Changes in Plasma Catecholamines during Fever Induced by Bacterial Endotoxin and Interleukin-1β

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Abstract We have examined whether or not the release of catecholamines into the blood circulation of rabbits during fever is mediated by prostaglandins. The plasma levels of catecholamines (epinephrine and norepinephrine) were measured in 2 ml of blood withdrawn from the marginal ear vein. At an ambient temperature of 21 ± 1°C, intravenous injection of either lipopolysaccharide (LPS, 4 μg/kg) or human recombinant interleukin-1β (rIL-1β, 1 μg/kg) produced a biphasic fever accompanied by an increase in the plasma level of catecholamines. Pretreatment with intravenous indomethacin (1 mg/kg) markedly suppressed the increase in catecholamines induced by LPS and rIL-1β. In contrast, although intracerebroventricular injection of rIL-1β (20 ng) produced fever, it did not produce a significant change in plasma catecholamine levels. Similarly, intrahypothalamic injection of prostaglandin E2 (200, 800 ng) induced fever, but did not cause a significant change in catecholamine concentrations. These results suggest that IL-1 acts via prostaglandins on the peripheral tissues to release catecholamines into the circulation.

Key words: interleukin-1, LPS, catecholamine, prostaglandin, fever.

Cytokines such as interleukin-1 (IL-1), interleukin-6, interferon, and tumor necrosis factor induce fever and a variety of acute-phase responses [1–3]. IL-1 also activates the hypothalamopituitary-adrenal axis by stimulating the secretion of corticotropin-releasing factor (CRF) in the hypothalamus [4, 5]. Furthermore, it has been reported that the plasma levels of catecholamine increase during IL-1- and endotoxin-induced fever in rats [6, 7]. However, the mechanisms mediating the release of catecholamines during fever and its physiological significance remain unclear. There is considerable evidence which suggests that prostaglandins (PGs) may play a role in the development of fever; this stems from the initial observations of Milton and Wendlandt [8] who showed that intracerebroventricular injection of prostaglandin E (PGE) resulted in a strong pyrogenic response. Furthermore, PGs...
are known to stimulate the release of CRF, since the blockade of cyclooxygenase inhibits the CRF release in vitro [6]. Since the activation of CRF neurons stimulates the sympathetic nervous system as well as the ACTH release, it is possible to suggest that PGs may be involved also in the release of catecholamines into the blood circulation during fever. In the present study, we examined whether the release of catecholamines into the blood circulation during fever is mediated by PGs, and if so, whether or not it is mediated by the central actions of PGs.

METHODS

The animals used in this study were 30 male New Zealand White rabbits, weighing 3.0–3.5 kg. Under general anesthesia (sodium pentobarbital, 30 mg/kg, i.v.), 8 animals were stereotaxically implanted with a steel guide cannula (0.8 mm o.d.) for microinjection of drugs into the third ventricle and 6 animals into the preoptic/anterior hypothalamus (PO/AH) according to the atlas of Sawyer et al. [9]. At least 2 weeks were allowed for recovery before the start of the experiments.

During the experiment, the animals were minimally restrained in conventional neck stocks. To minimize the effects of stress due to restraint, the rabbits were trained by placing them in the stocks for 6 h every other day for at least 10 d before the experiment. When individual rabbits were used for different treatments, the order in which the animals received each treatment was randomized. The rabbits were allowed a recovery period of at least 2 weeks between one experiment and another. The experiments were conducted in the animals having normal rectal temperature (38.7–39.2°C) at an ambient temperature of 21 ± 1°C between 0900 and 1700 h. A thermocouple (copper-constantan) was inserted into the rectum about 8 cm past the anus for measurement of rectal temperature. The volume of solutions injected into the ventricle and PO/AH was 2 µl, and this was performed with a microsyringe at a rate of 2 µl/min.

Lipopolysaccharide (LPS) (Salmonella typhosa, Difco) was dissolved in sterile saline at concentrations of 0.4 and 40 µg/ml and stored at 4°C until use. Recombinant human IL-1β (rIL-1β) produced by recombinant strains of Escherichia coli was supplied by Otsuka Pharmaceutical Co., Ltd. The rIL-1β was highly purified and endotoxin-free, as confirmed by the Limulus amoebocyte lysate test.

rIL-1β was dissolved in sterile saline. Prostaglandin E2 (PGE2, Sigma Chemical Co.) was dissolved in sterile saline containing 2% ethanol at concentrations of 100 and 400 µg/ml. Indomethacin (INDO), which inhibits the synthesis of PGs, was dissolved in saline containing 1% ethanol and 2% sodium bicarbonate at a concentration of 10 or 200 mg/ml. Intravenous injection was done through the subcutaneously implanted cannula into the marginal vein of the ear.

To measure the plasma levels of epinephrine and norepinephrine, 2 ml of blood was withdrawn from the marginal ear vein. The blood samples were taken at 90 min before and at 30, 60, 120, 180, and 300 min after intravenous or intracerebroventricular injections of LPS or rIL-1β. The blood was collected into heparinized
polyethylene tubes, and centrifuged immediately at 3,000 rpm for 15 min at 4°C. The plasma was collected into test tubes and stored at -40°C until assay. Epinephrine and norepinephrine levels were determined using HPLC (high-pressure liquid chromatography) followed by electrochemical detection, as described previously [10].

At the end of the experiment each animal was sacrificed by an overdose (50 mg/kg) of sodium pentobarbital. The thorax was opened and formaldehyde solution (10%) was infused into the brain through the left cardiac ventricle. The brain was removed, set in agar (5%), and cut in sections (50 μm) with a vibratome. The sections were stained with hematoxylin and eosin and used for the histological verification of the cannula tip locations.

The data were analyzed for statistical significance by a repeated measures (one-factor and two-factor) ANOVA. Differences were considered significant at p < 0.05.

RESULTS

Figure 1 shows the mean changes in rectal temperature (Fig. 1A) and in the plasma levels of epinephrine (Fig. 1B) and norepinephrine (Fig. 1C) after the intravenous injection of either saline or LPS. Changes in rectal temperature are expressed as deviation from the baseline (recorded at the time of injection). In agreement with our previous observations [11], a small dose of LPS (0.04 μg/kg) produced monophasic fever while a large dose (4 μg/kg) induced biphasic fever. Plasma levels of epinephrine and norepinephrine increased significantly 1, 2, and 3 h after LPS (4 μg/kg) injection when compared with the response observed after injection of saline. The small dose (0.04 μg/kg) of LPS did not affect the release of catecholamines.

Subsequently, we observed the changes in rectal temperature and plasma levels of epinephrine and norepinephrine when LPS (4 μg/kg, i.v.) was given 15 min after the injection of either INDO (1 mg/kg, i.v.) or an equal volume of its vehicle. Pretreatment with INDO inhibited the second phase of the biphasic fever, but not its first phase (Fig. 2A). The increases in plasma levels of epinephrine and norepinephrine induced by LPS (4 μg/kg) were also markedly suppressed by systemic pretreatment with INDO, suggesting that PG synthesis is required for the LPS-induced catecholamine releases (Fig. 2B, C).

Figure 3 shows the mean changes in rectal temperature (Fig. 3A) and in the plasma levels of epinephrine (Fig. 3B) and norepinephrine (Fig. 3C) when rIL-1β (1 μg/kg) was injected intravenously 15 min after the intravenous injection of either INDO (1 mg/kg) or its vehicle. The systemic pretreatment with INDO markedly inhibited the rIL-1β-induced elevation in plasma levels of epinephrine and norepinephrine and the biphasic fever which was induced by rIL-1β, again suggesting the involvement of PGs.

On the other hand, central injection of rIL-1β or PGE2 did not affect
catecholamine secretion. Although the intrathirdventricular injection of rIL-1β (20 ng) raised the rectal temperature by about 2°C, the plasma levels of epinephrine and norepinephrine did not change significantly (Fig. 4). Furthermore, when PGE_2 (200 and 800 ng), which is known as a mediator of actions of IL-1β, was micro-

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Fig. 2. Mean changes (±SEM) in the rectal temperatures (A) and the plasma levels of epinephrine (B) and norepinephrine (C) of rabbits (n = 8) after intravenous injections of LPS with systemic pretreatment with vehicle or indomethacin. Repeated measures ANOVA: *p < 0.05, **p < 0.01.

Injected into the PO/AH of 6 rabbits, a dose-dependent hyperthermia was produced but plasma levels of catecholamines did not change (Fig. 5). The sites of the cannulae implanted into these rabbits were verified to be located in the PO/AH as shown in Fig. 6.
Fig. 3. Mean changes (±SEM) in the rectal temperatures (A) and the plasma levels of epinephrine (B) and norepinephrine (C) of rabbits (n=8) after the intravenous injection of rIL-1β with systemic pretreatment with vehicle or indomethacin. Repeated measures ANOVA: *p < 0.05, **p < 0.01.

DISCUSSION

In the present study, the increases in the levels of plasma catecholamines

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Fig. 4. Mean changes (±SEM) in the rectal temperatures (A) and the plasma levels of epinephrine (B) and norepinephrine (C) of rabbits (n=8) after intracerebroventricular injections of saline or rIL-1β.

(epinephrine and norepinephrine) and rectal temperature in rabbits after an intravenous injection of LPS (4 μg/kg) or rIL-1β (1 μg/kg) were suppressed by pretreatment with INDO (1 mg/kg, i.v.), suggesting the involvement of PGs in the induction of catecholamine release and fever. It was also shown that an intrathirdventricular injection of rIL-1β (20 ng) or an intrahypothalamic injection of PGE2 (200 and 800 ng) failed to affect the secretion of catecholamines, although
Fig. 5. Mean changes (±SEM) in the rectal temperatures (A) and the plasma levels of epinephrine (B) and norepinephrine (C) of rabbits (n=6) after intra-hypothalamic injection of PGE₂.

these treatments were able to induce febrile responses which were comparable to those after peripheral injection of LPS or rIL-1β. These findings emphasize the likelihood that the increase in plasma catecholamines following peripheral injections of LPS or rIL-1β is mediated primarily by peripherally released, not centrally induced, PGs. This conclusion is contradictory to previous observations in rats, which suggest the involvement of PGs. In the rat, it has been reported that
intracerebroventricular injection of IL-1 caused an increase in plasma catecholamines [7], and that intrahypothalamic infusion of rIL-1β directly stimulates the release of CRF [5, 12], which subsequently stimulates the sympathetic nervous system [13]. Furthermore, it has been reported that the effect of IL-1 on the release of CRF is antagonized by inhibition of PG synthesis in rats [14]. At present, we have no explanation for this discrepancy.

Atkins [15] has proposed that the biphasic fever induced by intravenous LPS is due to endogenous pyrogen (EP) released at high levels in the circulation. EP is now known to include cytokines which are released from circulating and reticuloendothelial monocytes in response to a variety of pathogenic stimuli such as bacterial endotoxins [1, 2]. Thus, it is inferred that during LPS fever, the increase in plasma catecholamines is caused by the action of cytokines released peripherally at high concentrations.

Transection of the spinal cord (C-7) in dogs has been reported to abolish the LPS-induced secretion of catecholamines by the adrenal medulla, suggesting that LPS acts at some sites in the CNS above the level of the spinal cord transection and induces adrenal medullary secretion via a descending spinal pathway [16]. In the present study, there is a possibility that IL-1 acts centrally on secretion from the adrenal medulla via an interface provided by the organum vasculosum laminae terminalis (OVLT), whose fenestrated endothelial cells leak IL-1 which becomes accessible from both the blood side and the brain side [11]. Efferent signals from the OVLT to the hypothalamus would then induce catecholamine release through the descending spinal pathway. In the present study, rIL-1β injected intrans-
cerebroventricularly, which might act on the interface of the OVLT from the brain side, did not cause an increase in plasma catecholamines. Therefore, it is possible that the secretion of catecholamines from the adrenal medulla in rabbits is not caused by a central action of IL-1 acting via the descending spinal pathway.

We must also account for the possibility that the release of catecholamines during fever is triggered by afferent inputs from peripheral receptors, e.g. baroreceptors. It has been shown that systemic injection of IL-1 causes an increase in blood pressure and heart rate in rats [17]. However, IL-1β-induced cardiovascular changes are unlikely to induce catecholamine releases on the following grounds. A small dose of rIL-1 induces monophasic increases in blood pressure and heart rate during monophasic fever [17], which are smaller than those during the second phase of biphasic fever, but there was no increase in catecholamines even during monophasic fever in the present study. Another possibility is that the rise in body temperature itself brings about the increase in plasma catecholamines. However, in the present study, microinjection of PGE₂ into the PO/AH induced a rise in body temperature but this did not result in an increase in plasma catecholamines. Thus, it seems that elevation of body temperature does not directly lead to an increase in plasma catecholamines in the rabbit.

Since INDO is known to be a potent inhibitor of PG synthesis [18,19], the present results support the hypothesis that LPS and IL-1β cause an increase in plasma catecholamines during fever through the peripheral action of PGs. Indeed, it has been shown that injection of PGE₂ into the lumboadrenal artery of anesthetized dogs induces the secretion of epinephrine and norepinephrine from the adrenal gland [20].

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