Pharmacology Biochemistry and Behavior, Vol. 46, pp. 383–389, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Iloprost, a Stable Analogue of PGI_2, Potentiates the Hyperthermic Effect of PGE_2 in Rats

E. S. AKARSU AND I. H. AYHAN[1]

Department of Pharmacology, Faculty of Medicine, University of Ankara, Sihhiye 06339, Ankara, Turkey

Received 13 March 1992

AKARSU, E. S. AND I. H. AYHAN. *Iloprost, a stable analogue of PGI_2, potentiates the hyperthermic effect of PGE_2 in rats.* PHARMACOL BIOCHEM BEHAV 46(2) 383–389, 1993.—Centrally mediated effects of iloprost, a stable analogue of PGI_2, on rectal temperature have been investigated in conscious rats. ICV administration of iloprost (100–1,000 ng, ICV) produced a dose-dependent, monophasic hyperthermic response that was not inhibited by indomethacin. When injected into the preoptic anterior hypothalamic (POAH) region, iloprost (2–50 ng/POAH) induced a biphasic increase in rectal temperature. While the first phase was inhibited by AH 6809, an E_1-type prostaglandin (EP_1) receptor antagonist, the second phase was abolished by indomethacin pretreatment. Iloprost was found not to alter rectal temperature when injected into the ventromedial hypothalamic area. Administration of iloprost into the POAH in a dose that had no effect on rectal temperature significantly potentiated the hyperthermic effect of PGE_2 (50 ng, ICV). These findings suggest that the pyrogenic effect of iloprost is partly mediated by EP_1 receptors located on the POAH. Regarding the similarities of iloprost and PGI_2, it is further proposed that endogenous PGI_2 might act to modulate hyperthermic effects of PGE_2 released during arachidonic acid- or endogenous pyrogen-induced fever.

Iloprost PGI_2 Hyperthermia PGE_2 AH 6809 Rat

PROSTACYCLIN (PGI_2) is an unstable cyclooxygenase metabolite of arachidonic acid, being produced from prostaglandin (PG) endoperoxides primarily in the arterial wall (20). Recently, it was shown that PGI_2 is also synthesized in the CNS by both cerebral blood vessels and brain tissue (1). While the modulatory role of PGI_2 on various physiological processes such as cerebrovascular homeostasis and central cardiovascular regulation have been extensively discussed (12,23), little is known about the centrally mediated effects on body temperature.

It has been reported that ICV administration of PGI_2 produces hyperthermia in cats, rabbits, and anaesthetised rats (5,16,28) but hypothermia in guinea pigs and conscious rats (15,17). Due to the short biological half-life of PGI_2, being 3 min at physiological pH and temperature, in these studies relatively higher doses have been used that resulted in other pharmacological effects such as hypotension (17). This makes it difficult to evaluate the pure pyrogenic potency of the substance.

Iloprost (ZK 36374) is a new and stable analogue of PGI_2 that displays actions similar to the parent compound (24). It has been suggested that iloprost is a suitable tool for the investigation of various PGI_2-like activities in experimental conditions (25). Recently, it has been reported that intracerebral administration of iloprost produces anticonvulsant effect in rats (2) and hyperthermia in cats (13).

The following study is an attempt to investigate the intracerebrally mediated effects of iloprost on rectal temperature by administration into specific brain regions and evaluate the mechanisms of action in conscious rats.

METHOD

Male Wistar rats weighing 250–270 g were housed prior to experiments for 1 week in a laboratory environment with an ambient light (0700–1900 h)–dark (1900–0700 h) cycle at a temperature of 21 ± 2°C. Food and water were available ad lib.

One week before the experiments, rats, divided into four groups, were anaesthetised with sodium pentobarbital (50 mg/kg, IP) and 23-ga stainless steel cannulae were implanted stereotaxically (Stoelting) into various parts of the brain according to the coordinates (22) indicated in parentheses: a) right lateral cerebral ventricle (B, −0.7 mm; L, 1.5 mm; H, 4 mm); b) right preoptic anterior hypothalamus (POAH) (B, −1.6 mm; L, 0.7 mm; H, 9.0 mm); c) right ventromedial hypothala-

[1] To whom requests for reprints should be addressed.

mus (VMH) (B, −2.4 mm; L, 1.0 mm; H, 9.5 mm); d) both the right lateral cerebral ventricle and the right POAH were cannulated. Dental acrylic was used to secure the cannulae.

Thirty-gauge needles attached to a microsyringe (Hamilton Co., Reno, NV) by a polyethylene tubing were used for injection through the cannulae. The volume of injection was 5 μl for ICV and 1 μl for IC injections.

Rectal temperature was measured by a electrically operated thermometer (Ellab, Denmark), the probe being inserted for 4 cm into the rectum. Four control rectal temperature values were taken within the 90 min before administration of the drugs. Rectal temperature changes were observed over 300 min following drug injections and expressed as a difference from the mean of the control values. All the experiments were carried out at an ambient temperature 21 ± 2°C.

Iloprost (Schering AG, Berlin) was dissolved in apyrogenic distilled water and injected ICV or IC. Indomethacin (Merck, Darmstadt, Germany) was dissolved in 1 M Tris buffer solution (pH 8.4 at 25°C), diluted with 50 nM Trus buffer solution (pH 7.5 at 25°C), and injected SC 30 min before iloprost challenge. AH 6809, an EP_1 receptor antagonist (Glaxo Laboratories, Middlesex, UK), was dissolved in 0.9% apyrogenic saline + 1 % $NaHCO_3$ mixture and injected ICV 20 min before or 90 min after IC iloprost injections. PGE_2 (Sigma Chemical Co., St. Louis, MO) was dissolved in apyrogenic distilled water and injected ICV after 20 min of iloprost administration.

After histological verification of the injection sites, data were analyzed using the one-way analysis of variance followed by Bonferroni's test for multiple comparisons as appropriate and Student's t-test. A significance of $p < 0.05$ was considered significant (30).

RESULTS

ICV injection of iloprost (100–1,000 ng, ICV) resulted in a mild, dose-dependent increase in rectal temperature (Fig. 1). The effect was maximal at 30 min after iloprost injection and subsided after 60–90 min. The response seemed to be biphasic at a dose of 1,000 ng ICV but temperature values for 90–300 min were not significantly different from the control. ICV injection of iloprost in the same dose range was ineffective on the systemic blood pressure (data not shown).

Relatively smaller doses of iloprost injections into the POAH (2–50 ng/POAH) elicited a biphasic increase in rectal temperature (Fig. 2). The first phase was dose dependent and significant in the first 90 min. During this time period, the time-effect profile and maximum response were not different than those obtained with ICV administration. The second phase became significant at 180 min, reached the maximum at 240 min, and subsided 300 min in a dose-dependent fashion. Iloprost injections (2–100 ng/VMH) into the VMH did not cause any significant change in the temperature (Fig. 2). Unless otherwise mentioned, the maximal dose of iloprost (50 ng/POAH) was used as a test dose throughout the experiments.

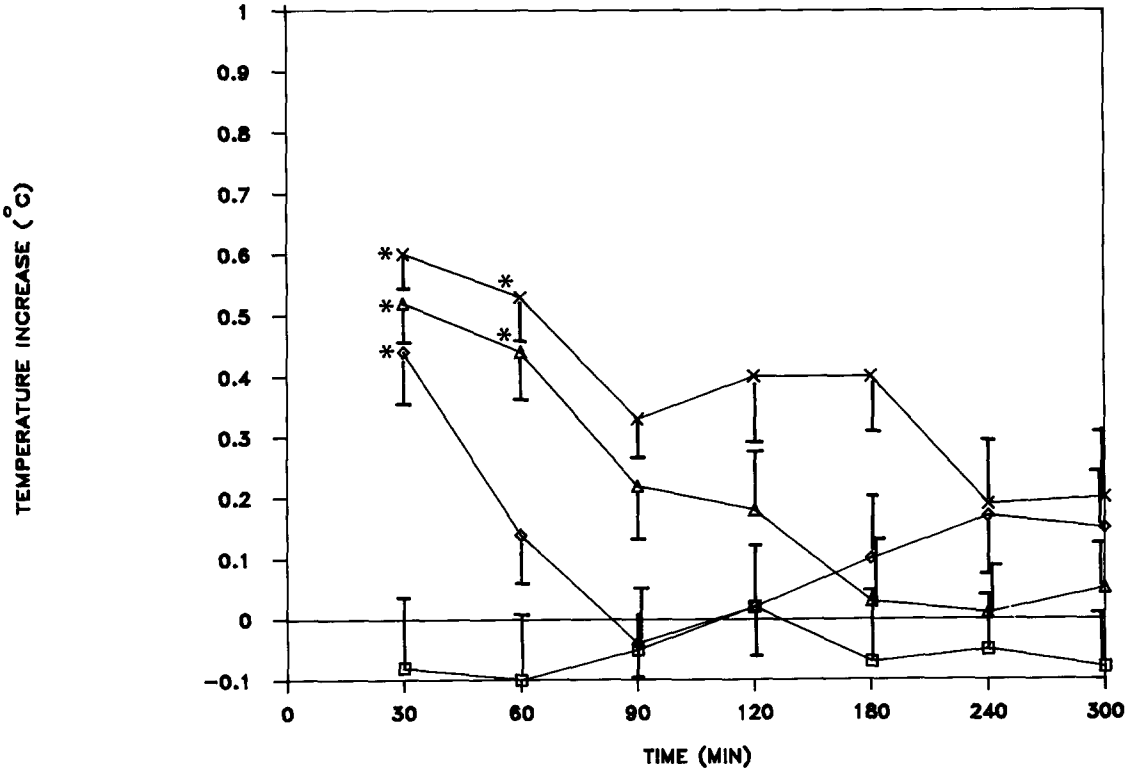

FIG. 1. Changes in rectal temperature induced by ICV injection of iloprost. (□), saline; (◇), 100 ng ICV; (△), 500 ng ICV; (X), 1,000 ng ICV. Each point represents the mean ± SE of seven observations. Injection at time 0. *$p < 0.05$ compared with saline.

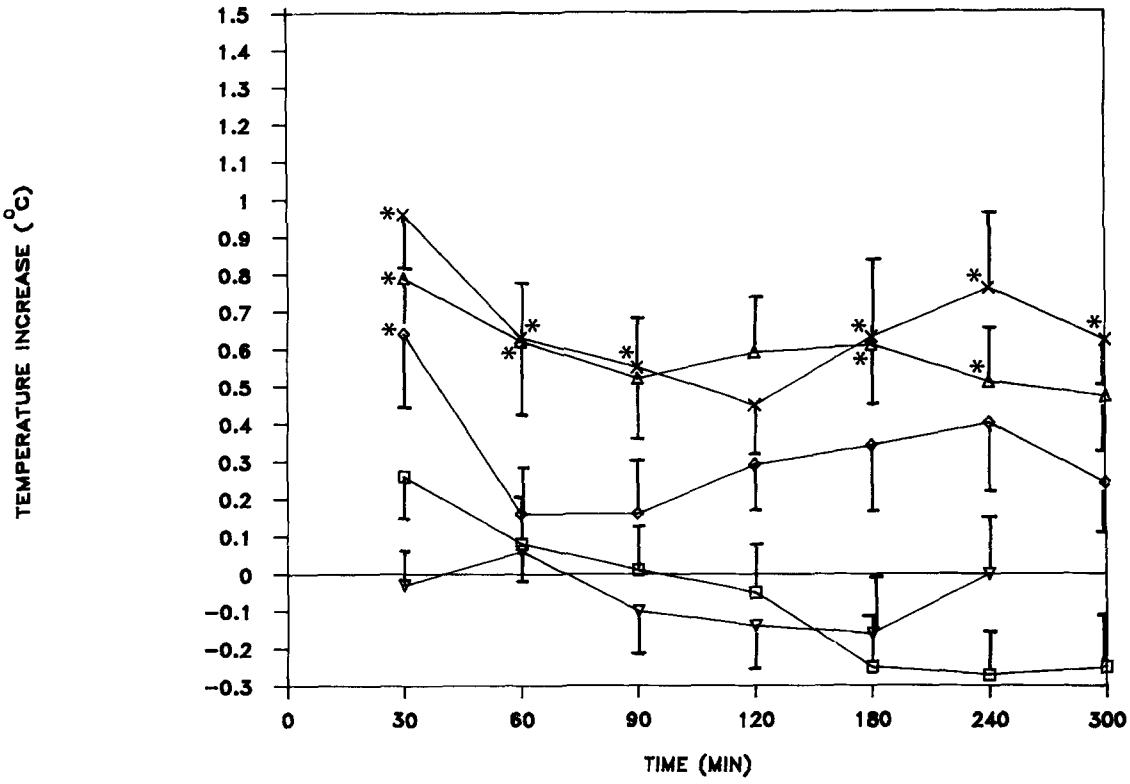

FIG. 2. Effects of iloprost on rectal temperature after injection into various hypothalamic regions. (□), saline/preoptic anterior hypothalamic area (POAH); (◇), 2 ng/POAH; (△), 10 ng/POAH; (X), 50 ng/POAH; (▽), 100 ng/ventromedial hypothalamus (VMH). Each point represents the mean ± SE of nine observations. Injection at time 0. *$p < 0.05$ compared with saline.

In control animals, indomethacin (5 mg/kg, SC) did not alter body temperature (Fig. 3). Iloprost-induced hyperthermia following ICV injection was not changed by indomethacin pretreatment (the temperature was 0.59 ± 0.09°C after 30 min of 500 ng/ICV dose; data not shown). Indomethacin was also ineffective on the first phase of the hyperthermia obtained by intrahypothalamic injection of iloprost but suppressed the second phase of the response (Fig. 3).

ICV administration of AH 6809, an EP_1 receptor antagonist, caused a hyperthermic response. But, the dose of 15 ng ICV was found to be ineffective on rectal temperature (Fig. 4). While the first phase of the hyperthermic response due to intrahypothalamic administration of iloprost was completely abolished by AH 6809 pretreatment (15 ng, ICV) injected 20 min before iloprost injection, the second phase remained unchanged. The same dose of AH 6809 administered 90 min after injection of iloprost significantly potentiated the second phase of the response (Fig. 4).

PGE_2 injection (50 ng, ICV) produced a mild hyperthermic response that was significant at 30 min and subsided after 60 min (Fig. 5). The hyperthermic effect of PGE_2 was significantly potentiated by iloprost pretreatment in a dose (0.5 ng/POAH) that was ineffective alone on rectal temperature when injected into the POAH. Both the magnitude and duration of the response to PGE_2 were potentiated (Fig. 5).

The PGE_2-induced hyperthermic response was also augmented by AH 6809 pretreatment (15 ng, ICV; Fig. 5). Thus, the maximal increase in temperature was significantly high and the effect prolonged up to 240 min.

DISCUSSION

It has been reported that ICV injection of arachidonic acid (AA) produced a hyperthermic response that was completely antagonized by indomethacin, a cyclooxygenase enzyme inhibitor, in rats, cats, and rabbits. Because indomethacin is known to be a potent antipyretic drug used for symptomatic treatment of certain inflammatory processes, this observation has been taken as evidence supporting a possible mediator role of cyclooxygenase metabolites for AA-induced fever (8,9).

PGE_2, being a cyclooxygenase metabolite of AA, has been widely accepted as a mediator of fever because of the findings that injections of PGE_2 into such thermoregulatory centers as the POAH or VMH caused a potent hyperthermic response that could not be blocked by indomethacin in various animal species, including rats, rabbits, and cats (6,7). Moreover, it has been demonstrated that the PGE_2 level is raised in cerebrospinal fluid or in the POAH during endogenous pyrogen-induced fever (8,27).

It is possible that some cyclooxygenase metabolites other than PGE_2 may also be involved in AA-induced fever because SC 19220, an E_1-type PG receptor (EP_1) antagonist, only partially inhibited the hyperthermic effect of PGE_2. But,

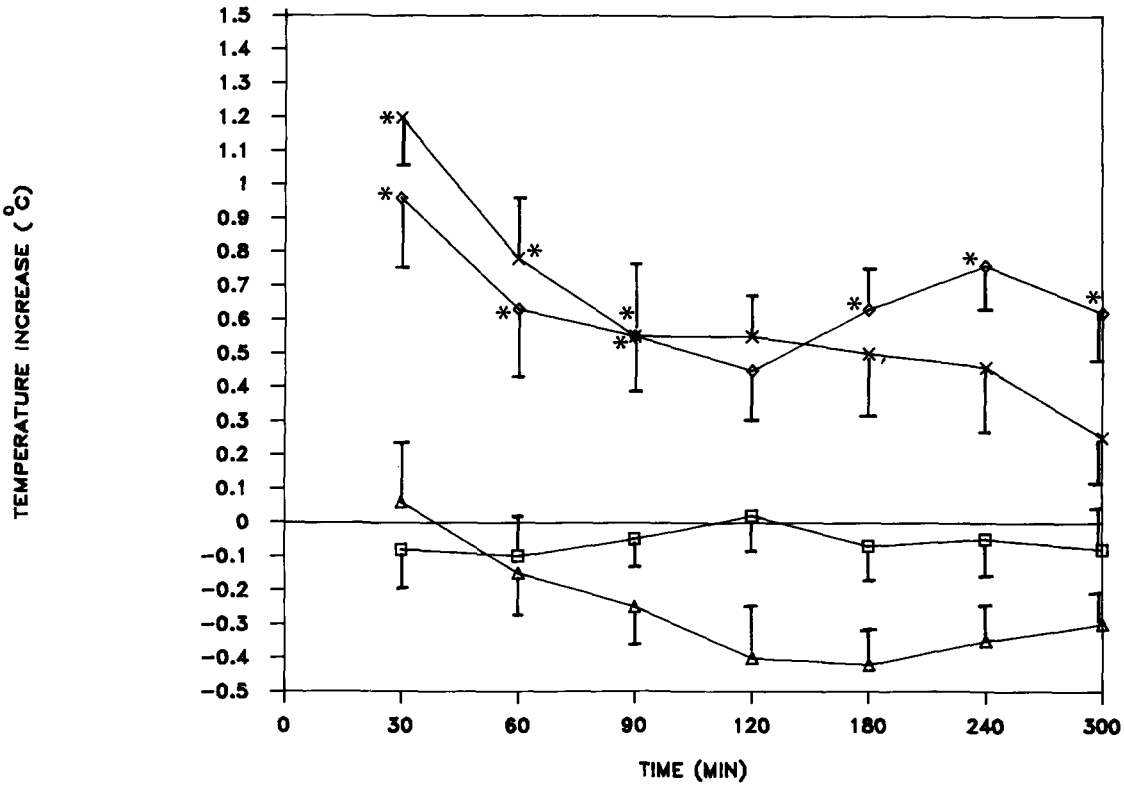

FIG. 3. Effects of indomethacin pretreatment on hyperthermia induced by intrahypothalamic injections of iloprost. (□), saline ICV; (△), indomethacin SC + saline ICV; (X), iloprost 50 ng/preoptic anterior hypothalamic area (POAH); (◇), indomethacin SC + iloprost 50 ng/POAH. Each point represents the mean ± SE of seven observations except (X), which represents nine observations. Iloprost injection at time 0. *$p < 0.05$ compared with saline.

pyrogenic potency of other endogenous prostanoids such as TxA_2, $PGF_{2\alpha}$, and PGD_2 were found to offer no support for an involvement of fever (8,13). The effects of PGI_2 on rectal temperature have been shown to be variable between species when administered ICV. Thus, it has been reported that ICV injection of PGI_2 causes a dose-dependent hyperthermia, rapid in onset, in anaesthetised rats (28), cats (5), and rabbits that can only be antagonized by protein synthesis inhibitors (16) but hypothermia in conscious rats (17) and guinea pigs that can be blocked by H_2 receptor antagonists (15).

In studies in which there was a hypothermic response, relatively higher doses have been used, and this may account for the above-mentioned discrepancy regarding species difference. Thus, it may be suggested that the possible centrally mediated hyperthermic effect of PGI_2 might have been masked by the hypothermic action due secondarily to its vasodilatory activity. The evidence mainly in support of this assumption is the antagonism of the hypothermic and hypotensive effects of PGI_2 by H_2 receptor antagonists (4).

It has been demonstrated that iloprost is a stable analogue that is a suitable tool reflecting PGI_2-like activity in certain experimental conditions (24,25). Moreover, the pyrogenic potency of iloprost has been found to be similar to PGI_2 when injected into the POAH in cats (13). Therefore, we evaluated the effects of iloprost on rectal temperature in conscious rats as a situation in which ICV injections of PGI_2 were shown to be hypothermic and hypotensive.

The results of our experiments showed that ICV injections of iloprost in a dose range that was ineffective on systemic blood pressure causes a mild but significant increase in rectal temperature that is not inhibited by indomethacin. The POAH was found to be a sensitive neuroanatomic site mediating the hyperthermic effect of iloprost. Indomethacin was also ineffective for suppression in the first phase of biphasic hyperthermic response to hypothalamic injections of iloprost. These observations led us to conclude that body temperature can be increased by a PGI_2-like activity in conscious rats in a similar manner as that observed for PGE_2.

We also observed that each phase of the hyperthermic response obtained by intrahypothalamic injections of iloprost is reduced by AH 6809 or indomethacin in different ways. The first phase, which is not affected by indomethacin, was completely abolished by AH 6809 pretreatment. Conversely, the second phase was reduced by indomethacin but potentiated by AH 6809 injected 90 min after iloprost. The results suggested that the hyperthermic effect of iloprost is mediated by different mechanisms in each phase.

AH 6809 appears to be a selective EP_1 receptor antagonist antagonizing the contractile response of PGE_1 and PGE_2 on isolated smooth muscle preparations (11). Iloprost displays potent EP_1- and I-type PG receptor agonistic activity (26). It has been suggested that PGE_1 and PGI_2 act on the same receptors in various tissues including neuronal hybrid cell lines (14,18). Suppression of the stimulant effects of PGI_2 and ilo-

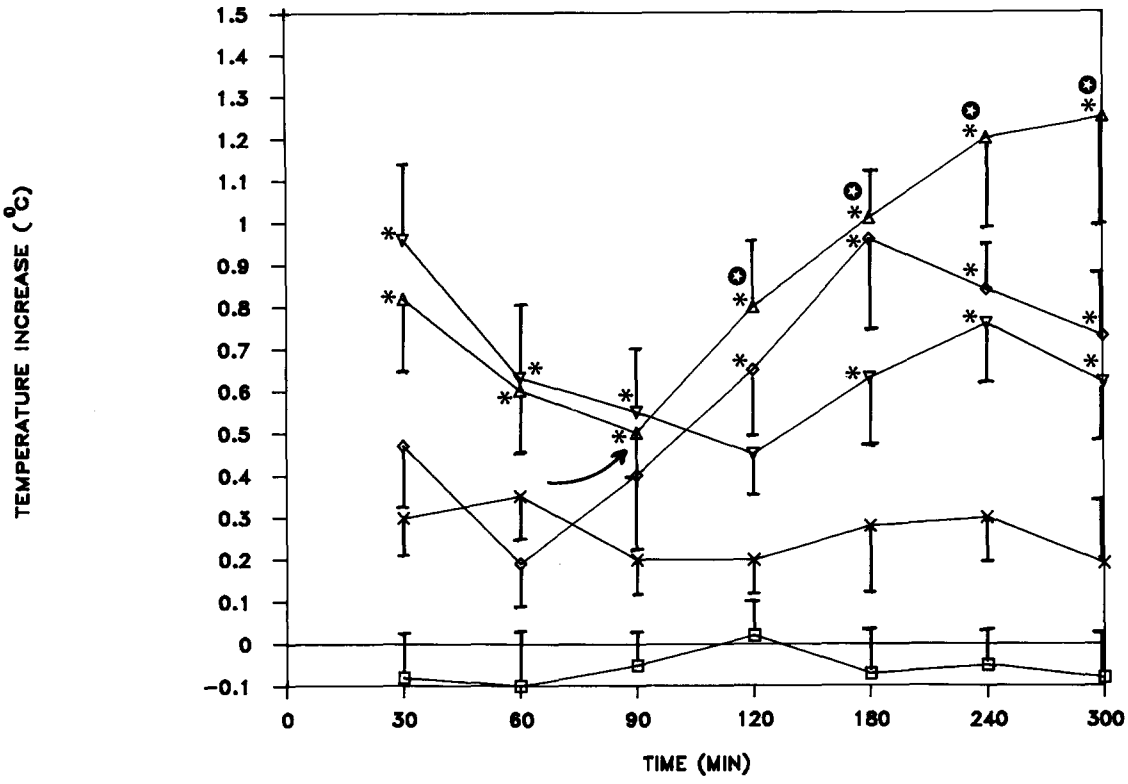

FIG. 4. Effects of AH 6809 administration on hyperthermic response induced by intrahypothalamic injections of iloprost. (□), saline ICV; (X), AH 6809 15 ng/ICV; (▽), iloprost 50 ng/preoptic anterior hypothalamic area (POAH); (◇), AH 6809 15 ng/ICV + iloprost 50 ng/POAH; (△), iloprost 50 ng/POAH + AH 6809 15 ng/ICV. Iloprost injection at time 0. Arrow indicates the injection time of AH 6809 on (△). Each point represents the mean ± SE of seven observations except (▽), which represents nine observations. $*p < 0.05$ compared with saline, $*p < 0.05$ compared with (▽).

prost on smooth muscle preparations by an EP_1 receptor antagonist has also been reported (10). Thus, it may be suggested that the first phase of the hyperthermia due to hypothalamic injections of iloprost is mediated by EP_1 receptors. Inhibition of the second phase by indomethacin pretreatment may indicate indirect pyrogenic potency of iloprost, which most likely promotes the release of PGs.

Because endogenous pyrogen is known to be a polypeptide, it is considered resistant to passage through the blood-brain barrier (BBB) (9). Thus, it has been speculated that endogenous pyrogen directly affects the endothelial cells of the BBB to produce and release PGE_2 (29). It has, though, been reported that endogenous pyrogen stimulates PGE_2 release without significantly altering PGI_2 formation in cerebral microvessels; under basal conditions, the release of PGI_2 in this preparation was found to be sixfold higher than that of PGE_2 (3). Thus, it may be suggested that relatively high levels of PGI_2 can modulate the PGE_2-induced hyperthermic response. To evaluate our suggestion, a dose of iloprost that did not modify a febrile rectal temperature was injected into the POAH prior to ICV PGE_2 administration. It was observed that the hyperthermic effect of PGE_2 was potentiated by iloprost pretreatment. One possible explanation, assuming that the thermosensitive neurons are involved in PGE_2 fever, is that these thermosensitive neurons in POAH may be sensitized by iloprost to the pyrogenic activity of PGE_2, allowing an adjustment of the set point to a higher level when compared with the effect of PGE_2 alone.

Regarding the lines of evidence that indicate that iloprost and PGI_2 act on the same receptors, it seems likely that endogenous PGI_2 is also able to modulate the response to PGE_2 without significantly rising above basal level during endogenous pyrogen stimulation.

The finding presented in this study that AH 6809 did not antagonize the hyperthermia induced by PGE_2 has been previously reported by Matsumura et al. (19). Because we observed that AH 6809 itself produces an increase in rectal temperature in doses higher than 15 ng ICV, it seems that AH 6809 has partial agonistic activity on the E-type PG receptor (EP) subtypes other than EP_1, such as EP_2 or EP_3. Potentiation of the PGE_2-induced hypothermic response by AH 6809 pretreatment may support that suggestion.

In contrast to the above, it has been reported that centrally mediated activities of PGE_2 on sleep-wake regulation and nociception were inhibited by AH 6809 (19,31). This has been evaluated as evidence that PGE_2 produces effects through different mechanisms than those responsible for hyperthermia. Although the interactions between PGE_2 and EP receptor antagonists might also indicate that different types of EP receptor subpopulations are involved in hyperthermia induced by PGE_2, the use of more specific receptor antagonists is necessary to explain exact mechanisms of action.

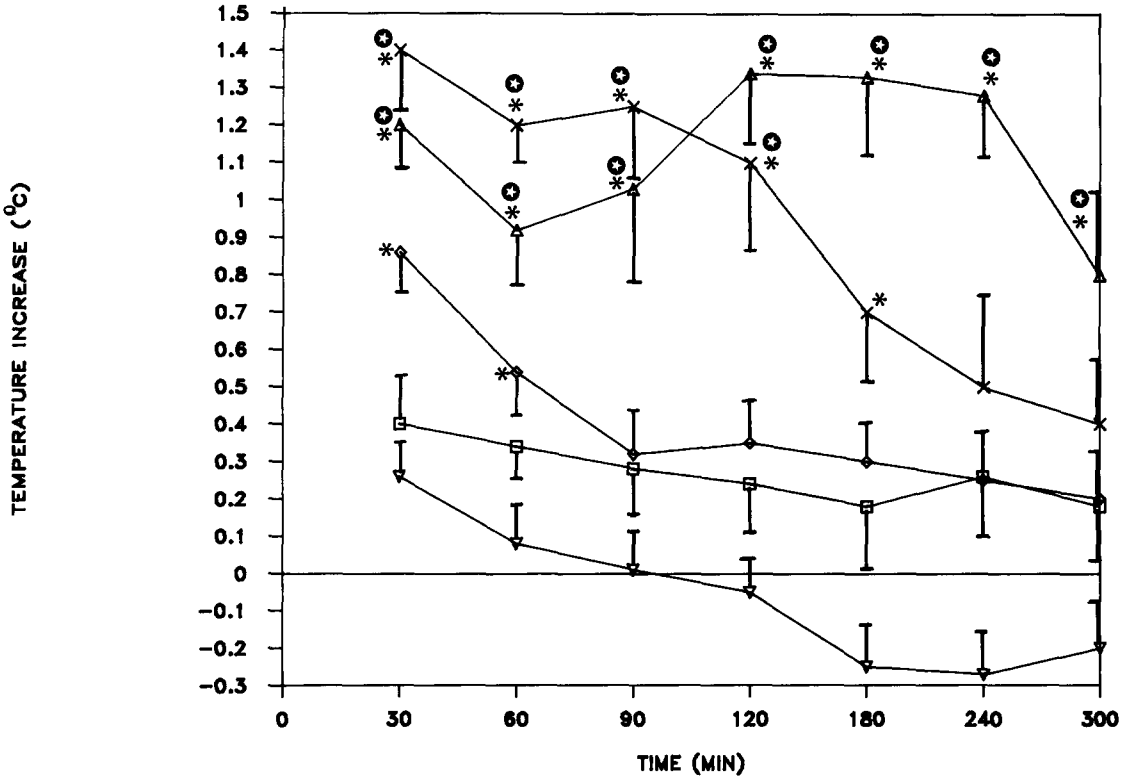

FIG. 5. Effects of iloprost or AH 6809 pretreatment on hyperthermic effect of PGE_2. (∇), saline preoptic anterior hypothalamic area (POAH); (□), iloprost 0.5 ng/POAH; (◇), PGE_2 50 ng/ICV; (△), iloprost 0.5 ng/POAH + PGE_2 50 ng/ICV; (X), AH 6809 ICV + PGE_2 50 ng/ICV. Each point represents the mean ± SE of seven observations except (◇), which represents nine observations. PGE_2 injection at time 0. AH 6809 was injected 20 min before PGE_2 administration. $*p < 0.05$ compared with saline, $*p < 0.05$ compared with (◇).

In conclusion, iloprost has pyrogenic potency mediated by specific PG receptors in conscious rats. Although these receptors seemed to be located in the POAH, it is not possible to define the site of action exactly, partly because the injected volume might have leaked to the other thermoregulatory sites such as the organum vasculosum lamina terminalis, which also contains PG receptors. The magnitude of the iloprost-induced response suggests that there is little if any contribution of PGI_2 to AA-induced fever. But, the results more importantly suggest that the hyperthermic effect of PGE_2 released during AA- or endogenous pyrogen-induced fever might be modulated by PGI_2. Thus, the roles of PGI_2 in fever pathogenesis need to be reevaluated.

ACKNOWLEDGEMENTS

The authors thank Dr. E. Schillinger, Schering AG, and Dr. R. K. Turker for their generous gift of iloprost. This study was supported by the Ankara Universitesi Research Fund (89.09.00.02 and 86.09.00.17) and the Eczacibasi Scientific Research and Award Fund (1989).

REFERENCES

1. Abdel-Halim, M. S.; Angaard, E. Regional and species differences in endogenous prostaglandin biosynthesis by brain homogenates. Prostaglandins 17:411–416; 1979.
2. Akarsu, E. S.; Ayhan, I. H. Role of GABAergic inhibitory mechanisms on the anticonvulsive effect of iloprost in rats. Eur. J. Pharmacol. 183:517–518; 1990.
3. Bishai, I.; Dinarello, C. A.; Coceani, F. Prostaglandin formation in feline cerebral microvessels: Effect of endotoxin and interleukin-1. Can. J. Physiol. Pharmacol. 65:2225–2230; 1987.
4. Brus, R.; Krzeminski, T.; Juraszczyk, Z.; Kurcok, A.; Felinska, W. Influence of H_2 receptors blockade upon some central effects of prostacyclin in rats. Pol. J. Pharmacol. Pharm. 37:831–839; 1985.
5. Clark, W. G.; Lipton, J. M. Hyperthermic effect of prostacyclin injected into the third cerebral ventricle of the cat. Brain Res. Bull. 4:15–16; 1979.
6. Coceani, F.; Bishai, I.; Lees, J.; Sirko, S. Prostaglandin E_2 in the pathogenesis of pyrogen fever: Validation of an intermediary role. In: Samuelsson, B.; Wong, P. Y.-K.; Sun, F. F., eds. Advances in prostaglandin, thromboxane and leukotriene research. vol. 19. New York: Raven Press; 1989:394–397.
7. Coceani, F.; Lees, J.; Bishai, I. Further evidence implicating prostaglandin E_2 in the genesis of pyrogen fever. Am. J. Physiol. 254:R463–R469; 1988.
8. Cooper, K. E. The neurobiology of fever: Thoughts on recent developments. Annu. Rev. Neurosci. 10:297–324; 1987.
9. Dascombe, M. J. The pharmacology of fever. Prog. Neurobiol. 25:327–373; 1985.

10. Dong, Y. J.; Jones, R. L.; Wilson, N. H. Prostaglandin E receptor subtypes in smooth muscle: Agonist activities of stable prostacyclin analogues. Br. J. Pharmacol. 87:97–107; 1986.
11. Eglen, R. M.; Whiting, R. L. The action of prostanoid agonists and antagonists on smooth muscle and platelets. Br. J. Pharmacol. 94:591–601; 1988.
12. Feuerstein, G.; Adelberg, S.; Kopin, I. J.; Jacobowitz, D. M. Central cardiovascular effects of prostacyclin. Neuropharmacology 20:1085–1090; 1981.
13. Gollman, H. M.; Rudy, T. A. Comparative pyrogenic potency of endogenous prostanoids and of prostanoid-mimetics injected into the anterior hypothalamic/preoptic region of the cat. Brain Res. 449:281–293; 1988.
14. Halushka, P. V.; Mais, D. E.; Mayeux, P. R.; Morinelli, T. A. Thromboxane, prostaglandin and leukotriene receptors. Annu. Rev. Pharmacol. Toxicol. 10:213–239; 1989 .
15. Kandasamy, S. B.; Kirlin, W. G.; Kaul, P. N. Prostacyclin-induced hypothermia: Involvement of central histamine H_2 receptors. Life Sci. 28:2553–2560; 1981.
16. Kandasamy, S. B.; Williams, B. A. Prostacyclin-induced hyperthermia: Implication of a protein mediator. Neuropharmacology 21:1065–1072; 1982.
17. Krzeminski, T.; Brus, R.; Juraszczyk, Z.; Kurcok, A.; Pogorzelska, T. Role of H_2 receptors in the central and peripheral effects of prostacyclin on circulatory system in rats. Biomed. Biochim. Acta 43:s199–s202; 1984.
18. MacDermot, J.; Hensby, C. N.; Blair, I. A. Neuronal prostacyclin receptors. In: Lewis, P. J.; O'Grady, J., eds. Clinical pharmacology of prostacyclin. New York: Raven Press; 1981:233–244.
19. Matsumura, H.; Honda, K.; Choi, W. S.; Inoue, S.; Sakai, T.; Hayaishi, O. Evidence that brain prostaglandin E_2 is involved in physiological sleep-wake regulation in rats. Proc. Natl. Acad. Sci. USA 86:5666–5669; 1989.
20. Moncada, S.; Vane, J. R. Prostacyclin and its clinical applications. Ann. Clin. Res. 16:241–252; 1984.
21. Murphy, S.; Pearce, B. Eicosanoids in the CNS: Sources and effects. Prostaglandins Leukotrienes & Essential Fatty Acids 31: 165–170;1988.
22. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. Syndey, Australia: Academic Press; 1982.
23. Saito, R.; Kamiya, H.; Ono, N. Role of the central muscarinic receptor of prostaglandin I2 in cardiovascular function in rat. Brain Res. 330:167–169; 1985.
24. Schillinger, E.; Krais, T.; Lehmann, M.; Stock, G. Iloprost. In: Scriabine, A., ed. New cardiovascular drugs. New York: Raven Press; 1986:209–231.
25. Schror, K.; Ohlendorf, R.; Darius, H. Beneficial effects of a new carbacyclin derivative ZK 36374 in acute myocardial ischaemia. J. Pharmacol. Exp. Ther. 219:243–250; 1981.
26. Senior, J.; Marshall, K.; Sangha, R.; Baxter, G. S.; Clayton, J. K. In vitro characterization of prostanoid EP receptors in the nonpregnant human myometrium. Br. J. Pharmacol. 102:747–753; 1991.
27. Sirko, S.; Bishai, I.; Coceani, F. Prostaglandin formation in the hypothalamus in vivo: Effect of pyrogens. Am. J. Physiol. 25: R616–R624; 1989.
28. Siren, A. Central cardiovascular and thermal effects of prostacyclin in rats. Prostaglandins 22:945–956; 1981.
29. Stitt, J. T. Prostaglandin E as the neural mediator of the febrile response. Yale J. Biol. Med. 59:137–149; 1986.
30. Tallarida, R. J.; Murray, R. B. Manual of pharmacological calculations with computer programs. New York: Springer-Verlag; 1981.
31. Uda, R.; Horiguchi, S.; Ito, S.; Hyodo, M.; Hayaishi, O. Nociceptive effects induced by intrathecal administration of prostaglandin D_2, E_2, or $F_{2\alpha}$. Brain Res. 510:26–32; 1990.