PGE₂ receptor subtype EP₁ antagonist may inhibit central interleukin-1β-induced fever in rats

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Oka, Kae, Takakazu Oka, and Tetsuro Hori. PGE₂ receptor subtype EP₁ antagonist may inhibit central interleukin-1β-induced fever in rats. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1762–R1765, 1998.—We have previously reported that central injection of PGE₂ induces hyperthermia through its actions on EP₁ receptors in rats. Because the increase in local synthesis of PGE₂ is assumed to be a necessary process in a fever caused by central injection of interleukin-1β (IL-1β), an EP₁ receptor antagonist (SC-19220) should inhibit the IL-1β-induced fever. To test this hypothesis, we observed the effect of SC-19220 on the fever produced by injection of recombinant human IL-1β (rhIL-1β) into the lateral cerebroventricle (LCV) in conscious rats. Administration of SC-19220 (100 µg) into the LCV 15 min before LCV injection of rhIL-1β (4 ng) suppressed an initial rise in colonic temperature for 30 min, producing a fever with a longer latency to onset and a longer time to peak elevation. SC-19220, given 60 min after the central administration of rhIL-1β, also suppressed the rhIL-1β-induced fever 15–60 min after its injection. These findings suggest that the central IL-1β-induced fever in rats is mediated, at least partly, by activation of EP₁ receptors by PGE₂.

prostaglandin E₂; SC-19220

INTERLEUKIN (IL)-1β is a principal component of endogenous pyrogens, which also include inflammatory cytokines such as IL-6, tumor necrosis factor-α, and interferon-α (see review in Ref. 9). Although it has been generally recognized that IL-1β released from immune cells peripherally produces fever by signaling the brain via various routes (2), there is evidence supporting the view that IL-1β in the brain is also responsible for a significant portion of fever during peripheral inflammation. IL-1β and its mRNA increase in brain tissues including the hypothalamus not only after various types of brain insults such as cerebral infarction and ischemia (7) but also after peripheral inflammation (20) and peripheral administration of endotoxin (19). Fever caused by peripheral injection of endotoxin is diminished by central injection of antiserum to IL-1β (8) and IL-1 receptor antagonist (11). Peripheral injection of both endogenous and exogenous pyrogens increases the concentration of PGE₂ in the brain (1, 5, 10), and the increase in PGE₂ is inhibited by a cyclooxygenase inhibitor (10). Furthermore, fever caused by peripheral injection of endogenous pyrogen is reduced by an intrahypothalamic injection of sodium salicylate (5). These findings, taken together, suggest that IL-1β and PGE₂ in the brain are responsible for at least part of the fever after peripheral infection and inflammation.

We have reported that hyperthermia caused by intracerebroventricular injection of rhIL-1β in rats is mediated through EP₁ receptors (15, 16). The PGE₂-induced hyperthermia is mimicked by an EP₁ receptor agonist, 17-phenyl-α-trinor-PGE₂, but neither by EP₂ nor EP₃ receptor agonist, and is inhibited by the simultaneous injection of an EP₁ receptor antagonist, SC-19220 (15, 16). Furthermore, the brain sites where PGE₂ and 17-phenyl-α-trinor-PGE₂ were microinjected to produce hyperthermia in rats are located in the preoptic hypothalamus and its neighboring basal forebrain including the anterior wall of the third ventricle (15), which correspond to those sites where IL-1β may produce fever in rabbits (13). These findings indicate the possibility that IL-1β in the brain induces fever by stimulating EP₁ receptors. In the present study, we investigated whether the fever following intracerebroventricular injection of recombinant human IL-1β (rhIL-1β) is inhibited by intracerebroventricular injection of SC-19220 in rats.

MATERIALS AND METHODS

Male Wistar rats (Kyudo, Tosu, Japan) weighing 270–300 g were housed two or three per cage at an ambient temperature of 23 ± 1°C on a 12:12-h light-dark cycle with lights on at 0800. Food and water were given ad libitum. Under anesthesia with pentobarbital sodium (50 mg/kg ip), rats were stereotaxically implanted with a 23-gauge stainless steel cannula containing 30-gauge stainless steel wire as a stylet in the lateral cerebroventricle (LCV). The coordinates were anterior, 0.8 mm posterior to the bregma; lateral, 1.5 mm from the midline; depth, 4.0 mm from the surface of the skull. The correct placement of the cannula in the LCV was confirmed by the rise of cerebrospinal fluid in the cannula. The cannula was then fixed to the skull with acrylic dental cement. After surgery, the rats were administered sulfamethoxazole (100 mg/rat ip), returned to the colony, and housed individually. During a postsurgical recovery period, the animals were placed in a cylindrical wire cage for a few hours every day to habituate them to the experimental environment.

Drugs. rhIL-1β and SC-19220 were generous gifts: rhIL-1β (Lot No. 9K77) from Drs. Y. Masui and Y. Hirai (Institute of Cellular Technology, Otsuka Pharmaceutical, Tokushima, Japan) and SC-19220 from Dr. R. A. Marks (Searle). rhIL-1β was dissolved in physiological saline and stored at −80°C and diluted with saline before use. SC-19220 was dissolved in DMSO before use, making 100 mg/3 ml of solution. All solutions were passed through a 0.22-mm Millipore filter (Millipore, Tokyo, Japan) before injection. All glassware, syringes, and injection needles were autoclaved before use.

Experimental procedures. Experiments were performed at least 7 days after surgery. On the experimental day, each rat was loosely restrained in the cylindrical wire cage at 0900.
The colonic temperature ($T_{co}$) of each animal was monitored automatically at 1-min intervals by a copper-constantan thermocouple inserted into the colon 4 cm beyond the anus. The temperature was allowed to stabilize for at least 2 h. To avoid the possibility that stress elicited by these procedures might affect the properties of $T_{co}$ responses to injected drugs, all the experiments started after $T_{co}$ had stabilized at normothermia and had shown no further changes for more than 20 min.

Then, the stylet was removed from the guide cannula and a 30-gauge injector cannula, connected to a 10-ml microsyringe, was inserted into the guide so that it protruded 0.5 mm beyond its tip. Drugs or the same volume of vehicles (3 or 5 µl) was delivered into the LCV over 60 s/µl. At the end of the injection, the injector cannula was kept in position for 30 s and then replaced by the stylet. There was no back flow of the fluid in this procedure.

In the experiments, SC-19220 (100 µg/3 µl) or the vehicle, DMSO (3 µl), was injected into the LCV 15 min before or 60 min after the intracerebroventricular injection of either rhIL-1β (4 ng/5 µl) or physiological saline (5 µl). The time when injection of rhIL-1β or saline was started was designated as time 0, and changes in $T_{co}$ were observed during the subsequent 4 h. All experiments were performed at a room temperature of 23 ± 1°C. Each rat was used for two experiments on different days at least 7 days apart.

Data analysis. The values are presented as means ± SE. Significant differences were assessed by one-way ANOVA followed by Scheffé’s test. Differences were considered to be significant at $P < 0.05$.

RESULTS

In the first series of experiment, we injected SC-19220 (100 µg/3 µl) or the same volume of DMSO into the LCV 15 min before or 60 min after the intracerebroventricular injection of either rhIL-1β (4 ng/5 µl) or physiological saline (5 µl). The mean $T_{co}$ at time zero of each group ranged from 37.87 ± 0.03 to 38.24 ± 0.09°C and did not differ significantly from each other. The rats treated with DMSO followed by rhIL-1β injection showed a rise in $T_{co}$ that became apparent 30 min after injection, reached a peak (1.06 ± 0.05°C) at 120 min, and then remained at this level until the end of the observation period (240 min) (Fig. 1). An administration of SC-19220 15 min before rhIL-1β injection suppressed an initial rise in $T_{co}$ for 30 min, producing a fever of similar magnitude (1.06 ± 0.15°C), but with slower onset (60 min) and longer time to reach a peak (165 min). Furthermore, pretreatment with SC-19220 significantly attenuated the rhIL-1β-induced rise in $T_{co}$ 75–90 min after rhIL-1β injection. No changes in $T_{co}$ occurred in the SC-19220 + saline-treated rats and the DMSO + saline-treated rats.

In the second series of experiment, we injected SC-19220 (100 µg/3 µl) or DMSO into the LCV 60 min after intracerebroventricular injection of either rhIL-1β (4 ng/5 µl) or saline. The mean $T_{co}$ at time zero of each group ranged from 37.91 ± 0.17 to 38.29 ± 0.08°C and did not differ significantly from each other. The rats that received rhIL-1β followed by injection of DMSO 60 min later produced fever with similar time courses and magnitude (Fig. 2) as that of rats that received DMSO followed by rhIL-1β (Fig. 1). The similar rise in $T_{co}$ in rhIL-1β + SC-19220-treated rats was significantly inhibited by SC-19220, when given 60 min after rhIL-1β injection, from 75 to 120 min (Fig. 2). The antipyretic activity of SC-19220 was maximal 30 min after its injection. Thirty minutes thereafter, $T_{co}$ returned to the level at the time of SC-19220 injection and then continued to rise maximally to a level that was similar to that of rhIL-1β + DMSO-treated rats. There was no change in $T_{co}$ in the saline + SC-19220-treated rats and the saline + DMSO-treated rats during the observation periods.

DISCUSSION

The present study demonstrates that SC-19220, a specific EP1 receptor antagonist (18), may inhibit fever...
induced by an intracerebroventricular injection of rhIL-1β in the rat. IL-1β is known to specifically increase the release of PGE2 from the hypothalamic explants (14). We have reported that the hyperthermia induced by the central injections of PGE2 is blocked by explants (14). We have reported that the hyperthermia is mediated, at least partly, through activation of EP1 receptors by PGE2 in the rat.

The present finding does not conform well with that of a previous study (3), which demonstrated the failure of SC-19220 (intracerebroventricularly) to affect the leukocyte pyrogen (intracerebroventricular)-induced fever in rabbits despite its ability to suppress the PGE2-induced hyperthermia. Although this discrepancy might be due to the difference of animal species, one may discuss the different protocols of two experiments as the possible causes, i.e., the timing of administration of SC-19220 in rats in the present study and ours is unlikely to be the cause of different results. Because an administration of SC-19220 either before or after injection of rhIL-1β suppressed the fever in rats in the present study, SC-19220 given immediately after injection of leukocyte pyrogen could have affected the fever.

The amount of SC-19220 (15 μmol) administered into the LCV in the previous study (3) is much greater than the amount given (~330 nmol) in our study, although the doses per body weight are similar in both experiments. Because of a low water solubility of SC-19220, it was dissolved in DMSO in both experiments. However, the antagonist dissolved in DMSO, when it is injected into the LCV, is likely to precipitate more or less out of solution (3). If the formation of precipitate of SC-19220 was dissolved in DMSO in both experiments. However, the central injection of the antagonist dissolved in DMSO, when it is injected into the LCV, is likely to precipitate more or less out of solution (3). If the formation of precipitate of SC-19220 was dissolved in DMSO in both experiments. However, the central injection of the antagonist dissolved in DMSO, when it is injected into the LCV, is likely to precipitate more or less out of solution. Because an administration of SC-19220 either before or after injection of SC-19220 suppressed the fever in rats in the present study, SC-19220 given immediately after injection of leukocyte pyrogen could have affected the fever.

Finally, the possible involvement of cytokines other than IL-1β and/or PGs other than PGE2 in the leukocyte pyrogen-induced fever deserves attention as a cause for the failure of PGE2 antagonists to affect it. Although the leukocyte pyrogen presumably contains IL-1β, other cytokines such as macrophage inflammatory protein-1 and IL-8, which are suggested to produce fever independently of the synthesis of prostanoids (12, 21), might contribute more to the development of fever by the leukocyte pyrogen. The involvement of other prostanoids such as PGF2α or PGD2, which were reported to exhibit hyperthermic actions in rabbits (13), might also be considered in the leukocyte pyrogen-induced fever in rabbits. In rats, however, PGD2 produces hyperthermia (17), and PGF2α is less potent in inducing hyperthermia than PGE2 (6).

Although some findings support the view that IL-1β is produced in the brain during the systemic inflammation (8, 11, 19, 20), it is not completely known how much concentration of IL-1β is induced and to what extent such centrally induced IL-1β contributes to the fever during the peripheral inflammation. In this regard, the experimental design (LCV injection of IL-1β) in the present study does not seem to be appropriate for determining what type(s) of EP receptors in the brain mediate the fever during systemic inflammation. However, the present result suggests the involvement of EP1 receptors in the central IL-1β-induced fever that is associated with various types of brain insults such as meningitis, brain injury, and cerebral ischemia (7).

This experiment was reviewed by the Committee of Ethics on Animal Experiments in the Faculty of Medicine, Kyushu University and was carried out under the control of the Guidelines for Animal Experiments of the Faculty of Medicine, Kyushu University, and the law (no. 105) and Notification (no. 6) of the government.

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