

ANGIOTENSIN II: ITS EFFECTS ON FEVER AND HYPOTHERMIA IN SYSTEMIC INFLAMMATION

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1. ABSTRACT

Angiotensin II (ANG II), a bioactive peptide that plays important roles in blood-pressure and body-fluid regulation, has recently been reported to be involved in normal thermoregulation and fever. In the case of thermoregulation, ANG II lowers body temperature when administered centrally or systemically (i.e. "exogenous" ANG II acts as a hypothermia-inducing agent). In contrast, "endogenous" ANG II is involved both in heat-loss responses in a hot environment and in thermogenesis in the cold. It therefore seems likely that endogenous ANG II is involved in maintaining body temperature at the set-point. In the case of fever, it has been reported that endogenous brain ANG II and its type 1 receptor mediate or modulate the fever induced by "restraint stress". At the final step in "pyrogen-induced" fever, brain ANG II facilitates the fever induced by prostaglandin E₂ (PGE₂) through its action on the type 2 receptor, whereas at its first step the lipopolysaccharide (LPS, 2 microg/kg, i.v.)-induced production of pyrogenic cytokines [such as interleukin-1 (IL-1)] involves an action of endogenous ANG II through its type 1 receptor. On the other hand, it is well known that

a very high dose of LPS (50-5000 microg/kg) injected systemically induces hypothermia in rodents. This hypothermia is presumably initiated by tumor necrosis factor (TNF). Since ANG II contributes to the LPS-induced production of cytokines such as IL-1beta, as described above, it is possible that the generation of TNF by LPS involves an action of ANG II, too, and that this TNF production leads to the LPS-induced hypothermia.

Together, these findings suggest that ANG II and its receptors make a number of contributions to normal thermoregulation, to fever, and to the hypothermia in systemic inflammation.

2. INTRODUCTION

Angiotensin II (ANG II), a bioactive peptide that plays important roles in blood-pressure and body-fluid regulation, may also be involved in body temperature regulation. First, this mini-review touches on the role of ANG II in normal thermoregulation. Then, we move onto

its importance in a pathologic thermal response, fever. Finally, we speculate on the role of ANG II in the development of the hypothermia in systemic inflammation.

3. ROLE OF ANG II IN NORMAL THERMOREGULATION

The intracerebroventricular (i.c.v.) administration of ANG II has been reported to lower resting body temperature in many species, such as rats, rabbits, and sheep (1-6), suggesting that ANG II is one of the hypothermia-inducing agents acting on the central nervous system. Furthermore, subcutaneous (s.c.) injection of ANG II induces hypothermia, too [both by enhancing heat-loss responses and by inhibiting heat-production] (7-10). This peripheral ANG II-mediated response might be at least in part due to its action on the ANG II type 1 (AT₁) receptor in the subfornical organ (SFO; 9), since the ANG II-containing SFO neurons transfer the peripheral ANG II signal to the brain. In previous studies by us and others, however, injection of ANG II into the medial and/or lateral preoptic area, a thermoregulatory center in the brain, failed to affect body temperature (11, 12). This leaves us with a question as to how brain ANG II lowers body temperature.

In fact, there are two papers in the literature suggesting that the ANG II-induced pressor response might hold the answer to this question. It should be noted that the studies mentioned above employed relatively high doses of ANG II (1-5 microg, i.c.v.), which would presumably have markedly increased blood pressure and thus produced a baroreceptor reflex. Indeed, Shido *et al.* (4) demonstrated that an i.c.v. injection of ANG II (5 microg) in rats induced a baroreflex bradycardia as well as hypothermia, and that sinoaortic denervation reduced the hypothermia. In addition, Hassinen *et al.* (13) noted that when the ANG II-induced pressor response is slow, body temperature rises, whereas a "rapid" increase in blood pressure is associated with hypothermia. These results suggest that inhibition of sympathetic nervous activity by the baroreceptor reflex - - which would lead both to a decrease in heat production in metabolic tissues and to an increase in heat loss via, for example, vasodilation - - is principally responsible for the ANG II-induced hypothermia. It therefore follows that the precise effect of "exogenous" ANG II on body temperature is still uncertain.

In contrast, a definite role for "endogenous" ANG II in thermoregulation in hot or cold environment has been suggested in rats. For example, i.c.v. injection of an AT₁-receptor antagonist increases body temperature in heat-exposed rats (14). Furthermore, in heat (40 °C)-exposed rats circumventricular structures, activated via circulating ANG II, seem to play a role in decreasing the threshold body temperature for salivation (15). Collectively this evidence indicates that in rats endogenous ANG II contributes to heat-loss responses in a hot environment. In addition, since heat-exposure frequently results in a loss of water (i.e., hypohydration and/or dehydration), it is reasonable to think that a hypohydration- or dehydration-induced production of ANG II may play an important role in heat-loss responses in rats. In fact, this idea is supported by a recent finding that systemic salt-loading increases heat-escape behaviour via

central AT₁ receptors in rats (16). Concerning the role of ANG II in thermoregulation in a cold environment, there is a report suggesting that ANG II contributes to cold-induced thermogenesis in rats (17). Collectively, the above evidence suggests that endogenous ANG II may help to maintain body temperature at the set point in rats, at least. However, it should be borne in mind that there may be a species difference in the role played by endogenous ANG II in thermoregulation. For example, in humans an ANG-converting-enzyme (ACE) inhibitor failed to alter the exercise-induced rises in skin and esophageal temperature seen in a hot environment (18), suggesting that ANG II may play no significant role in heat-loss responses in humans. Furthermore, in heat-exposed monkeys an i.c.v. injection of ANG II resulted in a decrease in sweat rate, which may indicate that ANG II actually increases body temperature in the heat in these animals (19).

From the above, we can see that the exact role of endogenous ANG II in normal thermoregulation is uncertain, and will require clarification in future research.

4. ROLE OF ANG II IN FEVER

Although the role of ANG II in normal thermoregulation has been investigated quite extensively, as described above, comparatively little effort has been made to elucidate whether ANG II is involved in the development of "fever". In addition to the classical pyrogen-induced fever, the rise in body temperature induced by stress needs to be considered as a type of fever, because this hyperthermia can be inhibited by administration of anti-pyretic drugs, such as sodium salicylate (20). In fact, we recently found that endogenous ANG II mediates or modulates the fever seen in animals exposed to restraint-stress (21) or pyrogenic substances (12).

In this section, we present our recent results demonstrating the involvement of endogenous ANG II and its receptors in fever in rats, and we try to place them in the context of the related papers to be found in the literature, although the latter are surprisingly limited in number.

4.1. Role of ANG II in stress-induced fever

It is well known that an animal reacts to stressful stimuli with stereotyped responses, including increases in blood pressure and body temperature. Previously, two reports have directly shown the involvement of ANG II in the blood pressure response to stress. According to these reports, blockade of the central AT₁ receptor resulted in a suppression, in rats, of the pressor response to heat-stress (22) or foot shock-stress (23). We repeated this experiment, and further examined the role of ANG II in the hyperthermic responses to restraint-stress (stress-induced fever), using spontaneously hypertensive rats (SHR) and their controls, Wistar-Kyoto (WKY) rats.

4.1.1. Stress-induced pressor and febrile responses are inhibited by treatment with the AT₁ antagonist, losartan, given intracerebroventricularly (i.c.v.)

The stress used in this experiment was restraint-stress, induced by placing the rat in a small cylindrical

restraining cage made of steel wire (7 x 12 cm; diameter x length) for 60 min. Throughout the experiment, each physiological variable was continuously monitored using a telemetric system. The i.c.v. injections were made into the third cerebral ventricle.

Figure 1A shows that immediately after the start of restraint-stress (at time 0) artificial cerebrospinal fluid (aCSF)-injected WKY and SHR had rapid increases in blood pressure, followed by a gradual decline throughout the period of stress. These changes were attenuated by the AT₁-receptor antagonist, losartan (10 microg and 100 microg, given i.c.v. at time 0) in a dose-related manner, its effects reaching significance only at the higher dose (100 microg; 21). In addition, as shown in figure 1B the restraint-stress-induced fever in both SHR and WKY was significantly attenuated by i.c.v. injection of losartan (given at the beginning of the stress exposure) (21). Losartan given i.c.v. alone had no effect on the resting body temperature in WKY and SHR (figure 1C). These results indicate that brain ANG II and AT₁ receptors contribute to the development of stress-induced pressor and febrile responses in rats.

We can not rule out the possibility that the effect of ANG II on the body temperature are simply an indirect response to changes in blood pressure regulation (vasodilation, etc). However, in our hands, losartan was effective in inhibiting the stress-induced fever in SHR at the lower dose (10 microg), that has no significant effect on the pressor response (figure 1A and B). Therefore, ANG II could have body temperature effects that are dissociated from blood pressure effects in SHR.

Furthermore, it is noted in this experiment that it required 100 microg to have an effect on the stress-induced response. Therefore, the effect of losartan at the lower dose (for example, 60 microg) should be examined on the stress-induced responses in the future experiments.

4.1.2. Stress responses are induced by activation of the sympathetic nervous system via the action of ANG II on brain AT₁ receptors

The aforementioned cardiovascular and temperature responses to stress are likely to be mediated, at least in part, by activation of the sympathetic nervous system. Indeed, we found that in both SHR and WKY, i.c.v. injection of losartan significantly attenuated the increases in plasma norepinephrine and epinephrine induced by restraint-stress (21). It is, therefore, very likely that activation of brain AT₁ receptors by endogenous ANG II is necessary for the full expression of the sympathoexcitatory responses to stress. This idea is supported by the finding that antagonism of central ANG II receptors attenuates the stress-induced elevation in splanchnic sympathetic neural activity (22). Furthermore, in the adrenal medulla the AT₁ receptor is the receptor-type directly related to catecholamine release (24). Thus, ANG II and AT₁ receptors would seem to play important roles in the stress-induced activation of the sympathoadrenomedullary system.

Collectively, the above evidence suggests that brain ANG II and AT₁ receptors contribute to the development of stress-induced pressor and febrile responses through their stimulatory effects on the sympathoadrenomedullary system. Furthermore, it should be interesting to examine the role of another ANG receptor, the AT₂ receptor in stress-induced fever as well.

4.2. Role of ANG II in IL-1beta- and PGE₂-induced fevers

It is widely accepted that a host reacts against pathogenic stimuli such as lipopolysaccharide (LPS) by producing several host-defence responses, including fever. LPS, once inside the body, stimulates leukocytes to produce pyrogenic cytokines, such as interleukin-1 (IL-1) or IL-6, and these cytokines are collectively called endogenous pyrogens (EP; 25). Furthermore, the febrile response to pyrogenic cytokines/EP is believed to be mediated by the central action of prostaglandin E (PGE; 25). Recently, we found that brain ANG II and type 2 ANG (AT₂) receptors (but not AT₁ receptors) contribute to the development of the febrile responses induced in rats by IL-1beta or PGE₂ (12).

4.2.1. IL-1beta- and PGE₂-induced febrile responses are inhibited by treatment with the AT₂ antagonist, CGP42112A (given i.c.v.), but not by the AT₁ antagonist, losartan.

Having found that the fever induced by restraint-stress is inhibited by an AT₁ antagonist, losartan (see above), we postulated that AT₁ receptors play an important role in pyrogen-induced fever, too. Surprisingly, however, i.c.v. treatment with losartan (60 microg) had no effect on the febrile response induced by either IL-1beta or PGE₂ (12). In this experiment, IL-1beta was injected intraperitoneally (i.p.), while PGE₂ was given i.c.v. The dose of losartan (60 microg) used is actually rather high, because less than 10 microg of the drug is sufficient to inhibit ANG II-induced responses such as increases in water intake (26) and arterial blood pressure (27) in rats. Thus, since it seemed that AT₁ receptors are not involved in IL-1beta- and PGE₂-induced fevers, although they participate in stress-induced fever, we wondered whether the other ANG receptor, the AT₂ receptor, contributes to the development of pyrogen-induced fever.

Figure 2 shows the effect of an AT₂ antagonist, CGP 42112A, on the fevers induced by i.p. IL-1beta and i.c.v. PGE₂ in rats (12). CGP42112A was administered i.c.v. just before the IL-1beta or PGE₂. Injection of IL-1beta (2 microg/kg, i.p.) in the aCSF-treated controls resulted in a biphasic increase in body temperature (figure 2A) which began after 5 min and reached its peak at 90-120 min. On the other hand, injection of PGE₂ (200 ng, i.c.v.) produced a monophasic fever in the aCSF-injected controls (figure 2B), the body temperature beginning to increase immediately and reaching peak at 30 min. Importantly, figure 2 shows that the IL-1beta-induced fever was attenuated by treatment with CGP42112A (5 or 20 microg, i.c.v.) in a dose-related manner, the effect being significant at $p < 0.05$ [CGP42112A (5 microg)+IL-1beta, 0-35 min; CGP42112A (20 microg)+IL-1beta, 0-115 min]. Likewise,

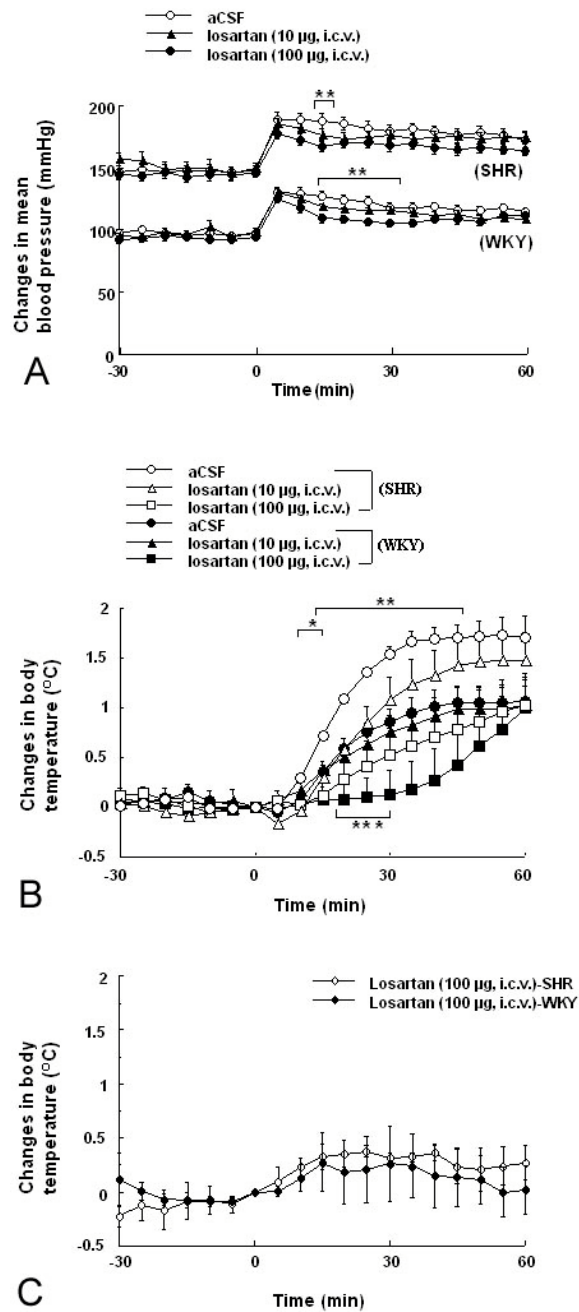


Figure 1. Effect of i.c.v. injection of losartan on restraint-stress-induced pressor or febrile response in WKY and SHR. A, Changes in mean arterial blood pressure (mmHg) in WKY and SHR induced by restraint-stress for 60 min. Losartan or artificial cerebrospinal fluid (aCSF) was given i.c.v. immediately before the period of stress (which began at time 0 min). In WKY, losartan [10 (n=8) or 100 microg (n=8)] or aCSF (n=7); in SHR, losartan [10 (n=8) or 100 microg (n=8)] or aCSF (n=8). ** $P < 0.05$ aCSF vs. losartan 100 microg. Data from Saiki *et al.* (21). B, Changes in body temperature (°C) in WKY and SHR induced by restraint-stress for 60 min. Losartan or aCSF was given i.c.v. immediately before the period of stress (which began at time 0 min). In WKY, losartan [10 (n=5) or 100 microg (n=6)] or aCSF (n=6); in SHR, losartan [10 (n=6) or 100 microg (n=5)] or aCSF (n=6). The resting body temperature at time 0 min of WKY [losartan (10 or 100 microg) or aCSF] or of SHR [losartan (10 or 100 microg) or aCSF] was $37.15 \pm 0.19^\circ\text{C}$, $37.08 \pm 0.18^\circ\text{C}$, $37.21 \pm 0.08^\circ\text{C}$, or $37.10 \pm 0.15^\circ\text{C}$, $37.20 \pm 0.07^\circ\text{C}$, $36.98 \pm 0.16^\circ\text{C}$, respectively. * $p < 0.05$ aCSF vs. losartan 10 microg in SHR; ** $P < 0.05$ aCSF vs. losartan 100 microg in SHR; *** $p < 0.05$ aCSF vs. losartan 100 microg in WKY. Data from Saiki *et al.* (21). C, Changes in body temperature (°C) in WKY and SHR following i.c.v. injection of losartan at time 0 min. In WKY, losartan [100 microg (n=3)]; in SHR, losartan [100 microg (n=3)]. The resting body temperature at time 0 of WKY or of SHR was $37.06 \pm 0.17^\circ\text{C}$, or $37.09 \pm 0.15^\circ\text{C}$, respectively.

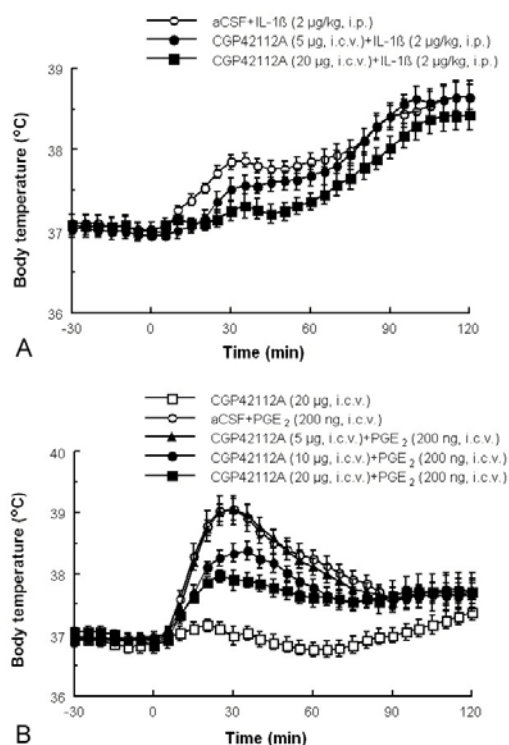


Figure 2. Effect of i.c.v. injection of CGP42112A on interleukin-1beta (IL-1beta)- and prostaglandin E₂ (PGE₂)-induced fevers in rats. A, Changes in body temperature (°C) in rats after intraperitoneal (i.p.) injection at time 0 of interleukin-1beta (IL-1beta, 2 microg/kg). CGP42112A [5 microg (n=7) or 20 microg (n=7)] or aCSF (n=10) was administered intracerebroventricularly (i.c.v.) immediately before the injection of IL-1beta. Data from Watanabe *et al.* (12). B, Changes in body temperature (°C) in rats after i.c.v. injection at time 0 of prostaglandin E₂ (PGE₂, 200 ng). CGP42112A [5 microg (n=9), 10 microg (n=7) or 20 microg (n=6)] or aCSF (n=11) was administered i.c.v. immediately before the injection of PGE₂. Also shown is the effect of CGP42112A alone (20 microg, i.c.v.; n=9) on resting body temperature. Data from Watanabe *et al.* (12).

the PGE₂ (200 ng, i.c.v.)-induced fever was reduced by CGP42112A (10 or 20 microg, i.c.v.) in a dose-related manner [$p < 0.05$: CGP42112A (20 microg)+PGE₂, 0-85 min], although at 5 microg the same drug had no effect. CGP42112A (20 microg, i.c.v.) given alone had no effect on resting body temperature (figure 2B). These results suggest the involvement of brain AT₂ receptors in the development of PGE₂-induced fever in rats.

The question then arises as to which part of the brain is responsible for the AT₂-receptor-mediated febrile response. Since it is believed that PGE₂ acts within the preoptic hypothalamic area to induce fever, we next examined the effect of intrahypothalamic (i.h.) injection of CGP 42112A on IL-1beta- and PGE₂-induced fevers in rats.

4.2.2. IL-1beta- and PGE₂-induced febrile responses are inhibited by treatment with the AT₂ antagonist, CGP42112A, given intrahypothalamically (i.h.)

In this experiment, IL-1beta was injected i.p., but PGE₂ was administered i.h. The results are shown in figure 3 (12). The febrile responses induced by IL-1beta (2 microg/kg, i.p.) or PGE₂ (100 ng, i.h.) were each attenuated by i.h. treatment with CGP42112A (2 and 5 microg) in a dose-related manner, its effect being significant at $p < 0.05$ [CGP42112A (5 microg)+IL-1beta, 0-80 min; CGP42112A (5 microg)+PGE₂, 0-40 min]. CGP42112A (5 microg, i.h.) given alone had no effect on resting body temperature (figure 3B). Since these findings indicated the involvement of hypothalamic AT₂ receptors in the development of PGE₂-induced fever in rats, we wondered what the effect of ANG II itself might be on resting body temperature.

4.2.3. An i.h. injection of ANG II has no effect on resting body temperature, but enhances the PGE₂-induced febrile response

In fact, ANG II, given i.h. at 25 ng or 5 microg, did not exert any significant effects on body temperature in rats (12). It therefore seems likely that ANG II does not mediate, but instead modulates PGE₂-induced fever in this species. To test this idea, we examined the effect of ANG II on the PGE₂-induced fever in rats. ANG II (25 ng) was given into the hypothalamus just before the i.h. injection of PGE₂ (25 ng). ANG II, which had no effect on resting body temperature, enhanced the PGE₂-induced fever (12), suggesting that ANG II has a positive modulating effect on such fever.

4.2.4. PGE₂-induced febrile response is inhibited by treatment with an ANG-converting-enzyme (ACE) inhibitor, given i.h.

When we administered an ACE inhibitor, lisinopril (5-10 microg), into the hypothalamus 15 min before the i.h. injection of PGE₂, the PGE₂-induced fever was significantly attenuated by the drug in a dose-related fashion (12).

Taken together, the above findings suggest that hypothalamic ANG II, which is synthesized and released in response to PGE₂, acts on AT₂ receptors in the rat hypothalamus to facilitate PGE₂-induced fever. Interactions between brain ANG II and catecholamines, serotonin and other transmitters have been suggested (28). For example, ANG II enhances field-stimulation-induced release of norepinephrine from rat brain tissue *in vitro* (29). Therefore, it is possible that central ANG II interacts in some way with transmitters involved in the mediation of fever induction. Furthermore, it has been reported that IL-1, given systemically, activates the renin-angiotensin system, and that the involvement of prostaglandins has been suggested in this activation (30). This evidence supports the above idea as well.

4.3. Role of ANG II in the LPS-induced production of pyrogenic cytokines and fever

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

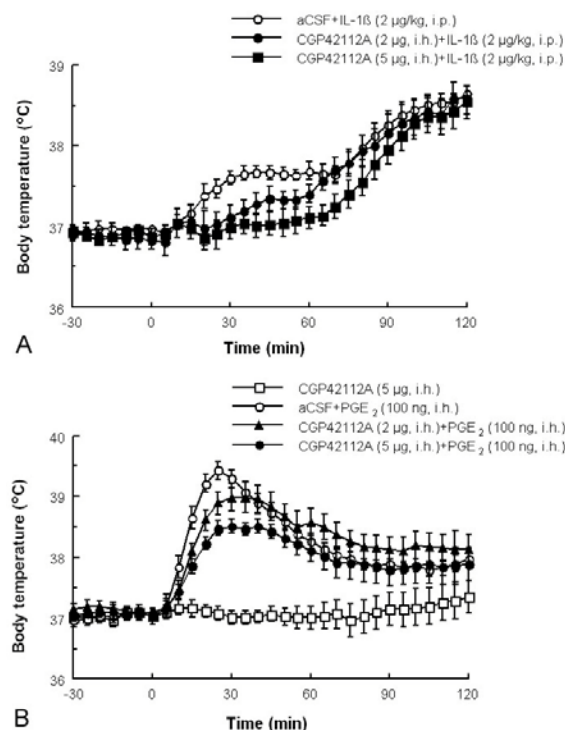


Figure 3. Effect of intrahypothalamic (i.h.) injection of CGP42112A on IL-1 β - and PGE $_2$ -induced fevers in rats A, Changes in body temperature ($^{\circ}$ C) in rats after i.p. injection at time 0 of IL-1 β (2 microg/kg). CGP42112A [2 microg (n=5) or 5 microg (n=6)] or aCSF (n=6) was administered i.h. immediately before the injection of IL-1 β . Data from Watanabe *et al.* (12). B, Changes in body temperature ($^{\circ}$ C) in rats after i.h. injection at time 0 of PGE $_2$ (100 ng). CGP42112A [2 microg (n=11) or 5 microg (n=8)] or aCSF (n=9) was administered i.h. immediately before the injection of PGE $_2$. Also shown is the effect of CGP42112A alone (5 microg, i.h. ; n=6) on resting body temperature. Data from Watanabe *et al.* (12).

dose of LPS induced a fever that was significantly greater in dehydrated rats than in euhydrated rats (31). However, dehydration had no effect on the fever induced by i.v. injection of IL-1 β (31), suggesting that the LPS-induced production of pyrogenic cytokines such as IL-1 β 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 next examined the effect of an ACE inhibitor on febrile responses in dehydrated rats.

4.3.1. LPS-induced febrile response is reduced by treatment with an ACE inhibitor given i.v., but the same inhibitor has no effect on IL-1 β -induced fever

Figure 4 shows the effect of an ACE inhibitor, lisinopril, on the fever induced in dehydrated rats by the i.v. injection of a relatively low dose of LPS (2 microg/kg; 31). When lisinopril was given i.v. just before the LPS (figure 4A), the LPS-induced fever was significantly attenuated,

suggesting that ANG II is involved in LPS-induced fever in dehydrated rats. By contrast, the same inhibitor had no effect on IL-1 β -induced fever (figure 4B), indicating that ANG II may act by contributing to the LPS-induced production of pyrogenic cytokines, such as IL-1 β . It is also noted in figure 4B that lisinopril given i.v. alone had no effect on the resting body temperature in dehydrated rats.

We have measured the plasma concentration of IL-1 β following i.v. injection of LPS (2 microg/kg) in dehydrated rats. However, IL-1 β was not detectable in the plasma after the injection of LPS (unpublished observation). For that reason, we next examined the role of ANG II in LPS-induced expression of IL-1 β in the liver, a representative organ of the reticuloendothelial system. It has been reported that LPS-induced production of IL-1 β in the tissues results in the local release of another cytokine, IL-6. Subsequently, this IL-6 enters the general circulation to cause fever (25).

4.3.2. LPS-induced production of IL-1 β in the rat liver is reduced by treatment with an ACE inhibitor, given i.v.

Figure 5 illustrates the effect of lisinopril on the expression of IL-1 β mRNA in the liver of dehydrated rats given an i.v. injection of LPS (32). Animals were sacrificed, the liver quickly removed, and the IL-1 β mRNA examined by Northern blot analysis. LPS induced a significant increase in the expression of hepatic IL-1 β mRNA at both 2 and 4 h after its injection, and this increase was reduced by treatment with lisinopril (figure 5A), an effect that, by quantitative analysis, was revealed to be statistically significant (figure 5B). This result suggests that ANG II contributes to the LPS-induced induction of IL-1 β at the mRNA level in dehydrated rats.

We also investigated the effect of lisinopril or losartan on the production of IL-1 β protein in the liver of dehydrated rats given an i.v. injection of LPS, the hepatic concentration of IL-1 β being measured by ELISA. In this experiment, the LPS-induced increase in the IL-1 β protein concentration was found to be significantly attenuated by these drugs (32).

Collectively, the above results support the idea that ANG II and AT $_1$ receptor are involved in the LPS-induced production of IL-1 β in dehydrated rats.

4.3.3. LPS-induced IL-6 response in rat plasma is reduced by treatment with an ACE inhibitor, given i.v.

In our next experiment, we examined the effect of lisinopril on the increase in plasma IL-6 seen in dehydrated rats given an i.v. injection of LPS. This effect was found to be significantly inhibited by lisinopril, suggesting the involvement of ANG II in the LPS-induced IL-6 response (32).

As mentioned above, the LPS-induced production of IL-1 β in the tissues leads to the local induction of IL-6, which enters the general circulation to cause fever (25). On this basis, IL-6 is now thought to be a candidate for a

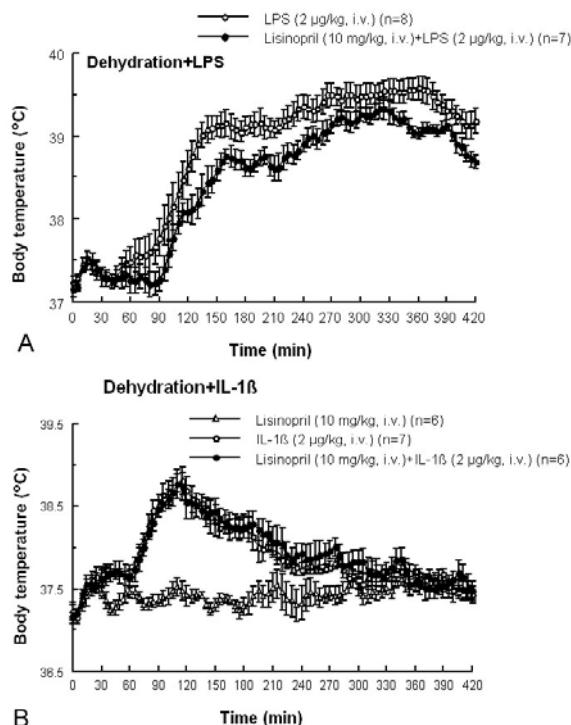


Figure 4. Effect of i.v. injection of lisinopril on LPS- and IL-1beta-induced fevers in dehydrated rats. A, Changes in body temperature (°C) in dehydrated rats after i.v. injection at time 0 of LPS (2 microg/kg). Lisinopril (10 mg/kg) was administered i.v. simultaneously with the LPS. Data from Watanabe *et al.* (31). B, Changes in body temperature (°C) in dehydrated rats after i.v. injection at time 0 of IL-1beta (2 microg /kg). Lisinopril (10 mg/kg), was administered i.v. simultaneously with the IL-1beta. Also shown is the effect of lisinopril alone (10 mg/kg, i.v. ; n=6) on resting body temperature. Data from Watanabe *et al.* (31).

circulating pyrogenic cytokine, particularly since IL-1beta cannot be detected in the blood of animals treated with LPS (25). A distinct possibility is that ANG II participates in the production of tissue IL-1beta in response to LPS, leading to the induction of IL-6, and that this IL-6, after entering the general circulation and acting on the brain as a circulating pyrogenic cytokine, participates in the development of the LPS-induced fever, especially in the dehydrated rats. It should be interesting to examine the role of ANG II in the induction of fever in response to IL-6 as well. Furthermore, the above idea seems to be applicable to the euhydrated rats as well, because we found that the i.v. injection of lisinopril significantly reduced the fever induced in euhydrated rats by i.v. injection of LPS (2 microg/kg) (31).

5. ROLE OF ANG II IN THE HYPOTHERMIA IN SYSTEMIC INFLAMMATION; A SPECULATION

It has been reported that the temperature responses induced by systemic administration of LPS depend very much on the dose of LPS. Actually, in rodents a very high dose of LPS (50-5000 microg/kg) injected either i.v. or i.p. induces either hypothermia or hypothermia followed by fever (33-39), whereas a low dose (2

microg/kg, i.v.) produced a fever in our study (see above; 31). It has been shown that the LPS-induced hypothermia involves a decrease in the threshold body temperature for thermogenesis, together with cold-seeking behavior (37). Thus, this hypothermia may be a well-regulated one. Furthermore, it has been suggested that the LPS-induced hypothermia is an adaptive thermoregulatory response to systemic inflammation (40). In fact, the LPS-induced hypothermia is mediated by PGs, because it is inhibited by cyclooxygenase inhibitors (34), and a recent study revealed that it is mediated especially by COX-1-produced PG (36). This raises the question as to whether ANG II is involved in the development of inflammation-related hypothermia, as it is in the development of fever.

It has been hypothesized that increased production of PG (presumably PGD₂) in the thermoregulatory center is one of the final events in the development of LPS-induced hypothermia. In fact, i.p. injection of LPS leads to the release of PGD₂ in the preoptic area of the rat brain, while microinjection of PGD₂ into the preoptic area causes hypothermia in this species (39). On the other hand, one of the cytokines, tumor necrosis factor (TNF), has been reported to be involved in LPS-induced hypothermia. Indeed, an antibody against TNF has been shown to inhibit LPS-induced hypothermia (33). Furthermore, after injection of a high dose of LPS TNF-receptor-knockout mice exhibit fever, while their control wild-type mice become hypothermic at an early stage (41). Since ANG II contributes to the LPS-induced production of such cytokines as IL-1beta, as described above, it seems likely that the generation of TNF by LPS involves an action of ANG II, too, and that this TNF production results in the synthesis of a hypothermia-inducing substance, namely PGD₂. If this is indeed the case, ANG II may participate not only in the development of the fever induced in rats by administration of LPS, but also in the hypothermia induced by the same agent. This possibility needs to be tested in detail in the near future.

6. CONCLUDING REMARKS

In this mini-review, we have discussed the role of ANG II in normal thermoregulation, in fever, and in the hypothermia in systemic inflammation.

ANG II has repeatedly been reported to induce hypothermia when injected either i.c.v. (1-6) or s.c. (7-10). However, it has been pointed out that the ANG II-induced rise in blood pressure may lead to an inhibition of sympathetic nervous activity via the baroreceptor reflex, and that this may be principally responsible for the ANG II-induced hypothermia (4). Thus, the precise effect of "exogenous" ANG II on body temperature is still unclear. By contrast, "endogenous" ANG II may help to maintain body temperature at the set-point by being involved in heat-loss responses in a hot environment (14) and in heat production in a cold environment (17).

Further, we have recently shown that ANG II contributes to fever induction, too. Indeed, brain ANG II may be involved in restraint-stress-induced fever in rats, an

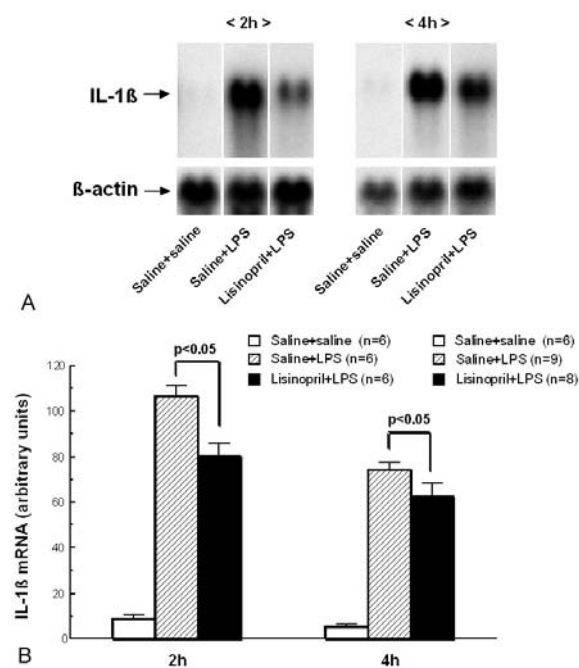


Figure 5. Effect of i.v. injection of lisinopril on LPS-induced increase in IL-1beta mRNA expression in the liver in dehydrated rats. IL-1beta mRNA expression in the liver of "Saline+saline", "Saline+LPS", and "Lisinopril+LPS" groups. Lisinopril (20 mg/kg) or saline was administered i.v. 30 min before a single i.v. injection (at time 0) of LPS (2 microg/kg) or saline in dehydrated rats. Data from Miyoshi *et al.* (32). A, Autoradiograms showing IL-1beta mRNA and beta-actin mRNA bands in a representative animal from each group at 2h and 4h after "time 0". B, Analysis of the density of the IL-1beta mRNA bands in each group. Mean values (\pm S.E.M.) obtained for IL-1beta mRNA in the liver are expressed in arbitrary units. For each sample, the density of the IL-1beta mRNA fraction was normalized with respect to the beta-actin density. Data from Miyoshi *et al.* (32).

action that may be exerted via its type 1 receptor (21). By contrast, it seems likely that brain ANG type 2 receptors, not type 1 receptors, play an important role in the development of the fever induced in rats by brain PGE₂ (12). Furthermore, ANG II and its type 1 receptor are, at least in part, responsible for the local production of pyrogenic cytokines in response to LPS in tissues such as the liver (32).

Finally, a high dose of LPS induces hypothermia (33-39), a response that has been reported to be dependent on TNF (33, 41) and PGD₂ (39). If ANG II participates in the LPS-induced production of TNF, as is the case for IL-1beta, then LPS-induced hypothermia may well involve an action of ANG II.

In conclusion, the available evidence suggests that endogenous ANG II acts to maintain body temperature at the set-point under afebrile, febrile, and hypothermic conditions.

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8. REFERENCES

- Huang BS, M.J. Kluger & R.L. Malvin: Thermal responses to central angiotensin II, SQ 20881, and dopamine infusions in sheep. *Am J Physiol* 248 (Regulatory Integrative Comp Physiol), R371-R377 (1985)
- Lin MT: Effects of angiotensin II on metabolic, respiratory and vasomotor activities as well as body temperatures in the rabbit. *J Neural Transmission* 49, 197-204 (1980)
- Lin MT, A. Chandra & J.J. Jou: Angiotensin II inhibits both heat production and heat loss mechanisms in the rat. *Can J Physiol Pharmacol* 58, 909-914 (1980)
- Shido O, A. Kifune & T. Nagasaka: Contributions of baroreceptor reflex to the hypothermic effect of intraventricular angiotensin II in rats. *Jpn J Physiol* 35, 301-309 (1985)
- Shido O & T. Nagasaka: Effects of intraventricular angiotensin II on heat balance at various ambient temperatures in rats. *Jpn J Physiol* 35, 163-167 (1985)
- Wilson KM & M.J. Fregly: Factors affecting angiotensin II-induced hypothermia in rats. *Peptides* 6, 695-701 (1985)
- Fregly MJ, T. Connor, M.J. Katovich & N.E. Rowland: Effects of chronic treatment with angiotensin II on thermoregulation in rats. *Ann N Y Acad Sci* 813, 211-215 (1997)
- Fregly MJ & N.E. Rowland: Effect of losartan potassium and deoxycorticosterone acetate on tail skin temperature response to acute administration of angiotensin II. *Pharmacol Biochem Behav* 43, 229-233, (1992)
- Fregly MJ & N.E. Rowland: Centrally mediated vasodilation of the rat's tail by angiotensin II. *Physiol Behav* 60, 861-865 (1996)
- Wilson KM & M.J. Fregly: Angiotensin II-induced hypothermia in rats. *J Appl Physiol* 58, 534-543 (1985)
- Sharpe LG, J.E. Garnett & N.S. Olsen: Thermoregulatory changes to cholinomimetics and angiotensin II, but not to the monoamines microinjected into the brain stem of the rabbit. *Neuropharmacology* 18, 117-125 (1979)

12. Watanabe T, Y. Saiki & Y. Sakata: The effect of central angiotensin II-receptor blockade on interleukin-1 β - and prostaglandin E-induced fevers in rats: possible involvement of brain angiotensin II receptor in fever induction *J Pharmacol Exp Ther* 282, 873-881 (1997)
13. Hassinen E, A. Pyornila & R. Hissa: Cardiovascular and thermoregulatory responses to vasotocin and angiotensin II in the pigeon. *Comp Biochem Physiol Part 1* 123, 279-285 (1999)
14. Mathai ML, T. Hubschle & M.J. McKinley: Central angiotensin receptor blockade impairs thermolytic and dipsogenic responses to heat exposure in rats. *Am J Physiol* 279 (Regulatory Integrative Comp Physiol), R1821-R1826 (2000)
15. Horowitz M, P. Kaspler, E. Simon & R. Gerstberger: Heat acclimation and hypohydration: involvement of central angiotensin II receptors in thermoregulation. *Am J Physiol* 277 (Regulatory Integrative Comp Physiol), R47-R55 (1999)
16. Konishi M, K. Nagashima & K. Kanosue: Systemic salt loading decreases body temperature and increases heat-escape/cold seeking behaviour via the central AT₁ and V₁ receptors in rats. *J Physiol (Lond)* 545.1, 289-296 (2002)
17. Cassis LA: Role of angiotensin II in brown adipose thermogenesis during cold acclimation. *Am J Physiol* 265 (Endocrinol Metab), E860-E865 (1993)
18. Mittleman KD: Influence of angiotensin II blockade during exercise in the heat. *Eur J Appl Physiol* 72, 542-547 (1996)
19. Owen MD, R.D. Matthes & C.V. Gisolfi: Effects of CSF ANG II and AVP on sweating in the heat-stressed patas monkey. *J Appl Physiol* 67(1), 134-140 (1989)
20. Kluger MJ, B. O'Reilly, T.R. Shope, & A.J. Vander: Further evidence that stress hyperthermia is a fever. *Physiol Behav* 39(6), 763-766 (1987)
21. Saiki Y, T. Watanabe, N. Tan, M. Matsuzaki & S. Nakamura: Role of central ANG II-receptors in stress-induced cardiovascular and hyperthermic responses in rats. *Am J Physiol* 272 (Regulatory Integrative Comp Physiol), R26-R33 (1997)
22. Kregel, KC, H. Stauss & T. Unger: Modulation of autonomic nervous system adjustments to heat stress by central ANG II receptor antagonism. *Am J Physiol* 266 (Regulatory Integrative Comp Physiol), R1985-R1991 (1994)
23. Cierco, M, & A. Israel: Role of angiotensin AT₁ receptor in the cardiovascular response to footshock. *Eur J Pharmacol* 251, 103-106 (1994)
24. Hano T, M. Mizukoshi, A. Baba, N. Nakamura, N. & I. Nishio: Angiotensin II subtype 1 receptor modulates epinephrine release from isolated rat adrenal gland. *Blood Pressure* 3 (Suppl), 105-108 (1994)
25. Kluger MJ: Fever: role of pyrogens and cryogens. *Physiol Rev* 71, 93-127 (1991)
26. Rowland NE, A. Rozelle, P.J. Riley & M.J. Fregly: Effect of nonpeptide angiotensin receptor antagonists on water intake and salt appetite in rats. *Brain Res Bull* 29, 389-393 (1992)
27. Hogarty DC, E.A. Speakman, V. Puig & M.I. Phillips: The role of angiotensin, AT₁ and AT₂ receptors in the pressor, drinking and vasopressin responses to central angiotensin. *Brain Res* 586(2), 289-294 (1992)
28. Phillips MI: Functions of angiotensin in the central nervous system. *Ann Rev Physiol* 49, 413-435 (1987)
29. Meldrum MJ, C.S. Xue, L. Badino & T.C. Westfall: Angiotensin facilitation of noradrenergic neurotransmission in central tissues of the rat: Effects of sodium restriction. *J Cardiovasc Pharmacol* 6, 989-995 (1984)
30. Bataillard A, A. del Rey, I. Klusman, G.M. Arditi & H.O. Besedovsky: Interleukin-1 stimulates aldosterone secretion: involvement of renin, ACTH, and prostaglandins. *Am J Physiol* 263, R840-R844 (1992)
31. Watanabe T, M. Hashimoto, M. Wada, T. Imoto, M. Miyoshi, D. Sadamitsu & T. Maekawa: Angiotensin-converting-enzyme inhibitor inhibits dehydration-enhanced fever induced by endotoxin in rats. *Am J Physiol* 279 (Regulatory Integrative Comp Physiol), R1512-R1516 (2000)
32. Miyoshi M, K. Nagata, T. Imoto, O. Goto, A. Ishida & T. Watanabe: ANG II is involved in the LPS-induced production of proinflammatory cytokines in dehydrated rats. *Am J Physiol* 284, R1092-R1097 (2003)
33. Derijk RH & F. Berkenbosch: Hypothermia to endotoxin involves the cytokine tumor necrosis factor and the neuropeptide vasopressin in rats. *Am J Physiol* 266 (Regulatory Integrative Comp Physiol), R9-R14 (1994)
34. Derijk RH, M.V. Kampen, N.V. Rooijen & F. Berkenbosch: Hypothermia to endotoxin involves reduced thermogenesis, macrophage-dependent mechanisms, and prostaglandins. *Am J Physiol* 266 (Regulatory Integrative Comp Physiol), R1-R8 (1994)
35. Dogan MD, H. Ataoglu & E.S. Akarsu: Effects of different serotypes of Escherichia coli lipopolysaccharides on body temperature in rats. *Life Sci* 67, 2319-2329 (2000)
36. Dogan MD, H. Ataoglu & E.S. Akarsu: Effects of selective cyclooxygenase enzyme inhibitors on lipopolysaccharide-induced dual thermoregulatory changes in rats. *Brain Res Bull* 57, 179-185 (2002)

37. Romanovsky AA, O. Shido, S. Sakurada, N. Sugimoto & T. Nagasaka: Endotoxin shock: thermoregulatory mechanisms. *Am J Physiol* 270 (Regulatory Integrative Comp Physiol), R693-R703 (1996)
38. Shemi D, & J. Kaplanski: Effect of different ambient temperature and lipopolysaccharide administration on the circadian rhythm of rectal temperature and hypothalamic PGE₂ production in aged male rats. *Mech Ageing Dev* 85, 65-72 (1995)
39. Ueno R, S. Narumiya, T. Ogorochi, T. Nakayama, Y. Ishikawa & O. Hayaishi: Role of prostaglandin D₂ in the hypothermia of rats caused by bacterial lipopolysaccharide. *Proc Natl Acad Sci USA* 79, 6093-6097 (1982)
40. Romanovsky AA & M. Szekely: Fever and hypothermia: two adaptive thermoregulatory responses to systemic inflammation. *Med Hypotheses* 50, 219-226 (1998)
41. Leon LR, W. Kozak, J. Peschon & M.J. Kluger: Exacerbated febrile responses to LPS, but not turpentine, in TNF double receptor-knockout mice. *Am J Physiol* 272 (Regulatory Integrative Comp Physiol), R563-R569 (1997)

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