by PGE$_2$ (Watanabe et al., 1997). In artificial cerebrospinal fluid (aCSF)-treated controls, injection of PGE$_2$ (100 ng, i.h.) resulted in a monophasic fever, the body temperature beginning to increase immediately and reaching peak at 25 min. This febrile response was attenuated by i.h. treatment with CGP42112A (2 and 5 µg) in a dose-related manner, the effect being significant at $P < 0.05$ [for CGP42112A (5 µg) + PGE$_2$, 0–40 min] (Fig. 4). CGP42112A (5 µg, i.h.) given alone had no effect on resting body temperature (Fig. 4).

These results suggest that AT$_2$ receptors in the rostral hypothalamus (PO/AH region) are involved in PGE$_2$-induced febrile responses in rats.

The question then arose as to whether ANG II mediates or, on the other hand, modulates febrile responses through its action on AT$_2$ receptors. To clarify this point, ANG II alone was administered into the rostral hypothalamus to observe its effect on resting body temperature. The results showed that ANG II, given i.h. at 25 ng or 5 µg, did not exert any significant effects on body temperature in rats (Watanabe et al., 1997). It therefore seems unlikely that ANG II mediates the induction of fever in rats; rather, ANG II is more likely to modulate the fevers induced by other agents. Having found that ANG II at a dose of 25 ng i.h. had no effect on resting body temperature, we used this dose to investigate whether ANG II modulates the fever induced by PGE$_2$. As shown in Fig. 5, this dose of ANG II, which had no effect on resting body temperature, enhanced the PGE$_2$-induced fever (Watanabe et al., 1997), suggesting that centrally acting ANG II modulates PGE$_2$-induced fever in rats.

Fig. 4. Effect of intrahypothalamic (i.h.) injection of CGP 42112A on prostaglandin E$_2$ (PGE$_2$)-induced fever in rats. Changes (mean ± SEM) in body temperature (°C) in rats after i.h. injection at time 0 of PGE$_2$ (100 ng). CGP 42112A (2 µg, n = 11; or 5 µg, n = 8) or aCSF (n = 9) was administered i.h. immediately before the injection of PGE$_2$. Also shown is the effect on resting body temperature produced by CGP 42112A (5 µg i.h., n = 6) given alone at time 0. Data from Watanabe et al. (1997).

Fig. 5. Effect of i.h. injection of ANG II on PGE$_2$-induced fever in rats. Changes (mean ± SEM) in body temperature (°C) in rats after i.h. injection at time 0 of PGE$_2$ (25 ng). ANG II (25 ng, n = 8) or aCSF (n = 8) was administered i.h. immediately before the injection of PGE$_2$. Data from Watanabe et al. (1997).
Finally, we investigated the effects of an i.h. injection of an ACE inhibitor, lisinopril, on PGE₂-induced fever in rats. Lisinopril (5 or 10 µg, i.h.) or aCSF was given to rats 15 min before an injection of PGE₂ (100 ng, i.h.). The results showed that treatment with lisinopril reduced the PGE₂-induced fever in a dose-related fashion (Watanabe et al., 1997). Lisinopril (10 µg, i.h.) given alone had no marked effect on the resting body temperature. Thus, inhibition of ANG II synthesis within the rostral hypothalamus resulted in an attenuation of PGE₂-induced fever.

Taken together, our results suggest that in the rat, hypothalamic ANG II, which is synthesized and released in response to PGE₂, acts on AT₂ receptors located within the hypothalamus to facilitate PGE₂-induced fever. Interestingly, there are published reports indicating that AT₂ receptors are present in the rostral hypothalamus (Lenkei, et al., 1996; Lenkei, et al., 1997). Moreover, using microdialysis (perfusion flow rate = 2 µL/min and recovery rate = 14.2%) we recently found that i.h. perfusion with PGE₂ (2 µg/2 µL perfusate/min) resulted in ANG II release (on average, 0.17 pg/2 µL perfusate/min; n = 3) within the rat’s rostral hypothalamus (unpublished observations).

**ANG II and Fever in Mice**

Recently, 2 laboratories developed mice deficient in AT₂ receptors that showed increased pressor responses to ANG II and a lower than normal body temperature (Hein et al., 1995; Ichiki et al., 1995). These mice also exhibit attenuated exploratory behavior and reduced spontaneous movements, suggesting that endogenous ANG II and AT₂ receptors regulate brain functions. We administered IL-1β intraperitoneally (i.p.) to AT₂-receptor-deficient mice to allow us to compare the febrile response in such mice with that in wild-type mice. As shown in Fig. 6, in the controls a single injection of IL-1β (10 µg/kg, i.p.) resulted in a biphasic fever which began after 5 min and reached its 1st peak at 15 to 20 min.
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(Watanabe et al., 1999). The 2nd peak occurred at around 60 min. The 1st phase involves an injection-stress-induced hyperthermia, while the 2nd phase is a true IL-1β fever. This IL-1β fever was suppressed in the AT2-receptor-deficient mice, the effect being significant at $P < 0.05$ (25–180 min), while there was no difference in the 1st phase of the hyperthermia between the 2 groups of mice. The body temperature of the mutant mice was similar to that of the controls from 150 min onwards.

These results suggest that in mice, the pathway distal to the point at which IL-1β acts involves an action of ANG II on AT2 receptors. Since in rats central injection of an AT2-receptor antagonist attenuates PGE2-induced fever [see above (Watanabe et al., 1997)], it is likely that the brain AT2 receptor is important in the induction of PGE2-induced fever in mice as well in rats. However, it should be noted in the above results that an attenuation of the IL-1β fever was observed only during the early stage of the hyperthermia (10–180 min). Therefore, it may be that the AT2 receptor is just one of a number of factors contributing to IL-1β fever, and that its important role is performed mainly during the rising phase of this fever.

Concluding Remarks

In this mini-review, we have focussed on the role of ANG II in the development of fever in rats and mice.

We have shown that an i.v. injection of LPS induces a fever that is significantly greater in “dehydrated” than in “euhydrated” rats (Morimoto et al., 1986; Watanabe et al., 1997). We have suggested i) that the LPS-induced production of pyrogenic cytokines such as IL-1β might be enhanced by dehydration and ii) that ANG II is involved in this enhanced production of pyrogenic cytokines, and ultimately in enhancing the fever seen in response to LPS. Indeed, an ACE inhibitor inhibited the LPS-induced production of hepatic IL-1β, as well as the fever, in dehydrated rats (Watanabe et al., 2000; Miyoshi et al., 2003).

It is widely accepted that PGE2 acts within the rostral hypothalamus to induce fever, as a final mediator (Blatteis and Sehic, 1997; Ushikubi et al., 2000). We found that i.h. injection of either an AT2-receptor antagonist or an ACE inhibitor inhibited the fever induced by an i.h. injection of PGE2. Furthermore, ANG II given i.h., which had no effect on the resting body temperature, facilitated PGE2-induced fever (Watanabe et al., 1997). These results suggest that hypothalamic ANG II, following its synthesis and release in response to PGE2, acts on AT2 receptors in the rat hypothalamus to facilitate PGE2-induced fever.

Finally, when we gave a single i.p. injection of IL-1β to AT2-receptor-deficient mice we found that the IL-1β-induced fever was significantly weaker than in the wild-type mice, suggesting that the brain AT2 receptor is involved in the development of such fever in mice as well.

Taken together, the observations reviewed here make it seem likely that ANG II contributes to the development of fever by acting at both the 1st and final steps in the pathway for fever-induction in rodents.

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