Pharmacology Biochemistry & Behavior, Vol. 39, pp. 535–539. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

RAPID COMMUNICATION

Hypothalamic Indomethacin Fails to Block Fever Induced in Rats by Central Macrophage Inflammatory Protein-1 (MIP-1)

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Received 13 March 1991

MIÑANO, F. J., M. VIZCAINO AND R. D. MYERS. *Hypothalamic indomethacin fails to block fever induced in rats by central macrophage inflammatory protein-1 (MIP-1).* PHARMACOL BIOCHEM BEHAV **39**(2) 535–539, 1991.—This investigation examined the extent to which the activity of a prostaglandin (PG) in the anterior hypothalamic, preoptic area (AH/POA) of the rat plays a role in the intense fever induced by macrophage inflammatory protein-1 (MIP-1) applied directly to this anatomical region. For the microinjection of both a PG synthesis inhibitor, indomethacin, and MIP-1 into sites within the AH/POA, guide cannulae were implanted chronically just above this pyrogen-reactive region. Postoperatively, the body temperature (T_b) of each rat was monitored in the unrestrained condition by means of a colonic thermistor probe. MIP-1 microinjected into the AH/POA in a 0.5-μl volume evoked a biphasic fever when given in a dose of 5.6 picograms (pg) and a monophasic fever in a dose of 28 pg. The latency of the febrile response was ordinarily 15 min with an asymptote of 1.5°C reached ordinarily within 2.0–2.5 h. When the cytokine-reactive site in the AH/POA was pretreated with indomethacin microinjected in an efficacious dose of 0.5 μg, the MIP-1 fever evoked by 5.6 pg was not inhibited. Further, pretreatment of AH/POA sites with indomethacin prior to the higher 28-pg dose of MIP-1 delayed the febrile response but did not block it. As a systemic control, indomethacin also was administered intraperitoneally in a dose of 5.0 mg/kg, again 15 min prior to the microinjection of MIP-1 into the AH/POA. In this case, indomethacin only partially attenuated but did not block the fever evoked by either dose of MIP-1. These results suggest that, although the synthesis of a PG in the periphery could contribute to the central pyrexic action of MIP-1, this cytokine exerts its febrile action within thermosensitive and pyrogen-reactive neurons independently of the local synthesis and/or release of a PG of the E type. Therefore, it is evident that a PGE-independent mechanism within the AH/POA participates in the sequence of cellular events underlying the pathogenesis of a centrally mediated febrile response.

Macrophage inflammatory protein-1 (MIP-1) Thermoregulation Fever Preoptic area Prostaglandin Hypothalamus Microinjection Indomethacin Cytokine Body temperature Thromboxane

ONE viewpoint concerning the mechanism whereby an endotoxin produces fever centers on a two-step process involving both interleukin 1 (IL-1) and a prostaglandin (PG) of the E type (1,20). In response to an endotoxin circulating systemically, mononuclear phagocytes are thought to release IL-1, which acts secondarily on cells of the anterior hypothalamic, preoptic area (AH/POA) to enhance the synthesis and/or release of a PGE (4). Although evidence supporting the central involvement of a PGE in fever has accumulated over the years (6), its precise role in the pathogenesis of leukocyte-induced and other febrile responses has been questioned repeatedly (19, 24, 28, 35).

Recently, a macrophage inflammatory protein (MIP) has been characterized molecularly (36) which, when administered by the systemic route, produces a robust febrile response (8) not unlike that elicited by IL-1 (2,12). MIP-1 arises from endotoxin-stimulated macrophages; it activates polymorphonuclear leukocytes (39) and is among the most potent of all endogenous physiological substances known. That is, when it is infused into the AH/POA in femtomolar concentrations, MIP-1 evokes an intense pyrexic response (14,16). Of special significance is the fact that the fever produced by MIP-1 administered systemically is not blocked by ibuprofen, an inhibitor of PGE synthesis (8). Thus the enigma surrounding the potential role of a PGE in the pathogenesis of fever is once again reinforced.

The present experiments were undertaken to clarify the possible role of the thromboxane-PGE pathway within the AH/POA

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in the pyrexic effect of MIP-1. In these experiments, anatomical sites were first identified in the AH/POA which react to trace doses of MIP-1 in evoking a fever. Then the prostaglandin synthesis inhibitor, indomethacin, was microinjected in an efficacious dose directly into the cytokine sensitive site in the AH/POA just prior to MIP-1. As a systemic control, indomethacin also was administered by the intraperitoneal route again just before the injection of MIP-1. By thus impairing the pathway of thromboxane-PGE synthesis, the potential contribution of a PGE at both central and peripheral levels was evaluated in relation to the hypothalamic mechanism of action of MIP-1 in eliciting fever.

METHOD

Individually caged male Wistar rats (n = 13) weighing 270–320 g were maintained at an ambient temperature of 22 ± 1.0°C and a 12-h light cycle (0700 to 1900 h) throughout the course of the experiments. The animals were provided Purina rat pellets and water ad lib throughout the course of the experiments. Food and water intakes as well as body weight of each animal were recorded daily.

Surgical Procedures

Following standard surgical procedures (23), a single 23-ga stainless steel thin-walled guide tube was implanted above the rat's AH/POA using the stereotaxic coordinates of Paxinos and Watson (30) as follows: AP, 7.5, 8.0 and 8.5; LAT, 0.3 to 1.5; and HOR, −7.0 to −8.0 below dura mater. Postoperatively, each rat was adapted for 4–5 consecutive days to the experimental situation so that the animals were tested entirely under unrestrained conditions. To record the body temperature of the rat, standard procedures were used in which a YSI 401 thermistor probe was inserted into the colon to a depth of 3–4 cm and held in place by Micropore surgical tape wrapped gently around the base of the animal's tail.

Preparation of MIP-1

Native mouse MIP-1 was isolated as described previously (7,36). Briefly, sequential anion exchange, heparin Sepharose and gel filtration was used to purify MIP-1 from macrophage-conditioned medium (7). Following aggregation, MIP-1 was isolated from the void volume of the gel filtration column at a purity of at least 95% as determined by SDS-PAGE and silver staining (8,36). In addition to the lack of effect of polymixin on the febrile response to MIP-1 (16) and antibody blocking studies, there was no biological activity when MIP-1 was tested in assays for other cytokines including tumor necrosis factor (TNF), IL-1 and IL-6. Additional affirmation of its purity was shown by the finding that the intrahypothalamic potency of MIP-1 is an order of magnitude greater than that of other thermogenic cytokines (16,17). After MIP-1 was sterilized by Millipore filtration, the cytokine was diluted with pyrogen-free 0.9% saline to a concentration of either 5.6 or 28 picograms (pg) per 0.5 μl. When applied directly to the AH/POA in either of these doses, MIP-1 evokes an intense and dose-dependent fever (16,17).

Administration of Indomethacin

Indomethacin (Sigma) was dissolved in 0.9% saline control vehicle containing 20% ethanol and 4.0% $NaHCO_3$. For its microinjection into the AH/POA, indomethacin was prepared in an efficacious dose of 0.5 μg in the same 0.5-μl volume. A dose of 0.4 μg indomethacin entirely blocks ovulation when the drug is infused directly into an homologous region of the AH/POA of the rat (11). Further, in a concentration of 1.0 μg/0.5 μl, indomethacin injected into the diffuse fluid spaces of the cerebral ventricle of the rat blocks TNF-induced thermogenesis (31) and other physiological responses (29). For its administration by the intraperitoneal route, indomethacin was prepared in 0.9% saline in a dose of 5.0 mg/kg, which also is efficacious in blocking the febrile response to different pyrogens (2, 10, 37). Indomethacin administered either centrally or peripherally always was given 15 min before the microinjection of MIP-1 in the AH/POA.

After the T_b of the rat had stabilized over a 1.5–2.0-h baseline interval, a 29-ga injector cannula attached to PE-10 tubing was lowered into the guide so as to protrude 1.0–2.0 mm beyond its tip. At 1000–1100 hours, a test injection of 5.6 or 28 pg of MIP-1 was microinjected at successive depths into the AH/POA. Each injection was given by means of a calibrated 10-μl Hamilton syringe over a period of 30 s and in a volume of 0.5 μl. Once a site reactive to either dose of MIP-1 was identified, then MIP-1 alone, indomethacin followed by MIP-1 or the saline control vehicle was microinjected into the AH/POA site following the same procedures. The T_b of each rat was recorded at 0.5-h intervals for 4.0 h.

Histological and Statistical Analyses

At the conclusion of the experiments, the position of each cannula track and locus of microinjection were verified histologically. Anatomical maps were then constructed of each site of reactivity to MIP-1. All data were analyzed statistically by the Stat-Mate computer software program. One-way classification analyses of variance were performed followed by Tukey or Student-Neuman-Keuls tests when appropriate for individual comparisons of a change in body temperature between and across each of the different treatment groups.

RESULTS

A composite anatomical mapping is presented in Fig. 1 of the sites in the AH/POA within which the microinjections of 5.6 and 28 pg MIP-1 were found to induce a biphasic and monophasic fever, respectively. At these reactive loci, microinjections in the same volume were given also of indomethacin plus MIP-1 as well as the saline vehicle control solution. The effect of the inhibitor of PGE synthesis given systemically also was tested on the fever evoked by MIP-1 microinjected at these loci. The anatomical region of maximum reactivity to the cytokine was shown to lie in the classical thermosensitive and homologous pyrogen-reactive region of the AH/POA (3, 24, 27) within coronal planes AP 7.5, 8.0 and 8.5. This region lies just dorsal to the optic chiasm and ventral to the border of the anterior commissure (Fig. 1), with the most sensitive sites bordering the ependymal wall of the third ventricle (14).

When microinjected into the AH/POA in a dose of 5.6 pg, MIP-1 evoked an intense biphasic fever in the rat, with the secondary rise commencing ordinarily within 1.0–2.0 h. As presented in Fig. 2, the resultant pyrexic response was essentially 1.2°C higher than the baseline T_b level of 37.7°C. Pretreatment of the microinjection site with 0.5 μg indomethacin 15 min prior to MIP-1 failed to attenuate the first phase of the febrile episode in that the two curves of core temperature were virtually congruent (mean S.E. values are given in the legend of Fig. 2). Following the decline in T_b at the end of the first phase (Fig. 2), the same asymptote was reached in 1.5 h as that following the microinjection of MIP-1 alone.

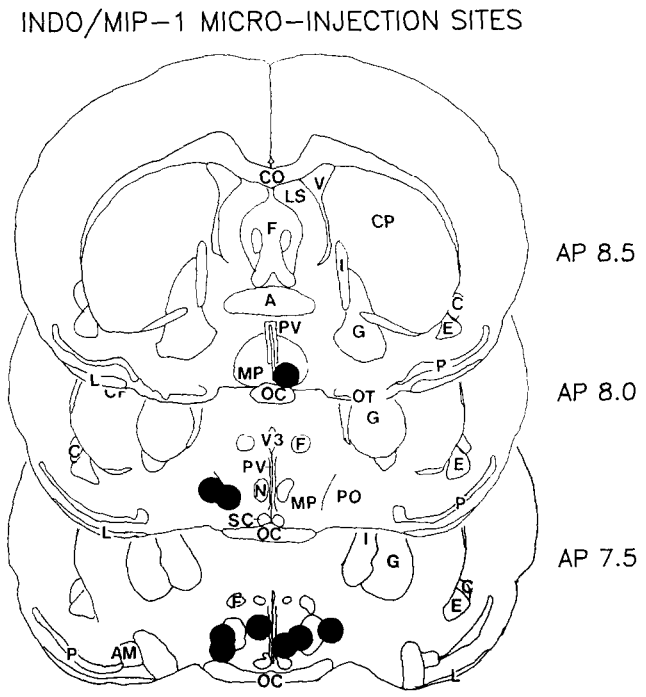

FIG. 1. Composite anatomical mapping of MIP-1-reactive sites (filled circles) within coronal planes AP 7.5, 8.0 and 8.5 at which microinjection of 0.5 μl MIP-1 in dose of either 5.6 or 28 pg induced a biphasic or monophasic fever. The positions of four loci of injection were identified as anatomically overlapping and thus are not depicted. Injection sites also were pretreated with 0.5 μg/μl indomethacin 15 min prior to MIP-1. Anatomical abbreviations are: A, anterior commissure; AM, amygdala; C, claustrum; CO, corpus callosum; CP, caudate-putamen; E, dorsal endopyriform nucleus; F, fornix; G, globus pallidus; I, internal capsule; L, lateral olfactory tract; LS, lateral septum; MP, medial preoptic area; N, medial preoptic nucleus; OC, optic chiasm; OT, tuberculum olfactorium; PO, lateral preoptic area; P, piriform cortex; PV, paraventricular hypothalamic nucleus; SC, suprachiasmaticus nucleus; V, lateral cerebral ventricle; V3, third ventricle.

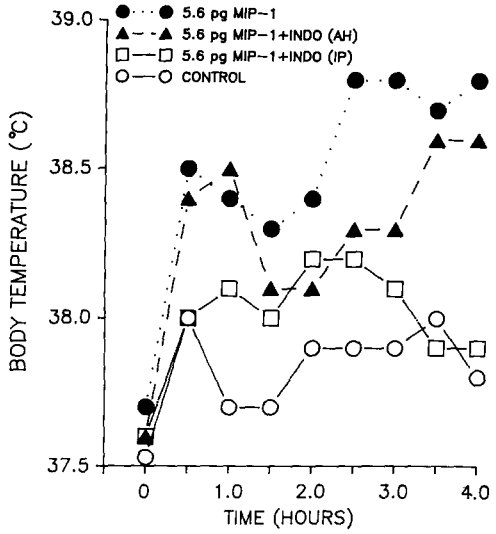

FIG. 2. Mean changes in T_b (°C) of rats after 0.5 μl microinjection in AH/POA of: 5.6 picograms (pg) MIP-1 alone; 0.5 μg indomethacin (INDO) 15 min before 5.6 pg MIP-1 at the same site (AH); 5.6 pg MIP-1 15 min after intraperitoneal administration (IP) of 5.0 mg/kg indomethacin (INDO); and saline vehicle (CONTROL). Mean S.E. values for MIP-1, INDO (AH), INDO (IP) and CONTROL curves are, respectively, 0.08, 0.19, 0.27 and 0.18. N = 6 experiments under each condition.

When the rat was pretreated with 5.0 mg/kg indomethacin given intraperitoneally 15 min prior to the microinjection of 5.6 pg MIP-1, the first phase of fever (Fig. 2) was diminished by 0.5°C at the 30-min interval when compared to the response to MIP-1 alone. The secondary rise in T_b following the microinjection of MIP-1 in the AH/POA also was reduced overall (Fig. 2) but significantly higher than that of the control, $F(1,106) = 11.98$, $p<0.01$. An analysis of variance of the data revealed a significant difference across treatment groups, $F(3,212) = 30.46$, $p<0.01$. However, no statistical difference was found between the T_b of rats in response to MIP-1 microinjected alone and that following pretreatment with intrahypothalamic indomethacin.

The microinjection into the AH/POA of 28 pg MIP-1 evoked an intense monophasic fever in the rat, with the asymptote ordinarily reached within 1.0–2.0 h. As illustrated in Fig. 3, the higher dose of the cytokine induced a pyrexic response which was 1.5°C greater than level of T_b following the saline control vehicle (mean S.E. values are given in the legend of Fig. 3). The microinjection of 0.5 μg indomethacin into the AH/POA prior to MIP-1 diminished the rate of rise in the T_b of the rat in that, by the third h, the asymptote coincided with that of the fever induced by MIP-1 alone (Fig. 3). When the rat was pretreated intraperitoneally with indomethacin given in the 5.0-mg/kg dose 15 min prior to the microinjection of 28 pg MIP-1, the increase in T_b was significantly higher than the control, $F(1,106) = 47.63$, $p<0.01$, with the febrile response reaching a peak increase of 1.0°C by the 3.5-h interval. An analysis of variance of the data showed significant differences among the temperature responses across the treatment groups, $F(3,212) = 9.69$, $p<0.01$. An overall comparison of the effects of the two doses of MIP-1 showed that the higher 28-pg dose of the cytokine induced a significantly greater rise in T_b than that produced by 5.6 pg after either intrahypothalamic, $F(1,106) = 17.57$, $p<0.01$, or peripheral pretreatment with indomethacin, $F(1,106) = 20.6$, $p<0.01$.

DISCUSSION

The present results confirm the potency of MIP-1 on cells of AH/POA in inducing a typical biphasic and monophasic febrile response by a minuscule dose of 5.6 and 28 pg, respectively (16,18). When neurons in this region are pretreated directly by indomethacin, the initial phase of the fever produced by MIP-1 injected into the AH/POA in the lower dose is not blocked. Following the higher dose of MIP-1, pretreatment with this inhibitor of PGE synthesis retards the rate of thermogenesis but also does not block the febrile response. The dose of indomethacin applied directly to the parenchyma of the AH/POA was not in the toxic range but clearly at a pharmacological level of efficacy. In comparison with other studies, when indomethacin is infused similarly into an anatomically homologous region of the AH/POA in a dose of 0.4 μg, the ovulatory mechanism in the rat is entirely blocked (11). Moreover, indomethacin injected into the cerebral ventricle in a concentration of only twice that used in the present study inhibits TNF-induced fever and specific endocrine responses in the rat (29,31) in spite of the manifold dilution of the drug within the ventricular fluid spaces in the brain (24). On the other hand, higher doses of indomethacin

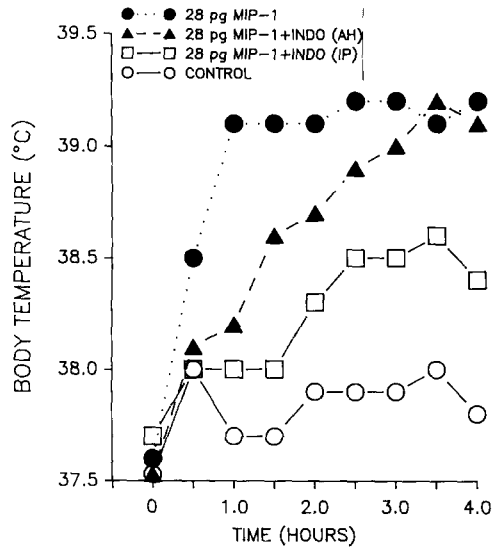

FIG. 3. Mean changes in T_b (°C) of rats after 0.5 μl microinjection in AH/POA of: 28 picograms (pg) MIP-1 alone; 0.5 μg indomethacin (INDO) 15 min before 28 pg MIP-1 at the same site (AH); 28 pg MIP-1 15 min after intraperitoneal administration (IP) of 5.0 mg/kg indomethacin (INDO); and saline vehicle (CONTROL). Mean S.E. values for MIP-1, INDO (AH), INDO (IP) and CONTROL curves are, respectively, 0.19, 0.31, 0.12 and 0.18. N=6 experiments under each condition.

given systemically can produce thermolytic effects (10) which may be due to a toxic action of the drug.

The present observations coincide with the earlier findings of Davatelis and coworkers (8), who found that the systemic administration of another inhibitor of PGE synthesis, ibuprofen, does not antagonize a fever produced by MIP-1 given parenterally. The fever evoked by MIP-1 administered by this route is essentially monophasic (8), whereas the fever elicited by intrahypothalamic MIP-1 is either biphasic or monophasic, depending on the dose of cytokine injected (14,16). The delay in the secondary phase of the fever generated by 5.6 pg MIP-1 following indomethacin pretreatment of the AH/POA indicates that both the initial component and the ultimate level of this pyrexic response are unrelated to the central synthesis and/or release of a PGE. Thus the cellular mechanisms underlying the stimulation and onset of the second phase of the centrally induced MIP-1 fever may, in part, be due to a transient but relatively immaterial action of a PGE within the AH/POA.

Indomethacin given intraperitoneally attenuates partially either the first or second or both phases of the pyrexic response produced by MIP-1 applied to the AH/POA in either a low or high dose. Therefore, one could envisage that receptor sites for MIP-1, as yet unidentified, are located ostensibly on cells reactive to MIP-1 both inside and outside of the blood-brain barrier. This assumption is in accord with the finding that indomethacin given intracerebroventricularly can suppress the second but not the first phase of a fever induced by a pyrogen (21). Consequently, MIP-1 causes a fever conceivably by a dual action on cellular systems in the periphery and on neurons within the AH/POA. In the case of the peripheral mechanism, the pyrexic response is partly contingent upon an intermediary action of a PGE. On the other hand, the activating mechanism in the diencephalon for the initial rise and the peak of the developing fever is not necessarily contingent on the action of a PGE on the function of nerve cells (10,22). In conjunction with these pharmacological findings, it is already known that a PGE does not underlie all pathophysiological responses evoked by pyrogenic factors including IL-1 (2,6).

If MIP-1 exerts its physiological effect on neurons of the AH/POA independently of a PGE, a major question arises as to the mechanism by which this cytokine acts. One possibility is that MIP-1 operates through an enhancement of the release or other activity of IL-1 within cells of the ventrobasal forebrain. Recent experiments have shown, for example, that cyclosporine A (CsA) microinjected into the AH/POA of the rat retards the first phase of the rise in T_b which commences immediately after the injection of MIP-1 into the same site (15,17). Moreover, the magnitude of the MIP-1-induced fever is likewise diminished by CsA applied directly to this thermosensitive region (15,17). In this case, CsA administered systemically is even more potent in delaying the onset of fever evoked by a low dose but not higher dose of MIP-1. CsA is an immunosuppressant agent which inhibits the synthesis and release of IL-2 from T-lymphocytes and subsequently the synthesis of IL-1 (9,38). Thus the febrile response to MIP-1 could be mediated through the central synthesis of another interleukin or other protein factor which possesses pyrexic properties (33). In either case, the factor presumably would be released by lymphocytes or other CsA-sensitive cells, mainly as the secondary phase of the fever evolves (15).

An alternative explanation centers on the possibility that MIP-1 possesses a unique and specialized affinity for receptors on membranes of serotonergic or catecholaminergic or both types of neurons within the AH/POA. It is well known that the activation of 5-HT receptors, for example, produces a dose-dependent hyperthermic response which is functionally analogous to that evoked centrally by an endotoxin or other pyrogen (24, 27, 34). Further, preliminary findings from this laboratory show that MIP-1 can alter differentially the activity of serotonergic and catecholaminergic neurons, as reflected by changes in release and/or turnover of 5-HT, norepinephrine and dopamine during the course of the febrile response (17). In this connection, the enhanced turnover of monoaminergic transmitters in the hypothalamus coincides with the activation of host defense responses by a pyrogen such as IL-1, which is believed to be due in part to stimulation of the arachidonic acid cascade (13).

Finally, the activity of Ca^{2+} ions in the hypothalamus as well as the Ca^{2+}-calmodulin complex (25–27) may play an important functional role in the febrile action of MIP-1 and other newly synthesized protein factors (33). This supposition is strengthened by the fact that CsA, which exerts an effect on MIP-1-induced fever (17), influences the endogenous activity of both calmodulin and Ca^{2+} ions in tissue (5,32).

ACKNOWLEDGEMENTS

This research was supported by National Science Foundation Grant BNS 84-10663 to R.D.M. and CICYT Spain Grant PB 88-0551 to F.J.M. We are particularly grateful to Drs. A. Cerami, B. Sherry and T. Fahey of the Laboratory of Medical Biochemistry, Rockefeller University, New York, for their generosity in supplying us with MIP-1 and for their kind contributions to this research. We thank also Amy K. Roscoe and M. F. Lankford for their excellent technical assistance and Drs. X. Paez and M. Sancibrian for constructive suggestions.

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