Hypothalamic interaction between macrophage inflammatory protein-1α (MIP-1α) and MIP-1β in rats: a new level for fever control?

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1. The microinjection of macrophage inflammatory protein-1 (MIP-1α; 5·0 and 25 pg) into the anterior hypothalamic, preoptic area (AHPOA) induced a slow onset, monophasic fever in rats that persisted for a long period. Microinjection of 25 pg MIP-1β into the AHPOA induced a fever of rapid onset, whereas 5·0 pg MIP-1β did not alter body temperature (T_b) significantly. When either MIP-1α or MIP-1β was heated to 70 °C for 30 min prior to their injection, no pyrexic response was produced.

2. The concurrent microinjection of 25 pg MIP-1α and 25 pg MIP-1β into the AHPOA attenuated the effects on T_b of either cytokine alone. However, pretreatment with either 5·0 pg MIP-1β or 5·0 pg MIP-1α suppressed the febrile response induced by 25 pg MIP-1α or 25 pg MIP-1β, given at the same site, respectively.

3. The present experiments show that MIP-1α and MIP-1β are active individually and possess distinct differences in their evocation of a febrile response. Further, our results suggest a functional antagonism between MIP-1α and MIP-1β that could represent a new level in the development of fever.

A number of well-characterized factors, including interleukin-1 (IL-1), IL-6, interferon (IFN) and tumour necrosis factor-α (TNFα), are known to be intrinsically pyrogenic and are now implicated as endogenous pyrogens (Davidson, Milton & Rotondo, 1990; Long, Otterness, Kunkel, Vander & Kluger, 1990; Myers, López-Valpuesta, Miñano, Wooten, Barwick & Wolpe, 1994). Apart from these factors, it is likely that several cytokines identified recently also play a role in the pathogenesis of the febrile response. For example, macrophage inflammatory protein-1 (MIP-1) has been hypothesized to play a role in the host defence response and act as an endogenous pyrogen. When administered intravenously (I.V.) or microinjected directly into the anterior hypothalamic, preoptic area (AHPOA), MIP-1 evokes a monophasic fever in rabbits and rats which is neither inhibited by pretreatment with I.V. ibuprofen nor intrahypothalamic indomethacin, respectively (Davatelis, Wolpe, Sherry, Dayer, Chicheportiche & Cerami, 1989; Miñano *et al.* 1990; Miñano, Sancibrián & Myers, 1991; Miñano, Vizcaino & Myers, 1991).

MIP-1 was purified initially from conditioned medium of endotoxin stimulated macrophages, and is a 8 kDa heparin-binding protein doublet comprised of two peptides, termed MIP-1α and MIP-1β (Sherry *et al.* 1988). These peptides are members of a new chemokine superfamily structurally related, with proinflammatory and growth regulatory activities; they have been purified separately and the corresponding cDNA has been cloned, sequenced and expressed in human lymphocytes (Wolpe *et al.* 1988; Widmer, Manogue, Cerami & Sherry, 1993). Although these highly homologous peptides, with 70% homology, do exert some overlapping biological effects such as inflammatory activity, native doublet MIP-1, MIP-1α and MIP-1β peptides are each active individually, with distinct differences in their biological activity. For example, MIP-1α and MIP-1β injected into the AHPOA produce two functionally different febrile responses, which indicate that MIP-1β could underlie the initial phase of a pyrogen-induced fever whereas MIP-1α mediates the secondary stage of a biphasic fever (Myers, Páez, Roscoe, Sherry & Cerami, 1993).

The present experiments were conducted to investigate the specific role that MIP-1α and MIP-1β peptides play in the development of fever when injected directly into the AHPOA of the rat. MIP-1α and MIP-1β were tested alone and in combination, to determine whether they associate and act as heterodimers *in vivo*, or act separately to evoke fever. Further, the effects of intrahypothalamic MIP-1α or MIP-1β were examined in rats pretreated with MIP-1β or MIP-1α given by the same route, respectively.

METHODS

Animals

Thirty male Wistar rats (Charles River, Inc.), weighing between 180 and 240 g at the start of the experiments, were housed in individual cages in a temperature-controlled room maintained at 26 ± 1 °C with a 12 h light–dark cycle (lights on at 07.00 h). Purina rodent chow and tap water were available *ad libitum* throughout. All experiments were started between 09.00 and 10.00 h to minimize the circadian variation in body temperature (T_b).

Implantation of cannula and radio transmitter

Under sodium pentobarbitone anaesthesia (35 mg kg^{-1}, I.P.) each animal was placed in a David Kopf stereotaxic instrument with the upper incisor bar set at 3·0 mm below the interaural line (Miñano *et al.* 1990). A sterilized stainless-steel 23 gauge cannula guide was placed just above the right or left AHPOA. The stereotaxic co-ordinates were: 0·4 mm anterior to bregma; 0·5 mm from mid-line and 6·0 mm ventral to the dura mater (Paxinos & Watson, 1986). A 30 gauge stylet of identical length was inserted into the guide tube to prevent its occlusion.

Measurement of body temperature

Calibrated battery-operated radio transmitters (Mini-mitter, Sunriver, OR, USA) were implanted intraperitoneally at least 4 days before the experiments began. The animals were then placed individually in recording cages in the temperature-controlled room. The output signals were monitored by a receiver (Mini-mitter, model RA 1010) placed under the cage of each animal and then fed into a peripheral microprocessor (consolidation matrix model BCM 100) connected to a Hewlett-Packard computer. Thus, the T_b could be monitored continuously and recorded at 5 min intervals without any disturbance to the animal.

Experimental protocol

On the second postoperative day, the patency of the guide cannula was verified using standard procedures (Myers *et al.* 1993). Pyrogen-free saline was microinjected in the AHPOA in a volume of 0·5 μl into each rat for each of 4 or 5 days before the experiments began. This procedure was followed to verify the fact that the injection itself was without effect on T_b. Further daily microinjections served to maintain the reactivity of the site to a chemical substance (Myers, 1974; McCaleb & Myers, 1982). On the following days, pyrogen-free saline or either a 5·0 or a 25 pg dose of MIP-1α or MIP-1β was microinjected in a volume of 0·5 μl. The cannula for microinjection was connected by sterilized polyethylene tubing to a 10 μl Hamilton syringe, which was mounted on a Harvard 22 syringe pump set at a flow rate of 1·0 μl min^{-1}. A 90 min stabilization period preceded all the experimental treatments. The order of the treatments was randomized, and no rat was used for more than three injections at intervals of 5–7 days.

In the first set of experiments, one of four procedures were used as follows: (1) the saline control vehicle was microinjected into the AHPOA in a volume of 0·5 μl, followed immediately thereafter by a second injection of saline in the same volume; (2) the saline vehicle was followed immediately by the microinjection of a 25 pg dose of either MIP-1α or MIP-1β injected similarly; (3) the microinjection of 25 pg MIP-1α was followed immediately by 25 pg MIP-1β; and (4) the microinjection of 25 pg MIP-1β was followed immediately by 25 pg MIP-1α injected similarly. In the second series of experiments, one of three procedures was used in which each microinjection into the AHPOA was separated by an interval of 15 min as follows: (1) both the microinjection of either 5·0 pg MIP-1α or 5 pg MIP-1β was followed by saline; (2) 5·0 pg MIP-1α was followed by 25 pg MIP-1β; and (3) 5·0 pg MIP-1β was followed by 25 pg MIP-1α. The final concentrations of the cytokines were selected on the basis of their actions on T_b in pilot experiments (Fernández-Alonso, Miñano, Sancibrián & Myers, 1993; Fernández-Alonso, Miñano & Myers, 1995).

Cytokines

Recombinant murine MIP-1α and MIP-1β were kindly provided by Dr S. D. Wolpe (Genetics Institute, Cambridge, MA, USA) and Dr B. Sherry (Picower Institute, Manhasset, NY, USA). All solutions were prepared using aseptic techniques and were dissolved in pyrogen-free saline (0·9% NaCl). Each aliquot of MIP-1α and MIP-1β was kept at −20 °C in sterile Eppendorf tubes and brought to the appropriate volume with pyrogen-free saline just prior to its microinjection. The possible pyrogenicity of solutions of MIP-1α and MIP-1β alone was tested in order to exclude endotoxin contamination. When the solutions of the cytokines were heated to 70 °C for 30 min, they lost all pyrogen activity, which confirmed any contamination of the solutions by a heat stable endotoxin.

Statistical and data analysis

Changes in the T_b of the rats following microinjections into the AHPOA were expressed in one of two ways: (1) as the mean ± S.E.M. degrees Celsius across the 4 h interval of recording; and (2) as the mean ± S.E.M. degrees Celsius maximum rise in the T_b of each of the individual rats, such that the highest point in the T_b of each rat differed across time points of the 4 h recording period. The changes in temperature were calculated by subtracting the baseline temperature of each animal (mean temperature at 5 min intervals over the last 30 min of the stabilization period) from its temperature at each subsequent time point. The significance of the changes in the T_b during the experiment between groups was evaluated by analysis of variance (ANOVA) with repeated measures. If a significant difference was found among the groups *post hoc*, all pairwise multiple comparisons of means were examined by the Scheffé's test to isolate the group or groups which differed from one another. A value of P less than 0·05 was considered statistically significant.

Histology

At the end of the experiments, the rats were given an overdose of sodium pentobarbitone, and perfused intracardially with isotonic saline followed by 10% buffered formalin. The brains were removed, stored in formalin for at least 48 h, and sliced on a freezing microtome. Each coronal section was mounted and stained with Cresyl Violet according to standard procedures, to verify the proper placement of the injection under light microscopy (Miñano *et al.* 1990).

RESULTS

A composite anatomical map of the individual sites of microinjection is presented in Fig. 1. Although some glial reaction occurred in the vicinity of the guide cannula, no gliosis was found at the sites of the microinjections, in accordance with the consistent functional reactivity of the tissue in the AHPOA to the two cytokines, as well as the lack of response to the control vehicle. The tips of the injector needle were distributed in coronal planes anterior–posterior (AP) −0·26, −0·4, −0·80 and −1·30

from bregma, within the region ventral to the anterior commissure, dorsal to the optic chiasma and medial to the lateral preoptic and hypothalamic areas. These sites encompassed the thermosensitive regions of the AH and medial POA.

Effects of intrahypothalamic injection of MIP-1α and MIP-1β on T_b

The time course of the changes in the T_b is presented in Fig. 2 from time zero after microinjection of 25 pg MIP-1α, 25 pg MIP-1β, and 25 pg MIP-1α plus 25 pg MIP-1β, directly into the AHPOA of the rat. The preinjection T_b did not differ significantly among the six groups ($P > 0.05$). A repeated measures ANOVA showed that each peptide acting on the AHPOA induced a rise in the T_b which was significant ($P < 0.01$). Scheffé's *post hoc* analysis of the treatment groups revealed significant increases in the T_b across the 4·0 h recording period in comparison with the saline control for both 25 pg doses of MIP-1α and MIP-1β ($P < 0.01$). The intrahypothalamic injection of 25 pg MIP-1β induced a febrile response with a rapid onset that began within 5·0 min after its injection. In contrast, 25 pg MIP-1α produced a gradual rise in T_b which reached a peak of 1.77 ± 0.17 °C at 4·0 h. Then T_b decreased gradually over the next 4 h (Fig. 2). Microinjection of 0·5 μl saline control vehicle produced an initial increase in the T_b of the rats during the first 30 min and then varied thereafter, falling below the baseline and then rising gradually until

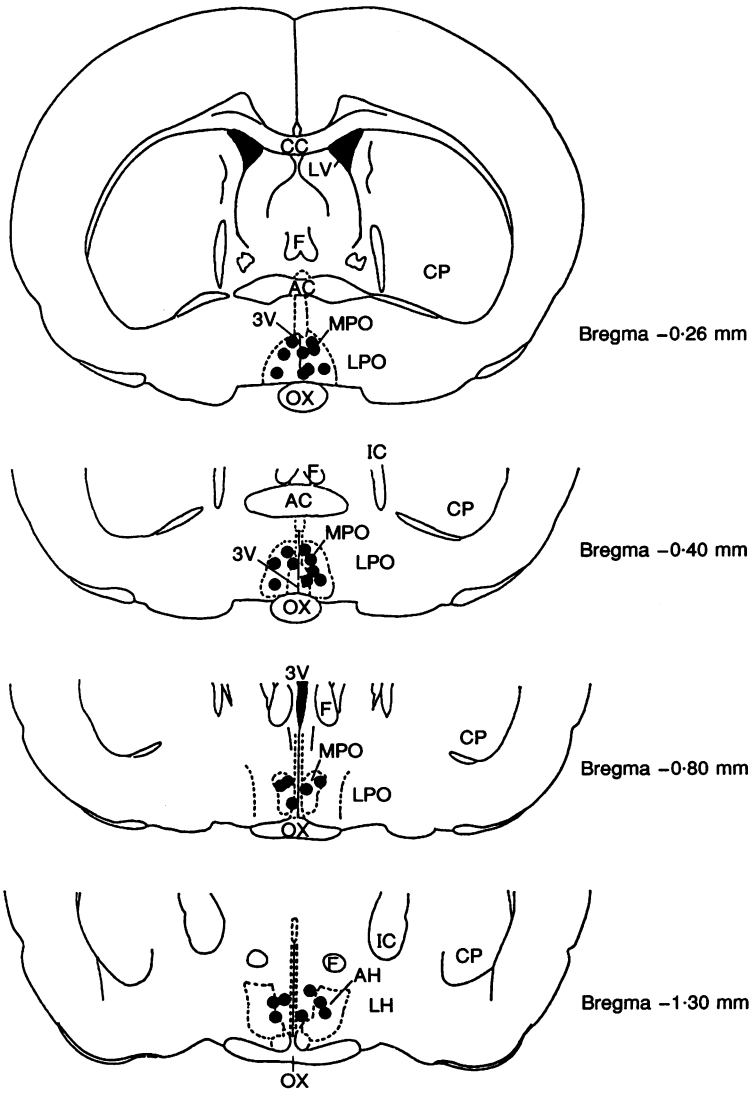

Figure 1. Anatomical mapping in successive frontal sections illustrating the distribution of individual sites of microinjections in the AHPOA, based on the anterior–posterior orientation of Paxinos & Watson (1986)

Anatomical abbreviations are: 3V, 3rd ventricle; AC, anterior commissure; AH, anterior hypothalamic area; CC, corpus callosum; CP, caudate putamen; F, fornix; IC, internal capsule; LH, lateral hypothalamic area; LPO, lateral preoptic area; LV, lateral ventricle; MPO, medial preoptic area; and OX, optic chiasma.

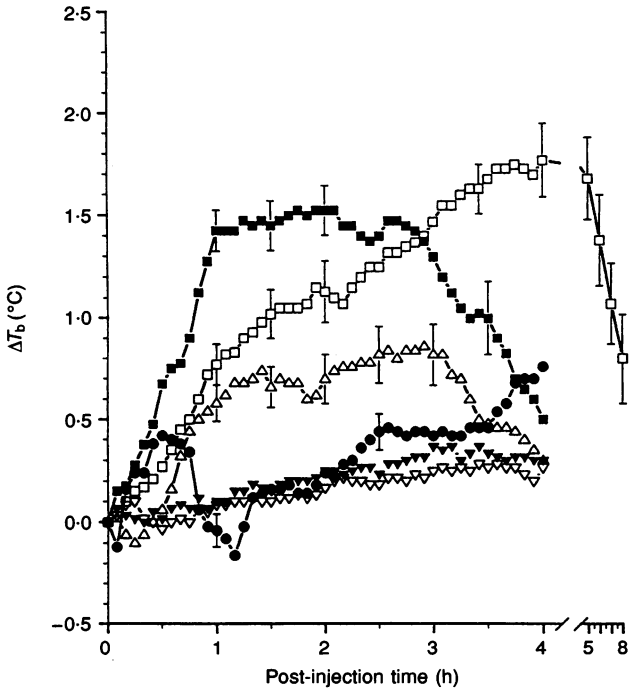

Figure 2. Changes (means ± S.E.M.) in body temperature (T_b) of rats

Concurrent microinjections into the AHPOA at time zero of: saline plus saline (37·08 ± 0·21 °C; ●); saline plus 25 pg MIP-1α (36·90 ± 0·22 °C; □); saline plus 25 pg MIP-1β (37·06 ± 0·19 °C; ■); 25 pg MIP-1α plus 25 pg MIP-1β (37·10 ± 0·19 °C; △); 25 pg MIP-1α heat-inactivated (37·13 ± 0·22 °C; ▽); or 25 pg MIP-1β heat-inactivated (37·03 ± 0·14 °C; ▼). $n = 6$ for each group. The order of injections was randomized. For clarity, S.E.M. bars are at 30 min intervals. Mean temperatures at time zero for each group are indicated in parentheses and were not significantly different from each other; $P > 0·05$, independent t test.

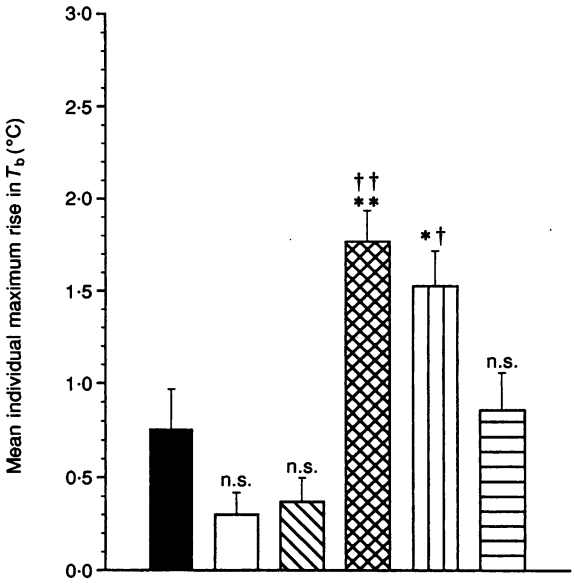

Figure 3. Maximum rise (means ± S.E.M.) in the T_b of each individual rat

Concurrent microinjections into the AHPOA at time zero of: saline plus saline, ■; 25 pg MIP-1α heat-inactivated, □; 25 pg MIP-1β heat-inactivated, ▨; saline plus 25 pg MIP-1α, ▩; saline plus 25 pg MIP-1β, ▥; or 25 pg MIP-1α plus 25 pg MIP-1β, ⊟. $n = 6$ for each group. Significance of saline: † $P < 0·05$; †† $P < 0·01$; n.s., not significant; significance of MIP-1β plus MIP-1α value: * $P < 0·05$; ** $P < 0·01$.

the end of the total 4·0 h period of recording. There was no significant difference in T_b between the group injected with saline and the groups injected with heat-inactivated MIP-1α or MIP-1β (Fig. 2). When 25 pg MIP-1α plus 25 pg MIP-1β were microinjected concurrently in the AHPOA, the rise in T_b was attenuated significantly ($P < 0{\cdot}01$) in comparison with the T_b response when the peptides were given separately (Fig. 2). That is, T_b gradually started to rise after 30 min to a level of less than 1·0 °C, and then merged toward the T_b response of the saline control rats (Fig. 2). When MIP-1α and MIP-1β were combined in the same solution, the effect was identical to that shown in Fig. 2 (F. J. Miñano & A. Fernández-Alonso, unpublished results).

As shown in Fig. 3, the mean maximum rise in T_b of the individual rats microinjected with either MIP-1α or MIP-1β was significantly higher ($P < 0{\cdot}01$) than that of rats given the same dose of heat-inactivated MIP-1α and MIP-1β, respectively. Microinjection of both of the chemokines simultaneously at the same site significantly reduced the individual mean maximal rise in T_b by 0·86 ± 0·20 °C in the individual rats when compared with their effect when administered alone ($P < 0{\cdot}05$; Fig. 3). The behavioural effects of MIP-1α and MIP-1β were similar to those of MIP-1, as described previously (Miñano *et al.* 1990; Miñano *et al.* 1991).

Effects of MIP-1α on fever induced by MIP-1β

As presented in Fig. 4, the febrile response of rats to the microinjection of 5·0 pg MIP-1α was significantly diminished in comparison with the rise in T_b produced by the higher dose of 25 pg ($P < 0{\cdot}01$; Fig. 2). When 5·0 pg of MIP-1α was injected into the AHPOA 15 min before 25 pg MIP-1β at the same site, the time course compared with that of the rats treated with 25 pg MIP-1β alone (Fig. 4) showed that rise in T_b was virtually blocked ($P < 0{\cdot}01$). The preinjection T_b values were not significantly different among the different groups ($P > 0{\cdot}05$). As shown in Fig. 5, the mean maximum increase in T_b in individual rats of 1·53 ± 0·19 °C was significantly greater ($P < 0{\cdot}001$) following the microinjection of MIP-1β alone, than in those animals pretreated with 5·0 pg MIP-1α prior to 25 pg MIP-1β (0·70 ± 0·10 °C). However, the difference in the mean maximal rise of individual animals given MIP-1β and MIP-1α was not significant (Fig. 5).

Effects of MIP-1β on fever induced by MIP-1α

The T_b of the rat was essentially unaffected by injection into the AHPOA of the lower dose of MIP-1β (Fig. 6). The effect of pretreatment with 5 pg MIP-1β injected directly into the AHPOA on the febrile response induced by microinjection of 25 pg MIP-1α into the same site (Fig. 6) showed that the β subunit completely blocked the rise in the

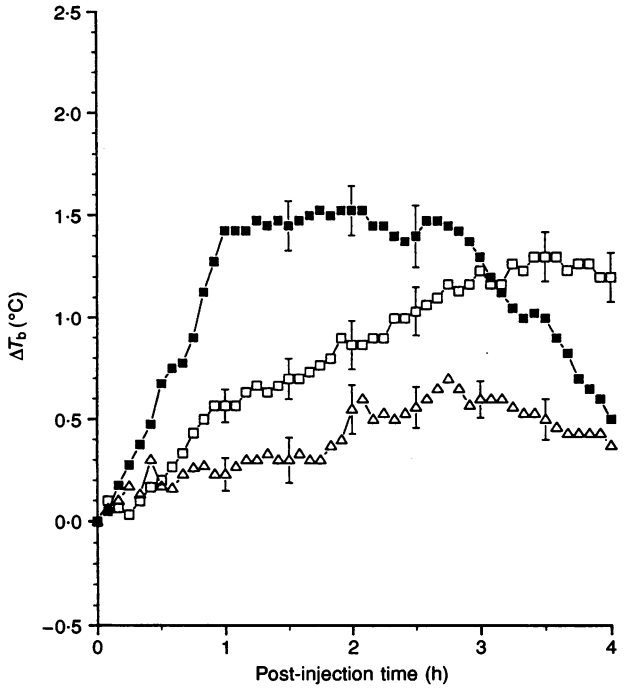

Figure 4. Changes (means ± S.E.M.) in T_b of rats

Microinjections, separated by an interval of 15 min into the AHPOA of: 5·0 pg of MIP-1α followed by saline (37·10 ± 0·16 °C; □); saline followed by 25 pg of MIP-1β (37·06 ± 0·19 °C; ■) and 5·0 pg of MIP-1α followed by 25 pg of MIP-1β (37·11 ± 0·13 °C; △). $n = 6$ for each group. For clarity, S.E.M. bars are at 30 min intervals. Mean temperatures at time zero for each group are indicated in parentheses and were not significantly different from each other; $P > 0{\cdot}05$, independent t test.

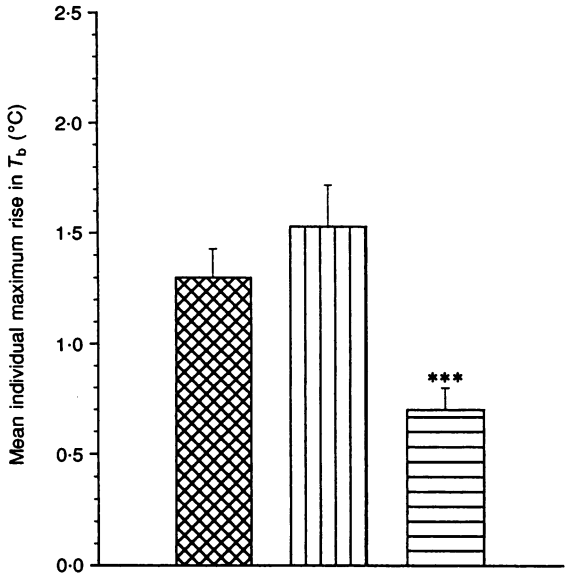

Figure 5. Maximum rise (means ± S.E.M.) in the T_b of each individual rat
Microinjections, separated by an interval of 15 min into the AHPOA of: 5·0 pg of MIP-1α followed by saline, ▩; saline followed by 25 pg of MIP-1β, ▥; and 5·0 pg of MIP-1α followed by 25 pg of MIP-1β, ⊟. $n = 6$ for each group. Significance of MIP-1β, ***$P < 0{\cdot}001$.

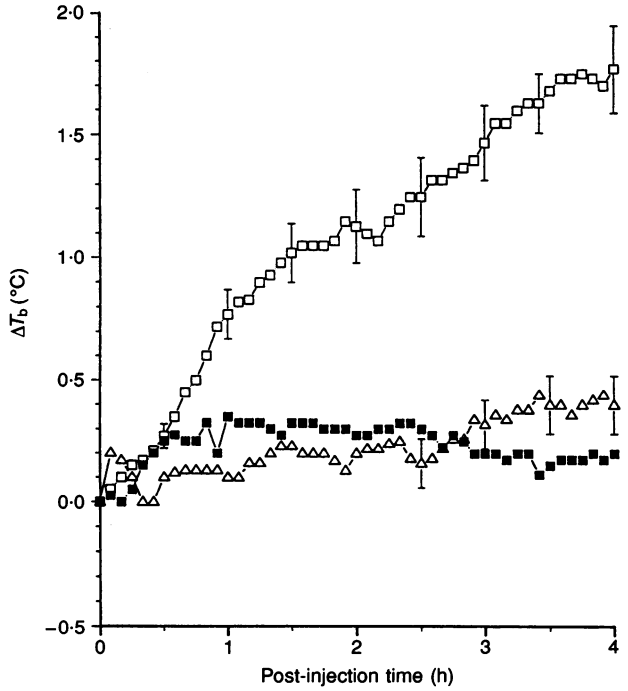

Figure 6. Changes (means ± S.E.M.) in T_b of rats
Microinjections, separated by an interval of 15 min into the AHPOA of: 5·0 pg of MIP-1β followed by saline (37·16 ± 0·3 °C, ■); saline followed by 25 pg of MIP-1α (36·90 ± 0·22 °C, □); and 5·0 pg of MIP-1β followed by 25 pg of MIP-1α (36·86 ± 0·3 °C, △). $n = 6$ for each group. For clarity, S.E.M. bars are at 30 min intervals. Mean temperatures at time zero for each group are indicated in parentheses and were not significantly different from each other; $P > 0{\cdot}05$, independent t test.

T_b induced by MIP-1α ($P < 0{\cdot}01$; Fig. 6). The preinjection T_b values were not significantly different among the different groups ($P > 0{\cdot}05$). As shown in Fig. 7, the mean increase in the T_b of individual rats induced by MIP-1α of $1{\cdot}77 \pm 0{\cdot}17$ °C was antagonized correspondingly by pretreatment of 5·0 pg MIP-1β, with a mean maximum rise of $0{\cdot}44 \pm 0{\cdot}11$ °C ($P < 0{\cdot}001$).

DISCUSSION

The present results, together with previous data, support a role for MIP-1α and MIP-1β in the pathogenesis of fever and uphold the hypothesis that endogenously produced MIP-1 plays a role in the development of fever (Miñano *et al.* 1990; Miñano *et al.* 1991; Myers *et al.* 1993). Previously, it was reported that native murine MIP-1, a mixture of the α and β subunits, induced a febrile response in the rabbit and rat. In contrast to the febrile response induced by an endotoxin, IL-1, IL-6 or IL-11 (Myers, Rudy & Yaksh, 1974; Davatelis *et al.* 1989; Davidson *et al.* 1990; Long *et al.* 1990; Miñano *et al.* 1990; López-Valpuesta & Myers, 1994), the MIP-1 fever is independent of the cyclo-oxygenase pathway and not blocked by a PGE inhibitor (Miñano *et al.* 1991).

It is apparent that the pyrogenic response induced by doublet MIP-1 occurs in response to an aggregate MIP-1α–β since each peptide is active individually; however, on the basis of weight (e.g. 25 pg), these two highly homologous proteins injected into the AHPOA in the same concentrations produce functionally different febrile responses. Following intrahypothalamic injection, MIP-1β produces a fever which is characterized by a rapid rise and steady-state level of fever lasting ~2 h. In contrast, MIP-1α produces a more gradually rising fever that persists longer. The reason for the differences in action of the two cytokines combined is unclear, and the proportion of each in the doublet MIP-1 is unknown. Previously, it was suggested that MIP-1β underlies the initial phase of a pyrogen-induced fever whereas MIP-1α could mediate the secondary phase of a phasic fever (Myers *et al.* 1993). In addition, the prolonged fever associated with injection of MIP-1α into the AHPOA supports the hypothesis that this cytokine may act through the release of other intermediary substances or endogenous pyrogens within the hypothalamus (Fernández-Alonso *et al.* 1993).

In agreement with this hypothesis, a protein synthesis inhibitor administered intrahypothalamically can block the febrile responses to MIP-1 and MIP-1α, but does not block the pyrogenic response induced by MIP-1β (Fernández-Alonso *et al.* 1993; Zawada, Ruwe & Myers, 1993; Fernández-Alonso *et al.* 1995). Therefore, the fever induced by MIP-1β alone could be due to a direct action of the cytokine on receptors on the thermosensitive cells of the AHPOA. Alternatively, in view of the findings of Fahey *et al.* (1992), the pyrogenic response induced by MIP-1 and MIP-1α may also be due to the release of other chemokines of the interleukin family within this region of the hypothalamus. These findings are in agreement with a previous report which showed that the fever produced by MIP-1 injected into the AHPOA is attenuated by a microinjection into the same site of the potent immunosuppressant agent cyclosporin A. This suggests that an

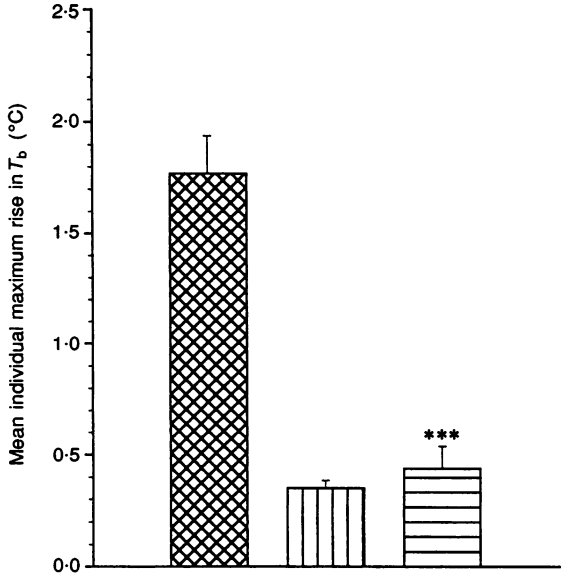

Figure 7. Maximum rise (means ± S.E.M.) in the T_b of each individual rat

Microinjections, separated by an interval of 15 min into the AHPOA of: 5·0 pg of MIP-1β followed by saline, ▥; saline followed by 25 pg of MIP-1α, ▩; and 5·0 pg of MIP-1β followed by 25 pg of MIP-1α, ⊟. Significance of MIP-1α, ***$P < 0{\cdot}001$.

interleukin released in the hypothalamus may contribute to the febrile response induced by native MIP-1 (Miñano, Vizcaino & Myers, 1992). In accordance with this viewpoint, MIP-1 or MIP-1α, but not MIP-1β, stimulates the secretion of other pyrogenic cytokines, particularly TNFα, IL-1α and IL-6; however, MIP-1β acts to antagonize the effect of MIP-1α in inducing the secretion of TNF (Fahey *et al.* 1992). Recently, the presence of abundant MIP-1 receptors has been demonstrated on both human and murine K562 cells, and in spite of the differences in their primary structures, both MIP-1α and MIP-1β compete for binding sites on the surface receptors of FDCP mix cells (a stem cell-like murine progenitor cell line) with identical affinity (Graham *et al.* 1993). Based on each of these effects, therefore, Widmer *et al.* (1993) have suggested that 'specialized' cells may regulate the expression of MIP-1α and MIP-1β differentially. Taken together, these observations thus suggest that the MIP-1 peptides play a role in modulating the host-defence febrile response, either directly or indirectly, by stimulating the secretion of one or more endogenous pyrogens.

Of significance is the fact that the concurrent microinjection of MIP-1β and MIP-1α into the AHPOA produces a smaller increase in the T_b than either MIP-1β or MIP-1α administered alone at the same hypothalamic site and in the same doses. Moreover, the pretreatment of the AHPOA with a low dose of MIP-1α or MIP-1β , followed by high doses of MIP-1β or MIP-1α, respectively, results in a reduced level of T_b similar to that following a control saline injection. Thus, the biological actions of MIP-1 are presumably receptor mediated, as demonstrated by Fahey *et al.* (1992). Consequently, the fever induced by MIP-1β could be mediated centrally by a specific receptor which is different from a MIP-1α receptor, and in combination, both MIP-1α and MIP-1β apparently compete for the same receptor protein. Recently it was reported that human and murine recombinant MIP-1α and MIP-1β bind to the same receptor molecule with a similar affinity (Graham *et al.* 1993) and, in fact, as little as eightfold excess of the MIP-1β blocks the induction of TNF by MIP-1α to a significant degree (Fahey *et al.* 1992). Hence, when acting in combination, the two chemokines block the febrile effect of each other. This divergence with respect to the pyrexic activity of MIP-1α and MIP-1β may offer an additional level for fever control, and according to other authors it is suggested that a network of cytokines participates in the complex processes underlying a fever, which involve synergistic as well as inhibitory interactions (Kluger, 1991; Bluthé, Dantzer & Kelley, 1992).

In conclusion, the regulatory mechanisms for MIP-1 and its interactions with other cytokines are exceedingly complex. Nevertheless, it is likely that the peptide components of MIP-1 which elicit a febrile response in the rat are receptor mediated. Since MIP-1α and MIP-1β act differentially on cells of the AHPOA, the febrile response induced by MIP-1α may be the result of the activation of other mechanisms, including the synthesis of IL-1, IL-6 or TNFα. However, the functional antagonism between MIP-1α and MIP-1β observed in the present study suggests that their pyrogenic properties may be due to a competition for either one or more classes of receptor molecule. Indeed, our observations demonstrate that these two cytokines oppose each other by acting directly within the AHPOA, either on the same neurons or on different, but functionally interconnected neurons. Thus, specialized hypothalamic cells may serve to regulate the expression of MIP-1α and MIP-1β differentially, in a manner similar to the autocrine effects of these peptides on peritoneal macrophages (Fahey *et al.* 1992). Finally, these findings are consistent with the hypothesis that endogenously produced MIP-1α and MIP-1β may provide a unique mechanism/level for the control of fever.

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