Angiotensin-converting enzyme inhibitor inhibits dehydration-enhanced fever induced by endotoxin in rats

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It has been reported that a host develops a marked fever under dehydrated conditions compared with normally hydrated conditions (11). The present study was carried out to investigate whether ANG II is involved in the enhancement seen in dehydrated rats of the fever induced by bacterial endotoxin. The results showed that intravenous injection of bacterial endotoxin produced a fever in dehydrated rats (rats deprived of water for 24 h) that was significantly greater than that seen in normally hydrated rats. In contrast, dehydration had no effect on the fever induced by intravenous interleukin-1β (IL-1β). Under dehydrated conditions, the enhanced endotoxin-induced fever was significantly inhibited by the angiotensin-converting enzyme inhibitor lisinopril, but the IL-1β fever was not. These results suggest that the dehydration-induced enhancement of endotoxin fever is due, at least in part, to the action of ANG II, which elicits an increased production of pyrogenic cytokines such as IL-1.

Indeed, Morimoto et al. (11) demonstrated that although dehydrated rats exhibited a more severe fever than normally hydrated rats after intravenous injection of bacterial endotoxin, there was no difference between the fevers induced in the two groups of rats by intravenous injection of EP. However, the mechanism underlying the supposed increased production of EP under dehydrated conditions remains to be elucidated.

It is well known that dehydration results in an enhancement of ANG II secretion. Moreover, angiotensin-converting enzyme (ACE) inhibitors have been shown to have anti-inflammatory effects both in vitro and in vivo (2, 13, 15). Interestingly, the in vitro studies suggested an involvement of ANG II in the production of cytokines from leukocytes stimulated with bacterial endotoxin (13, 15). From the above pieces of evidence, it seems highly likely that dehydration-induced ANG II production elicits an increased production of pyrogenic cytokines/EP and that these are actually responsible for the augmentation seen under dehydrated conditions of the fever induced by bacterial endotoxin.

To test this hypothesis, we investigated the effect of an ACE inhibitor on the fevers separately induced by bacterial endotoxin and a pyrogenic cytokine, IL-1. The results revealed an enhancement under dehydrated conditions of the fever induced by intravenous injection of bacterial endotoxin; however, dehydration had no effect on the fever induced by intravenous IL-1β. Under dehydrated conditions, the enhanced endotoxin-induced fever was markedly suppressed by the ACE inhibitor lisinopril, whereas the IL-1β fever was not. These results suggest that the dehydration-induced event...

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enhancement of endotoxin fever is due, at least in part, to the action of ANG II, which elicits an increased production of pyrogenic cytokines such as IL-1.

METHODS

Animals

The animals used in this study were male Wistar rats, weighing 270–350 g. They were housed in individual plastic cages (40 × 25 × 25 cm; length × width × depth) with wood chip bedding in a room maintained at 26 ± 1°C, a temperature within the thermoneutral zone for rats. They experienced a 12:12-h light-dark photoperiod, with lights coming on at 0700. All animals had ad libitum access to drink and standard laboratory rat chow. The protocols were reviewed by the Committee on the Ethics of Animal Experiments in Yamaguchi University School of Medicine, and the experiments were carried out in accordance with the Guidelines for Animal Experiments at Yamaguchi University School of Medicine and with the Federal Law (No. 105) and Notification (No. 6) of the Japanese Government.

This study comprised two experiments, all on freely moving rats. Each rat took part in only one experiment. Details of the experimental protocols are given below.

Surgery

Body temperature was measured using a biotelemetry system (Data Science, St. Paul, MN) (9). Each rat was anesthetized with pentobarbital sodium (50 mg/kg ip), and a battery-operated transmitter (model TA100TFA40) was implanted intraperitoneally. The transmitter included a sensor and a radio-frequency transmitter. The output of the transmitter was monitored by antennae mounted in a receiver board (model CTR86) placed under each animal’s cage. The data were fed into a peripheral processor (matrix model BCM100) connected to a Sanyo MBC-17J AX computer (IBM compatible). The implantation of the transmitter was performed at least 1 wk before the implantation of a venous cannula for intravenous injections.

Rats with intraperitoneal biotelemetry transmitters were again anesthetized with pentobarbital sodium (50 mg/kg ip), and a polyvinyl tube was inserted into the jugular vein so that its tip lay in the superior caval vein near the right atrium (6). The free end of the catheter was passed subcutaneously to the midscapular 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 3 days before the start of the experiment.

All rats were handled for 15 min each day for at least 5 days to accustom them to the experimenters.

Drugs

The bacterial endotoxin used was the lipopolysaccharide (LPS) of Salmonella typhosa endotoxin (Difco), which was dissolved in sterile saline. Human recombinant IL-1β, supplied by Otsuka Pharmaceutical, was produced from recombinant strains of Escherichia coli. The activity of the IL-1β was found to be 2 × 10^5 units/μg by a thymocyte coproliferation assay. The IL-1β preparation was shown to be free of significant endotoxin contamination by a Limulus amoebocyte assay (<0.05 pg/μg protein). For injection purposes, the recombinant IL-1β was dissolved in sterile saline, the solution being divided between several vials and stored at −40°C until needed. We used the entire contents of a given vial within the 2 days after thawing and thus avoided repeated freezing and thawing. Lisinopril (Sigma) was dissolved in sterile saline. The doses injected in each experimental group are given below.

Experimental Protocols

Experiment 1. Changes in body temperature were examined in conscious rats after intravenous injection of LPS (2 μg/kg). Each rat received only one injection of LPS, because repeated injections of LPS result in febrile tolerance. The animals used in experiment 1 were divided into four groups.

GROUPS 1 AND 2: The effect of dehydration was examined on the LPS-induced febrile responses. Dehydration was achieved by deprivation of drinking water for 24 h (group 1), whereas control rats had ad libitum access to water (group 2).

GROUPS 3 AND 4: Simultaneous injection of an ACE inhibitor, lisinopril (10 mg/kg iv), and LPS (2 μg/kg iv) was performed in each rat under dehydrated (group 3) or normally hydrated conditions (group 4).

On the day of the experiment, each rat was gently picked up, and its transmitter was switched on with a magnet. The body temperature was then allowed to stabilize for a period of 90 min before any injections. The injectate (LPS solution or lisinopril mixed with LPS solution) was given intravenously to each animal in a volume of 1 ml/kg over a period of 30 s. To minimize the influence of the rat’s own circadian rhythm, LPS was always given between 1100 and 1200.

Experiment 2. The effect on body temperature produced by an intravenous injection of IL-1β (2 μg/kg) was examined in conscious rats. The animals used in experiment 2 were divided into three groups.

GROUPS 1 AND 2: Rats received an intravenous injection of IL-1β (2 μg/kg) under either dehydrated (group 1) or normally hydrated (group 2) conditions.

GROUP 3: The effect of an intravenous injection of lisinopril (10 mg/kg) was investigated on the fever induced by IL-1β (2 μg/kg) in dehydrated rats.

The injectate (IL-1β solution or lisinopril mixed with IL-1β solution) was given intravenously to each animal in a volume of 1 ml/kg over a period of 30 s.

The other procedures were essentially the same as those described for experiment 1.

Statistical Analysis

All results are expressed as means ± SE. The data were analyzed for statistical significance by a repeated-measures ANOVA (Macintosh, StatView 4.0) to assess the overall effect. Details of the results of the repeated-measures ANOVA are given in Figs. 1–4. Differences between the groups were considered significant at P < 0.05 (treatment effect).

RESULTS

Effect of Dehydration on LPS-Induced Fever in Rats

Figure 1 shows changes in body temperature induced in rats by intravenous injection of LPS (2 μg/kg) under normally hydrated or dehydrated conditions. Normally hydrated rats showed a biphasic fever after a latency of 90 min. The small increase in body temperature seen immediately after the injection represents an injection stress-induced hyperthermia, because saline injection elicited a similar response (data not shown). In contrast, under dehydrated conditions, the body temperature started to rise 40 min after the
injection of LPS, and the febrile response was significantly greater than that seen in the normally hydrated rats. It should be noted that there was no difference in the resting body temperatures at time 0 between the two groups of rats (dehydrated group, 37.24 ± 0.08°C; normally hydrated group, 37.22 ± 0.20°C).

**Effect of an ACE Inhibitor, Lisinopril, on the LPS-Induced Fever Under Dehydrated or Normally Hydrated Conditions**

When lisinopril (10 mg/kg iv) was administered simultaneously with the LPS (2 μg/kg iv), the enhanced LPS fever induced in dehydrated rats was significantly attenuated, as shown in Fig. 2A. In particular, lisinopril injection delayed the onset of the LPS fever from 40 (control group) to 90 min (lisinopril injection group) (Fig. 2A), the same latency as that of the LPS-induced fever in the normally hydrated rats (see Fig. 1). Under normally hydrated conditions, lisinopril (10 mg/kg iv) inhibited the LPS fever as well, this inhibition being significant but relatively small (Fig. 2B). Lisinopril had no effect on latency in this group.

The intravenous injection of lisinopril alone had no effect on the resting body temperature in the dehydrated rats (data not shown; n = 6).

**Effect of Dehydration on IL-1β-Induced Fever in Rats**

Both the dehydrated and normally hydrated rats that received an intravenous injection of IL-1β (2 μg/kg) exhibited a marked fever with a rapid onset (Fig. 3). There was no significant difference between the fevers induced in the two groups.

**Effect of an ACE Inhibitor, Lisinopril, on the IL-1β-Induced Fever Under Dehydrated Conditions**

Figure 4 shows that lisinopril (10 mg/kg iv) had no effect on the IL-1β (2 μg/kg iv)-induced fever in dehydrated rats.

**DISCUSSION**

The present results revealed an inhibition by an ACE inhibitor of the LPS-induced fever in both dehydrated and normally hydrated rats, although the inhibitory effect was greater under dehydrated conditions (see Fig. 2). Furthermore, the control LPS fever was more severe in the dehydrated rats than in the normally hydrated animals. It is well known that dehydration results in an increase in ANG II release. Hence, these findings suggest that ANG II contributes to the enhancement of the LPS-induced fever seen in dehydrated rats. The question then arises as to how ANG II might be involved in this enhancement. In our hands, systemic administration of the ACE inhibitor had no effect on the fever induced in dehydrated rats by an intravenous injection of IL-1β. Moreover, dehydration itself did not elicit any alteration in the IL-1β-induced fever. Taken together, these results suggest 1) that the effector system for fever induction, just down-
stream to the point of action of IL-1, does not undergo any changes as a result of either dehydration or administration of the ACE inhibitor and 2) that the increased secretion of ANG II seen during dehydration leads to an enhanced production of pyrogenic cytokines (such as IL-1) in response to LPS, and it is this that produces the enhancement of the LPS fever.

It has been reported that ANG II has proinflammatory properties. For example, ANG II increases the expression of such proinflammatory enzymes as phospholipase A₂ (16) and NAD(P)H oxidase (5), and it is involved in certain types of cardiovascular inflammation (17). Moreover, ACE inhibitors have anti-inflammatory effects (10, 14). Interestingly, cytokine production from LPS-stimulated peripheral blood mononuclear cells is decreased by ACE inhibitors in vitro (13, 15). These findings strongly support the present suggestion of an involvement of ANG II in LPS-induced cytokine production in vivo. In future studies, IL-1 production (assessed by, for example, measuring the IL-1 content of different brain loci or of the blood) should be examined in rats that have received an intravenous injection of LPS and an ACE inhibitor.

In this study, the resting body temperature in dehydrated rats, which was similar to that of the normally hydrated rats, showed no change after intravenous administration of the ACE inhibitor alone (data not shown). This indicates that any dehydration-induced increase in the secretion of ANG II does not induce cytokine production from leukocytes not stimulated with LPS. Furthermore, it also suggests that the dehydration-induced secretion of ANG II does not participate in normal thermoregulation. However, it has previously been reported that subcutaneous injection of a relatively large dose of ANG II (50–200 μg/kg) results in a rapid and transient hypothermia (4, 18). Therefore, it is likely that ANG II acts as a temperature-lowering substance in a normal state (if its secretion is markedly increased above the resting level for any reason).

Because LPS-induced fever was inhibited by the ACE inhibitor, it seems likely that ANG II enhances cytokine production only in leukocytes stimulated with LPS. How then might ANG II contribute to the LPS-stimulated production of pyrogenic cytokines? It has been reported that LPS activates a proinflammatory transcription factor, nuclear factor-κB (NF-κB), in monocytes (1, 12). Furthermore, the expression of cytokines is controlled at the transcriptional level through NF-κB (1, 12), and ANG II has been shown to activate NF-κB in monocytes (8). Taken together, this suggests the possibility that the activation of NF-κB by LPS is enhanced by ANG II, leading to an increase in cytokine production. To test this idea, the precise mechanisms underlying the action of ANG II in enhancing cytokine production will need to be examined in detail in future research.

Finally, we used the ACE inhibitor lisinopril to inhibit the production of ANG II. However, it is well known that ACE not only promotes production of ANG II, but also inactivates bradykinin. Thus ACE inhibitors elevate bradykinin levels. Bradykinin is important in inflammation and may also mediate the elevation in febrile temperature. To strengthen our hypothesis that ANG II is involved in LPS-induced fever, all the experiments performed in this study should be repeated, using ANG receptor blockers, before reaching any final conclusions. Furthermore, a continuous infusion of ANG II by means of miniosmotic pumps may be a useful way of examining its effect on the fever induced in rats by intravenous injection of LPS. These studies should be performed in the near future.

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