Role of prostaglandin D₂ in the hypothermia of rats caused by bacterial lipopolysaccharide

(Proc. Natl. Acad. Sci. USA Vol. 79, Neurobiology) Lipopolysaccharide from Salmonella typhimurium (1 mg/kg) caused a fall in the rat colonic temperature of about 2°C at an ambient temperature of 22 ± 3°C. The hypothermia induced by the lipopolysaccharide was abated in a dose-dependent manner by the administration of indomethacin. Other inhibitors of prostaglandin synthetase such as aspirin, flufenamic acid, and phenylbutazone had effects similar to those of indomethacin. When various prostaglandins were injected intracerebroventricularly, only prostaglandin D₂ caused a dose-dependent fall in the colonic temperature at doses between 1.2 and 6 nmol/kg. Microinjection of prostaglandin D₂ into the preoptic area caused hypothermia of about 1°C. However, injection of prostaglandin D₂ into the posterior hypothalamus had little effect on the colonic temperature. The hypothermia caused by prostaglandin D₂ was not abated by the administration of indomethacin. The amount of prostaglandin D₂ increased significantly in the preoptic/hypothalamic region of rat brain 1 hr after the intraperitoneal administration of the lipopolysaccharide, whereas such increase was not observed in rats pretreated with indomethacin. The in vitro incubation of the preoptic/hypothalamic slices with the lipopolysaccharide also increased the amount of prostaglandin D₂. These results suggest that the intraperitoneal administration of the lipopolysaccharide induces the release of prostaglandin D₂ in the preoptic/hypothalamic area of rat brain and that the latter compound is involved in the hypothalamic response of rats to the lipopolysaccharide.

Since Milton and Wendlandt found that prostaglandin (PG) E₁ is a potent pyrogenic agent in cats in 1970 (1) and Vane's discovery that antiinflammatory and antipyretic drugs inhibit PG synthesis (2), the rise in body temperature caused by pyrogens has been considered to be mediated by the formation of PG(s) in the brain (3–8). The E series PGs have been identified in the brain as well as in the cerebrospinal fluid and suggested as a mediator of hyperthermia by Milton and co-workers (5–8), although Cranston et al. reported results contradicting this hypothesis (9–11). Bacterial endotoxin [lipopolysaccharide (LPS)] is a pyrogen that causes fever in a variety of mammals. However, in rodents, such as rats, guinea pigs, and mice, it causes hypothermia instead of hyperthermia (12–17). Recently the PG profile in the brain of these animals was clarified and PGD₂ was found to be the major PG (18–20). The synthesis and degradation of PGD₂ in the brain have been studied extensively in our laboratory (20, 21). In this communication, we demonstrate that indomethacin and other inhibitors of PG synthesis abate the hypothermia of rats induced by the intraperitoneal administration of LPS from Salmonella typhimurium and that PGD₂ in the brain is at least in part responsible for the hypothermia.

ABSTRACT

MATERIALS AND METHODS

Intraventricular Injection of PGs. Male Wistar rats weighing 350–420 g were anesthetized with intraperitoneal injection of pentobarbital sodium (35 mg/kg). The rats were mounted on a stereotaxic instrument (Takashahi, Tokyo) with the head fixed according to the Pellegrino and Cushman coordinate system (22). For the injection into the third ventricle, a stainless steel guide tube (0.9-mm outer diameter) was implanted in the brain 6 mm anterior (A) on the midline (L) and at the depth of 1 mm (H) from the stereotaxic zero point. For the injection into the lateral ventricle, a guide tube was placed at a position of A, 5.8 mm; L, 2.0 mm; H, 4.0 mm in the left brain. The tubes were fixed by using dental cement. In both cases, the injection cannulas (0.4-mm outer diameter) were adjusted to protrude 2.0 mm beyond the guide tubes. The animals were permitted to recover from surgery for at least 5 days before experiments and were randomly used twice, with an interval of at least 7 days. The colonic temperature was measured every 5 min with thermistor probes (Takara, Yokohama, Japan) introducing 6 cm into the rectum of rats placed in a box (9 × 20 × 9 cm) at an ambient temperature of 22 ± 3°C. After the colonic temperature was stabilized (1–2 hr), PGs were injected at the rate of 3 μl/min around 1000–1100. The volume of injections was 3 μl and 10 μl for the administration into the third and the lateral ventricles, respectively. After the experiments, thionine dye (0.4%) was injected and the site of injection was examined postmortem.

Microinjection of PGs. Male Wistar rats weighing 350–420 g were used. While the animals were under pentobarbital anesthesia (50 mg/kg), a guide tube was implanted into the preoptic area (A, 7.4 mm; L, 1.0 mm; H, −0.5 mm) or into the posterior hypothalamus (A, 4.8 mm; L, 1.5 mm; H, −1.0 mm) of the left half of the brain. The injection cannula was adjusted to protrude 1.0 mm beyond the guide cannula. A week after the surgery, the rat was removed from its home cage at 1000 and placed on an unsteady small platform 1.2 m high (23). The device was set in a soundproof and electrically sealed room maintained at 25 ± 2°C. Tail skin temperature at 10 cm from the base and colonic temperature at 7 cm from the anus were measured every 1 min by means of thermocouples and a recording potentiometer (Okura, Tokyo). After the rat became accustomed to the device and colonic temperature was stabilized (about 40 min), the indwelling stylet occluding the guide tube was removed and replaced with an injection cannula. The volume of injection was 3 μl and the sample was injected at the rate of 3 μl/min. Animals used once were not used again. At the termination of the series of experiments, 3 μl of 0.5% pontamine sky blue was

Abbreviations: PG, prostaglandin; LPS, lipopolysaccharide; ME medium, minimal essential medium.
been stabilized for measured (0).

Injection of LPS and Antiinflammatory Drugs. The LPS from *S. typhimurium* was administered intraperitoneally. Indomethacin was dissolved in 0.3 M Tris-HCl buffer, pH 8 (2 mg/ml), and administered intraperitoneally. Aspirin, flufenamic acid, and phenylbutazone were dissolved in dimethyl sulfoxide (150 μl) and injected intraperitoneally. The administration of Tris buffer (1 ml) or dimethyl sulfoxide (150 μl) alone did not change the colonic temperature significantly.

Quantification of PGD<sub>2</sub> in the Preoptic/Hypothalamic Region of Rat Brain. Male Wistar rats (200–250 g) were sacrificed by microwave radiation (4.5 kW, 1.2 sec). The brains were carefully removed and chilled. The preoptic/hypothalamic region was dissected according to the method of Glowinski and Iversen (24) with the modification that the anterior limit of the region was made by transverse section at the level of the anterior margin of the olfactory tuberculum. The mean wet weight for 1 preoptic/hypothalamic region was 135.1 ± 2.6 mg (mean ± SEM, n = 32). The preoptic/hypothalamic regions obtained from eight rats were collected as one pool and the amount of PGD<sub>2</sub> in a pool was determined as one sample by a radioimmunoassay specific for PGD<sub>2</sub> (25).

Incubation of Preoptic/Hypothalamic Slices. Male Wistar rats (200–250 g) were anesthetized by intraperitoneal injection of pentobarbital (35 mg/kg), perfused with 200 ml of ice-cold sterile saline, and decapitated. The preoptic/hypothalamic regions were quickly removed as described above and the slices of about 1 × 1 × 2.5 mm were prepared with a razor. The slices (0.4 g wet weight) were placed in Teflon tubes (2-cm outer diameter) the bottoms of which were covered with nylon mesh (0.2 × 0.2 mm). The Teflon tubes were placed in test tubes (3.5-cm outer diameter) containing 15 ml of sterile minimal essential medium (ME medium) at pH 7.2 under 5% CO<sub>2</sub>/95% O<sub>2</sub>. After incubation of the slices for 5 min at 37°C, the sample medium was changed to ME medium containing LPS from *S. typhimurium* at 10 μg/ml. The medium of the control slices was changed to fresh ME medium. The amounts of PGD<sub>2</sub> released into the media were determined by the radioimmunoassay. No materials in ME medium and LPS crossreacted with the anti-PGD<sub>2</sub> antiserum used.

Chemicals. PGs were gifts from Ono Central Research Institute (Osaka, Japan). PGE<sub>1</sub>, PGE<sub>2</sub>, and PGD<sub>2</sub>, stored at −20°C, were weighed and dissolved in sterile saline 30 min before the injection. PGD<sub>2</sub> (sodium salt), stored at −80°C, was weighed and dissolved in sterile saline adjusted to pH 9 with NaOH immediately before use. Indomethacin, aspirin, flufenamic acid, phenylbutazone, and LPS from *S. typhimurium* were obtained from Sigma. ME medium was purchased from Nissui Seiyaku (Tokyo).

**RESULTS**

Effect of Indomethacin on the Hypothermia of Rats Induced by the Intraperitoneal Administration of LPS. The LPS from *S. typhimurium* was administered intraperitoneally to rats and the change in the colonic temperature was monitored (Fig. 1). The colonic temperature began to fall 1 hr after injection, reached the maximal change (−2°C) around 2 hr after injection, and returned to the initial level within 4 hr. Rats appeared to be sedated during this hypothermic period. The intraperitoneal injection of indomethacin 30 min prior to the LPS injection abated this hypothermia in a dose-dependent manner; in the rats pretreated with indomethacin at 2 mg/kg both the extent and duration of hypothermia decreased significantly, and the pretreatment with indomethacin at 5 mg/kg almost completely blocked the hypothermic effect of LPS (Fig. 1A). In addition, the injection of indomethacin 90 min after the LPS administration interrupted the temperature fall and the colonic temperature began to rise immediately after the treatment with this drug (Fig. 1B). The injection of indomethacin alone did not affect the body temperature significantly. The pretreatment with other nonsteroidal antiinflammatory drugs such as aspirin, phenylbutazone, and flufenamic acid showed the similar inhibitory effect on the fall in the colonic temperature caused by LPS. The fall in the colonic temperature was inhibited by about 50% with doses of 100 mg/kg, 20 mg/kg, and 20 mg/kg of aspirin, phenylbutazone, and flufenamic acid, respectively. However, because these drugs alone caused hypothermia in rats with doses above the indicated amounts, complete inhibition was not attained.

**Intraventricular Administration of Various PGs into Conscious Rats.** Various PGs were injected into the third ventricle of conscious rats and the change in the colonic temperature was measured (Table 1). Injection of PGE<sub>1</sub>, PGE<sub>2</sub>, or PGF<sub>2α</sub> 6 mmol/kg increased the colonic temperature by about 2°C, and the

**Fig. 1.** Effects of indomethacin on hypothermia of rats induced by injection of LPS from *S. typhimurium*. After the colonic temperature had been stabilized for 2 hr, LPS (1 mg/kg) was injected intraperitoneally at an ambient temperature of 22 ± 3°C, and the colonic temperature was measured (○). (A) Pretreated with indomethacin at 2 (●) and 5 mg/kg (▲) 30 min before the injection of LPS. Each point represents the mean ± SD of four observations. (B) Indomethacin (5 mg/kg) was administered intraperitoneally 90 min after the injection of LPS (arrow). The time course after the injection of indomethacin is shown by ●. Each point represents the mean ± SD of four observations.
maximal change was attained within 30 min. Injection of sterile saline or PGI₂ (6 nmol/kg) had no effect on the colonic temperature. On the contrary, PGD₂ at the same dose induced a gradual fall in the colonic temperature, and the maximal fall of about 0.8°C was reached around 1 hr after the injection. The time courses of the temperature falls induced by PGD₂ are shown in Fig. 2. The PGD₂-induced hypothermia began with a small and transient fall of about 30-min duration which was then followed by a major fall of about 2-hr duration. The extent of the temperature fall was dependent on the dose of PGD₂ administered; the falls of 0.4°C and 0.8°C were caused by the doses of 1.2 nmol/kg and 6.0 nmol/kg, respectively (Fig. 2). The doses of PGD₂ exceeding 6.0 nmol/kg showed no further effects on the colonic temperature. Injection of PGD₂ into the lateral ventricle induced similar changes in the colonic temperature in conscious rats. The dose dependency was observed with PGD₂ between 7 and 21 nmol/kg in these experiments, and the extent of the temperature change induced by the latter dose was about the same as that obtained by PGD₂ injected into the third ventricle at 6 nmol/kg. Pretreatment of animals with indomethacin did not affect the hypothermic effects of PGD₂.

Microinjection of PGs into Preoptic Area and Posterior Hypothalamus. In order to clarify the site and mode of action of PGD₂, we microinjected various PGs into the preoptic area as well as posterior hypothalamus and we measured the changes in the colonic and tail temperatures in conscious rats. As shown in Table 2, the injection of PGD₂ (6 nmol/kg) into the preoptic area caused a fall in the colonic temperature of about 1°C; there was an initial lag period of about 30 min and then a gradual fall in the colonic temperature was observed. The temperature reached its minimum at 2.5 hr after the injection with this dose and the hypothermia lasted for at least 3 hr after the injection. The injections of PGE₂ and PGF₂α into the preoptic area caused a rise in the colonic temperature as reported (26–28), and these responses were of short duration (Table 2). The injection of sterile saline alone into the same area induced a slight increase (0.5°C) in the colonic temperature. In contrast to the above results, PGD₂ injected into the posterior hypothalamus also decreased the colonic temperature, but the change was much smaller than that induced by the preoptic injection (Table 2), indicating that the preoptic area is the site of action of PGD₂ in regulating the body temperature.

At an ambient temperature of 25 ± 2°C, 18 out of 21 rats maintained their tail temperatures 4–7°C above room temperature. When PGD₂, PGE₂, or PGF₂α was injected into the preoptic area, a decrease in tail temperature almost to the level of room temperature was observed consistently for the initial 0.5 hr, indicating the occurrence of peripheral vasoconstriction. The duration of such vasoconstriction was 35.0 ± 7.7 min (mean ± SD) for PGE₂ (n = 6) and 21.6 ± 5.3 min for PGD₂ (n = 5). Although the peripheral vasoconstriction was usually associated with the rise in the colonic temperature as shown in the case of PGE₂, no rise in the colonic temperature was observed with the vasoconstriction caused by PGD₂. The injection of saline into the preoptic area or the injections of PGs into the posterior hypothalamus showed no vasoconstricting effects.

Effect of LPS on the PGD₂ Formation in Brain. To examine the effect of the intraperitoneal injection of LPS on the amount of PGD₂ in the preoptic/hypothalamic region of rat brain, we sacrificed LPS-treated and control rats by microwave radiation (4.5 kW, 1.2 sec). Under these conditions, both PGD₂ synthetase (20) and PGD₂ dehydrogenase (21) were completely inactivated. As shown in Table 3, the level of 1.43 ng of PGD₂ per g wet weight was observed in the preoptic/hypothalamic region of the

![Fig. 2](image-url) Changes in colonic temperature induced by injection of PGD₂ into the third ventricle of conscious rats. PGD₂ was injected at an ambient temperature of 22 ± 3°C. PGD₂ at 6 nmol/kg (○) or 1.2 nmol/kg (△) or sterile saline (□) was injected. Each point represents the mean ± SD of four observations. Significant differences (P < 0.05) between control and test injections were found for PGD₂ at 6 nmol/kg between 30 and 100 min and for PGD₂ at 1.2 nmol/kg between 40 and 70 min.

<table>
<thead>
<tr>
<th>PG</th>
<th>ΔTₘₐₓ, °C</th>
<th>Time required to reach ΔTₘₐₓ, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGD₂</td>
<td>-0.79 ± 0.20 (n = 6)</td>
<td>58 ± 12</td>
</tr>
<tr>
<td>PGE₁</td>
<td>+1.73 ± 0.12 (n = 3)</td>
<td>27 ± 4.7</td>
</tr>
<tr>
<td>PGE₂</td>
<td>+1.95 ± 0.10 (n = 3)</td>
<td>29 ± 4.1</td>
</tr>
<tr>
<td>PGF₂₀</td>
<td>+1.63 ± 0.12 (n = 3)</td>
<td>29 ± 3.1</td>
</tr>
<tr>
<td>PG₁</td>
<td>0 ± 0.2 (n = 3)</td>
<td>—</td>
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</tbody>
</table>

All PGs were injected at 6 nmol/kg. ΔTₘₐₓ represents the maximal change in the colonic temperature. Results are presented as mean ± SD.

Table 2. Microinjections of PGs into the preopti area and posterior hypothalamus of conscious rats

<table>
<thead>
<tr>
<th>PG</th>
<th>Injection site*</th>
<th>ΔTₘₐₓ, °C</th>
<th>Time required to reach ΔTₘₐₓ, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGD₂</td>
<td>PO</td>
<td>-0.96 ± 0.49 (n = 4)</td>
<td>120–240</td>
</tr>
<tr>
<td>PGD₂</td>
<td>PH</td>
<td>-0.18 ± 0.11 (n = 3)</td>
<td>30–90</td>
</tr>
<tr>
<td>PGE₂</td>
<td>PO</td>
<td>+2.40 ± 0.54 (n = 4)</td>
<td>15–25</td>
</tr>
<tr>
<td>PGE₂</td>
<td>PH</td>
<td>+1.10 ± 0.32 (n = 3)</td>
<td>30–45</td>
</tr>
<tr>
<td>PGF₂₀</td>
<td>PO</td>
<td>+1.35 ± 0.20 (n = 2)</td>
<td>25–30</td>
</tr>
<tr>
<td>Saline</td>
<td>PO</td>
<td>+0.53 ± 0.45 (n = 7)</td>
<td>30–80</td>
</tr>
<tr>
<td>Saline</td>
<td>PH</td>
<td>± 0.10 (n = 3)</td>
<td>—</td>
</tr>
</tbody>
</table>

*PO, preoptic area; PH, posterior hypothalamus.

Table 3. Increase in the amount of PGD₂ by LPS in the preoptic/hypothalamic region in vivo

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of samples</th>
<th>PGD₂, ng/g wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>1.43 ± 0.13</td>
</tr>
<tr>
<td>LPS (1 mg/kg)</td>
<td>8</td>
<td>2.32 ± 0.14*</td>
</tr>
<tr>
<td>Indomethacin, then LPS</td>
<td>4</td>
<td>1.47 ± 0.20</td>
</tr>
</tbody>
</table>

Rats were treated with LPS or sterile saline (control) 60 min prior to sacrifice by microwave radiation. Indomethacin was given intraperitoneally (2 mg/kg) 30 min before the injection of LPS. Each sample is composed of the preoptic/hypothalamic region from eight rats. Results are presented as mean ± SEM.

* Significant difference (P < 0.001) compared to control.
control rats, and this level increased significantly to 2.32 ng/g wet weight (P < 0.001) at the beginning of the fall of the colonic temperature—i.e., 1 hr after the administration of LPS at 1 mg/kg. The pretreatment with indomethacin blocked this increase in the amount of PGD_2 as well as abated the hypothermia caused by LPS as shown in Fig. 1. On the ascending phase of the colonic temperature (2.5 hr after the injection of LPS), however, the amount of PGD_2 decreased to 0.74 and 0.80 ng/g wet weight (n = 2), which was significantly lower than the control values. The effect of LPS on increasing the amount of PGD_2 was further confirmed by the in vitro incubation of the preoptic/hypothalamic slices (Fig. 3). The incubation with LPS was started after 5-min preincubation. The control and sample tissues released the same amount of PGD_2 during the preincubation. When LPS was added at 10 μg/ml, the amount of PGD_2 released to the incubation media increased by about 50% within 10 min in comparison to the control.

**DISCUSSION**

In the central nervous system of rats, PGD_2 is identified as the major PG among various PGs and thromboxanes (18–20). The biosynthesis and degradation of PGD_2 in the brain have been well studied (20, 21). The highest activity of PGD synthetase in rat brain is found in the hypothalamic region (29). Further, PGD_2 at 0.1 μM stimulates the adenylate cyclase (29) and depolarizes the membrane potential in cultured neuroblastoma cells (30). However, its physiological roles in the central nervous system remain unclear. In this study, we attempted to elucidate the possible role of PGD_2 during the hypothermia of rats caused by LPS.

Almost all species of mammals respond to the administration of endogenous pyrogens such as LPS as well as to endogenous pyrogens with an increase of body temperature, and PGs of the E series have been suggested as mediators of such febrile responses (4–8). However, hypothermia was observed in rodents such as rats, mice, and guinea pigs in response to the intraperitoneal administration of LPS (12–16). In addition, hypothermia was also observed in rats treated with pig endogenous pyrogen (17). Because the intraventricular or intracerebral injection of LPS causes fever in these species (31, 32), Feldberg and Saxena (33) postulated that endotoxin does not pass through their blood–brain barriers, and Spawinski et al. (34) hypothesized the presence of a detoxifying system for endotoxin in those species. However, either theory could not fully explain hypothermia caused by LPS injected intraperitoneally. In this study we confirmed that rats responded to the intraperitoneal administration of LPS from *S. typhimurium* with a fall in the colonic temperature, and we further found that several inhibitors of PG synthesis such as indomethacin and aspirin (2, 3) abated this fall in a dose-dependent manner. These results suggested the involvement of PG(s) in the hypothermic response in rats. When various PGs were injected intraventricularly, only PGD_2 elicited the hypothermia in rats. The microinjection study revealed that the site of action of this PG in brain was the preoptic area. Another candidate for the hypothermia-inducing agent was PGI_2, because Kandasamy et al. (20) reported that it produced hypothermia in guinea pigs (35). However, these authors injected about two orders of magnitude higher amounts of PGI_2 intraventricularly, and we found no response with the PGI_2 dose of 6 mmol/kg in this study. Thus, PGD_2, if formed in the brain in response to LPS, is likely to act as a hypothermic mediator. To corroborate our interpretation, we determined the amount of PGD_2 in the brain, particularly in the preoptic area, during the hypothermic response. The level of PGD_2 in this region increased significantly to 2.32 ng/g wet weight in comparison to the control level of 1.43 ng/g wet weight 1 hr after LPS injection, and pretreatment with indomethacin blocked this increase. Furthermore, the in vitro incubation of the preoptic/hypothalamic slices with LPS released significantly more PGD_2 to the incubation medium, suggesting the possibility that LPS could act directly on the selected area of brain in vivo to increase the formation of PGD_2. The hypothermia of rats caused by LPS may not be explained entirely by the action of PGD_2 formed in the brain. The contribution of the peripheral effects caused by LPS such as vasodilatation (34) should also be taken into account. However, our results suggest that the formation of PGD_2 in brain is at least in part responsible for the hypothermic response to LPS in the rats.

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