Activation of central melanocortin-4 receptor suppresses lipopolysaccharide-induced fever in rats

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Sinha, Partha S., Helgi B. Schioth, and Jeffrey B. Tatro. Activation of central melanocortin-4 receptor suppresses lipopolysaccharide-induced fever in rats. Am J Physiol Regul Integr Comp Physiol 284: R1595–R1603, 2003; 10.1152/ajpregu.00581.2002.—Activation of central melanocortin receptors (MCR) inhibits fever, but the identity of the MCR subtype(s) mediating this antipyretic effect is unknown. To determine whether selective central melanocortin receptor-4 (MC4R) activation produces antipyretic effects, the MC4R selective agonist MRLOB-0001 (CO-His-D-Phe-Arg-Trp-Dab-NH2) was administered intracerebroventricularly to rats treated with Escherichia coli lipopolysaccharide (LPS, 30 µg/kg ip). Treatment with MRLOB-0001 (150 ng icv) did not lower core body temperature (Tc) in afebrile rats but did suppress LPS-induced increases in Tc and associated decreases in tail skin temperature (Ts), an indicator of vasomotor thermoeffector function. In contrast, systemic treatment with MRLOB-0001 (150 ng iv) did not produce similar antipyretic effects. Coadministration of the selective MC4R agonist HS014 (1 µg icv) blocked the antipyretic effects of MRLOB-0001. HS014 alone (1 µg icv) had no significant effect on LPS-induced increases in Tc or decreases in Ts and in afebrile rats had no significant effects on Tc or Ts. We conclude that pharmacological activation of central MC4R suppresses febrile increases in Tc and that inhibition of heat conservation pathways may contribute to this effect. These findings suggest that the central MC4R may mediate the long-recognized antipyretic effects of centrally administered melanocortins.

antipyretic; thermoregulation; thermoeffector; peripheral vasoconstriction; HS014

FEVER IS A HALLMARK of the acute phase response to infection that is orchestrated by the central nervous system (CNS) and is believed to have adaptive value in optimizing host immune responses against microbial invasion (17). However, because excessive fever can prove dangerous to the host, it is believed that counterregulatory systems have evolved to modulate the duration and magnitude of the febrile response (20, 29). Among these systems, the central melanocortinergic system is a prominent pharmacological target capable of suppressing fever.

Activation of central melanocortin receptors (MCRs) suppresses fever. For example, the endogenous melanocortin α-melanocyte stimulating hormone (α-MSH), a 13-amino acid peptide derived from proopiomelanocortin, has well-documented antipyretic activity. Administration of exogenous α-MSH, which acts as a nonselective MCR agonist, suppresses experimental fevers induced by immune stimuli, including the bacterial endotoxin lipopolysaccharide (LPS; see Refs. 6 and 29). It has been proposed that the MCR-3 (MC3R) and/or MCR-4 (MC4R), both of which are CNS-associated MCR subtypes, mediate these antipyretic effects, because intracerebroventricular administration of SHU-9119, a nonselective synthetic MC3R/MC4R antagonist, blocked suppression by α-MSH of LPS-induced fever in rats (12, 13). However, the specific roles of MC3R and MC4R in mediating these effects are unknown.

It is of fundamental interest to determine the pharmacological mechanisms involved in mediating the antipyretic actions of melanocortins. The MC4R, which has been the focus of recent interest as a key central regulator of energy homeostasis (4), is expressed widely among thermoregulatory and other autonomic centers in the CNS (21, 30, 31). Its role in regulation of fever remains unknown, and it is unknown whether selective MC4R activation is sufficient to produce antipyretic effects. The recent development of novel synthetic ligands having improved selectivity for MC4R has enhanced the prospects for investigating the specific role of this receptor in regulation of various CNS functions, including fever.

The aim of this study was to test the hypothesis that pharmacological activation of central MC4R signaling suppresses febrile responses. The effect of central infusion of a novel selective MC4R agonist, MRLOB-0001 (CO-His-d-Phe-Arg-Trp-Dab-NH2) (Dab: 2,4-di-amino butyric acid; see Ref. 3), on core body temperature (Tc) in LPS-challenged febrile rats was determined in the

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presence and absence of coadministration of the selective MC4R antagonist, HS014 (16). The potential contribution of general motor activity and heat-conserving thermoeffectors to these responses was assessed by measuring gross motor activity and changes in local skin temperature of the tail, the major thermoeffector organ controlling radiant heat loss in rats, reflecting alterations in peripheral vasomotor tone (11). The results indicate that selective central MC4R activation by MRLOB-0001 suppresses fever and that this suppression is associated with inhibition of fever-associated increases in peripheral vasomotor tone.

MATERIAL AND METHODS

Animals and Surgical Procedures

Adult male Sprague-Dawley rats (Taconic, Germantown, NY) initially weighing 250–300 g were used. Rats were initially housed three per cage in a room with temperature maintained at 21 ± 1°C and a 14-h light cycle (lights on 0600–2000). Standard rodent chow (Harlan, Madison, WI) and tap water were provided ad libitum throughout the experiment. All procedures were approved by the Institutional Animal Care and Use Committee of Tufts University Medical School and New England Medical Center, and these studies comply with the guiding principles for research of the American Physiological Society (2).

Before experiments (7–10 days), each rat was anesthetized with pentobarbital sodium (45 mg/kg ip) and implanted intraperitoneally with a radiotelemetry transmitter (E-Mitter 4000 System; Mini Mitter, Bend, OR) for monitoring Tc and gross motor activity. A 22-gauge stainless steel guide cannula (Plastics One, Roanoke, VA) was permanently implanted in the right lateral ventricle for intracerebroventricular injections (Plastics One, Roanoke, VA) was permanently implanted in the right lateral ventricle for intracerebroventricular injection, as described earlier (12). Coordinates for right lateral ventricular cannulation, determined using a rat brain atlas (22), were as follows (incisor bar set 3.3 mm below the horizon plane): 0.8 mm posterior to bregma, 1.4 mm lateral, and 3.4 mm ventral to the skull surface. After surgery, the rats were housed individually in cages in a separate room maintained at 28 ± 1°C, within the thermoneutral range for rats (11), and with a 12:12-h light-dark cycle (lights on 0600–1800). Examination of the diurnal Tc, and motor activity profiles for the day preceding experiments confirmed that the rats were entrained to the 12:12-h light-dark cycle (data not shown). Correct placement of intracerebroventricular cannulas was verified at the end of the experiment by injection of 10 μl of 0.1% trypan blue followed by postmortem brain dissection. Data obtained from rats with misplaced cannulas were excluded from analysis. For studies involving intravenous injections, 7 days before experiments, rats were anesthetized using ketamine (40 mg/kg)-xylazine (8 mg/kg) and implanted with permanent intravenous catheters in the right jugular vein followed immediately by radiotelemetry transmitter implantation, as described above. Catheters were constructed of an 8.2-cm length of Micro-Renathane tubing (0.04 in. OD × 0.025 in. ID; Braintree Scientific, Braintree, MA) fused via a short length of 22-gauge tubing to a 2.5-cm length of medical-grade silicone tubing (Silastic, 0.047 in. OD × 0.025 in. ID; Dow-Corning, Midland, MI). The silicone tubing was inserted to the level of the right atrium, and the catheter was anchored in place with ligatures. The Micro-Renathane end was fitted with a subcutaneous lug constructed of a short length of intravenous set tubing affixed with silicone cement, and the proximal end was exteriorized at the nape of the neck, secured in place with wound clips, and closed with a 22-gauge stainless steel obturator. Catheters were flushed with 0.4 ml heparinized saline daily to maintain patency. Heparinized saline was prepared by rinsing a 1-ml tuberculin syringe with heparin sodium (10,000 U/ml; Pharmacia & Upjohn, Kalamazoo, MI) and mixing the residual solution in the syringe with 1 ml pyrogen-free 0.9% NaCl.

Animal Handling and Intracerebroventricular Injections

Studies were performed on conscious, unrestrained rats. Each rat was subjected to only one experiment. To minimize the potential influence of stress on results, each rat was conditioned daily for five consecutive days before experiments to a regimen that included gentle handling and a simulated intracerebroventricular injection, performed by removing the dummy cannula and connecting the injection device to the intracerebroventricular guide cannula, or flushing of intravenous catheters. Intracerebroventricular injections were administered via an injection cannula designed to protrude 1 mm from the guide cannula and connected via PE-50 tubing to a 1-ml Hamilton syringe driven by a microinfusion pump (Bee, MF-9090; Bioanalytical Systems, West Lafayette, IN). Injections were delivered at a speed of 2.5 μl/min over a 2-min time period for a final intracerebroventricular injectate volume of 5 μl. Internal cannulas were left in place for 1 min after injection to prevent reflux of injectate.

Tc, Motor Activity, and Tail Skin Temperature Measurements

The radiotelemetry signals emitted by each implanted transmitter were monitored continuously via a receiver (Mini Mitter) placed under each cage. These signals were transmitted to a personal computer (Dell, Round Rock, TX), integrated over 5-min intervals, and converted to Tc values according to the frequency-temperature calibration curves using the Vitalview software package (Mini Mitter). General motor activity was also measured using the Mini Mitter system. In this system, activity is detected as changes in signal strength arising from changes in position or orientation of the transmitter and are recorded as motor activity counts.

To monitor vasomotor thermoeffector responses noninvasively in the conscious, unrestrained rats, tail skin temperatures (Tsk) were determined using an infrared pyrometer device (Omega Engineering, Stamford, CT). Permanent black ink marks were placed bilaterally on the skin overlying the lateral tail vein ~1 cm from the base of the tail. For determination of Tsk, the infrared pyrometer was held in a horizontal position, perpendicular to the tail skin surface, and aimed at the marked skin surface from a distance of ~8 mm. Bilateral Tsk readings were recorded and averaged at the indicated times.

Experimental Protocol

Before experiments (1 day), rats were weighed and arbitrarily assigned to body weight-matched experimental groups. On the day of the experiment, each rat received an intraperitoneal injection of either 200 μl vehicle (pyrogen-free 0.9% NaCl) or LPS (30 μg/kg in 200 μl vehicle) at a time (designated time 0) between 0900 and 1000. Thirty minutes after the intraperitoneal injection (time 0.5 h), rats received intracerebroventricular injections with either artificial cerebrospinal fluid (aCSF) in mM): 138 NaCl, 3.37 KCl, 1.5 CaCl2, 1.15 MgCl2, 1.45 Na2HPO4, and 4.85 NaH2PO4, pH 7.4), varying doses of MRLOB-0001 [50, 150 (180 pmol), or...
500 ng, and/or 1 µg (600 pmol) HS014 diluted in aCSF. In one experiment, rats received similar intraperitoneal treatments with LPS or 0.9% NaCl, followed 30 min later by intravenous injections of MRLOB-0001 (150 ng in 0.15 ml of 0.9% NaCl) via intrajugular catheters. Tc and motor activity were recorded at 5-min intervals starting at 0900 the day before the experiment and ending at 0900 the day after the experiment. MC3R (AUC Tc), and total motor activity between treatment groups were determined by one-way ANOVA using SigmaStat software (SPSS Science, Chicago, IL) followed by post hoc tests using the Bonferroni correction for multiple comparisons. Differences showing a P value <0.05 after Bonferroni correction were deemed significant.

RESULTS

Effects of MC4R Agonist on LPS-Induced Changes in Tc, Tail Skin Vasomotor Response, and Motor Activity

Tc. A small rise in Tc (<0.5°C), peaking at time 1–1.5 h, was observed in all groups (Fig. 1A) because of the minor stress of handling, injections, and associated increase in motor activity (Fig. 1D). After this, LPS (30 µg/kg ip)-treated rats exhibited a biphasic rise in Tc, with the first peak occurring during phase 1 (time 0–3.5 h) and the second peak occurring during phase 2 (time 4–8 h). During both phases, the AUC Tc for LPS-treated rats was significantly higher than that of vehicle-injected rats (Fig. 1B).

The dose-response relationship for effects of intracerebroventricular MRLOB-0001 on LPS-induced fever was determined. At a dose of 50 ng icv MRLOB-0001 had no effect on the LPS-induced rise in Tc (n = 2, data not shown), but MRLOB-0001 treatment at 150 ng (180 pmol) significantly suppressed the LPS-induced rise in Tc during both phases (Fig. 1A and B). In rats receiving 500 ng MRLOB-0001, the LPS-induced rise in Tc was suppressed to an extent that was virtually identical to that seen after the 150-ng dose (n = 4, data not shown), indicating that doses of MRLOB-0001 ≥150 ng icv were maximally effective. To determine whether the antipyretic effects of centrally administered MRLOB-0001 were mediated within the CNS, we tested the effect of systemic injection of an antipyretic dose of MRLOB-0001 (150 ng). In contrast with the marked antipyretic effect of intracerebroventricular injection of MRLOB-0001, LPS-induced Tc profiles were virtually identical in rats receiving intravenous MRLOB-0001 and in those receiving 0.9% NaCl for at least 5 h (data not shown), and there were no significant treatment effects on Tc during either phase 1 (LPS/NaCl, 2.6 ± 0.6, n = 5; LPS/MRLOB-0001, 2.9 ± 0.6°C·h, n = 5) or phase 2 (LPS/NaCl, 7.1 ± 1.0, n = 5; LPS/MRLOB-0001, 5.9 ± 1.3°C·h, n = 5).

Tail skin vasomotor response. LPS (30 µg/kg ip)-treated rats exhibited a marked, significant reduction in Tsk values vs. those of vehicle-injected controls that persisted during phases 1 and 2, indicative of a heat-conserving vasomotor response activated during LPS-induced fever (Fig. 1, C and D). Intracerebroventricular treatment with MRLOB-0001 (150 ng) prevented the LPS-induced drop in Tsk during both phases 1 and 2 (Fig. 1, C and D).

Motor activity. During phase 1, all groups exhibited a brief increase in gross motor activity that lasted ~1.5 h after intraperitoneal and intracerebroventricular injections (Fig. 1E). There were no significant differences.
in motor activity among groups during phase 1 (Fig. 1F). During phase 2, LPS/aCSF-treated rats receiving only aCSF intracerebroventricularly exhibited significant decreases in total motor activity compared with rats receiving intraperitoneal vehicle/intracerebroventricular aCSF (Fig. 1, E and F). Treatment with MRLOB-0001 (150 ng icv) had no significant effect on the LPS-induced decrease in total motor activity during phase 2 (Fig. 1F). These results indicate that the LPS and MRLOB-0001-induced changes in Tc could not be attributed to corresponding changes in gross motor activity.

Effect of Central MC4R Blockade by Intracerebroventricular HS014 on Antipyretic Actions of MC4R Agonist

Tc. To determine whether the antipyretic action of MRLOB-0001 was mediated specifically via the MC4R, we tested the ability of a selective MC4R antagonist to inhibit the effect. Coadministration of the MC4R antagonist HS014 (1 μg, 600 pmol icv) with the lower effective dose of MRLOB-0001 determined above (150 ng icv) prevented the suppression by MRLOB-0001 of the LPS-induced increase in Tc during phase 1. During phase 2, Tc responses of LPS/(MRLOB-0001 + HS014)-treated rats were not significantly different from those observed in either LPS/aCSF- or LPS/MRLOB-0001-treated rats (Fig. 1, A and B). In the absence of coadministered MRLOB-0001, HS014 had no significant effect on Tc responses in either LPS-treated or NaCl-treated rats (Fig. 2, A and B).

Skin vasomotor response and motor activity. Coadministration of HS014 prevented the suppression by MRLOB-0001 of the LPS-induced decrease in Tk during phase 1 of fever (Fig. 1, C and D). During phase 2, the integrated changes in tail skin temperature (AUC ΔTsk) seen in LPS/(MRLOB-0001 + HS014)-treated rats were not significantly different from those observed in either LPS/aCSF- or LPS/MRLOB-0001-treated rats (Fig. 1D). Nevertheless, the time course of ΔTsk indicated that the blockade of MRLOB-0001 effects on Tk responses by HS014 persisted at least through 4.5 h (Fig. 1C). In the
Absence of coadministered MRLOB-0001, HS014 had no significant effect on Tsk responses in either LPS-treated or NaCl-treated rats (Fig. 2, C and D). Coadministration of HS014 with MRLOB-0001 had no significant effect on total motor activity vs. that in LPS-treated rats that received only aCSF intracerebroventricularly (Fig. 1, E and F). In the absence of coadministered MRLOB-0001, HS014 had no significant effects on Tsk or gross motor activity (Fig. 2, C–F).

Effect of Selective MC4R Agonist in Afebrile Rats

Tc. Administration of MRLOB-0001 (150 ng icv) in rats treated intraperitoneally with vehicle caused a rise in Tc that was significantly greater than that seen in intraperitoneal NaCl/intracerebroventricular aCSF-treated controls during the period 0.5–3.5 h (corresponding to phase 1 in LPS-treated rats; Fig. 3, A and B). No differences in Tc profiles were seen among these two groups during the 4- to 8-h period (corresponding to phase 2; Fig. 3, A and B).

Skin vasomotor response and motor activity. There were no significant differences in Tsk profiles between NaCl/MRLOB-0001- and NaCl/aCSF-treated rats during either the 0.5- to 3.5-h or 4- to 8-h periods (Fig. 3, C and D), although there was a trend toward increased motor activity (P = 0.09) in the NaCl/MRLOB-0001 rats during the 0.5- to 3.5-h period (corresponding to phase 1: Fig. 3, E and F). During this period, all NaCl/MRLOB-0001-treated rats, but not NaCl/aCSF-treated rats, exhibited a marked increase in grooming behavior. Grooming, a classic behavioral response to intracerebroventricular administration of melanocortins (8), commenced within 3–5 min of MRLOB-0001 administration and lasted ~2 h. Because it was not a formal objective of this study to assess grooming responses, the induction of this marked and reproducible behavioral response was noted qualitatively but was not quantified. None of the rats in the LPS-treated groups exhibited this response.
DISCUSSION

These findings support the hypothesis that central activation of the MC4R suppresses the febrile response. Intracerebroventricular administration of the selective MC4R agonist MRLOB-0001 suppressed LPS-induced increases in Tc, but did not lower Tc in afebrile rats. LPS administration caused a decrease in Tsk, reflecting reduced blood flow to the skin via the lateral tail vein, a well-established heat-conserving thermoeffector response associated with the elevated body temperature set point during fever (11). Treatment with MRLOB-0001 prevented this LPS-induced peripheral vasoconstriction. In contrast, in afebrile rats MRLOB-0001 treatment did not lower Tc or raise Tsk, indicating that its suppression of LPS actions reflected an antipyretic, rather than a cryogenic effect, consistent with the well-documented antipyretic effects of centrally administered exogenous melanocortins (29). Furthermore, MC4R blockade by coadministered HS014 completely blocked the antipyretic effects of MRLOB-0001 during the first phase of fever. In addition, a dose of MRLOB-0001 that was antipyretic when given intracerebroventricularly failed to inhibit fever when injected systemically, indicating that its antipyretic action was mediated by activation of MC4R within the CNS. Therefore, the antipyretic effect of the selective MC4R agonist and its blockade by a selective MC4R antagonist strongly suggest that the antipyretic effect of MRLOB-0001 is mediated by MC4R within the CNS. Previous studies showed that centrally administered α/MSH exerts an antipyretic effect in rats that is qualitatively similar to the presently observed effect of MRLOB-0001, and which is blocked by coadministered SHU-9119, an MCR antagonist having equivalent potencies at the MC3R and MC4R (12). Therefore, considered together with the previous findings, the present findings further suggest a probable role of MC4R in mediating the long-recognized antipyretic actions of centrally administered α-MSH, a nonselective MC4R agonist.

To determine whether the antipyretic effects of MRLOB-0001 were mediated specifically via the central nervous system, the effect of MRLOB-0001 on Tc, Tsk, and gross motor activity was assessed in afebrile rats treated ip with saline. Data in C–F are from the same rats shown in A and B. Also, NaCl/aCSF data are identical to those shown in Fig. 1, and are included here for display purposes. All treatment groups shown in Figs. 1 and 3 were part of the same experiment series and were subjected to simultaneous statistical analysis. Times of ip and icv injections are indicated (arrows). Baseline Tc in respective groups were as follows (°C): NaCl/aCSF, 37.5 ± 0.05 (n = 5); NaCl/MRLOB-0001, 37.5 ± 0.03 (n = 5). A: time course of Tc responses. B: ∆Tc AUC during phase 1 (0.5–3.5 h) and phase 2 (4.0–8.0 h). *Significant vs. NaCl/aCSF (P = 0.003; phase 1 only). C: time course of Tsk responses. D: ∆Tsk AUC during phases 1 and 2. No significant differences occurred between groups. E: time course of gross motor activity responses. Note brief rise in gross motor activity associated with injections in all treatment groups. F: summation of gross motor activity during phases 1 and 2. No significant differences occurred between groups.

Fig. 3. Effect of MRLOB-0001 (150 ng icv) on Tc (A and B), Tsk (C and D), and gross motor activity (E and F) in afebrile rats treated ip with saline. Data in C–F are from the same rats shown in A and B. Also, NaCl/aCSF data are identical to those shown in Fig. 1, and are included here for display purposes. All treatment groups shown in Figs. 1 and 3 were part of the same experiment series and were subjected to simultaneous statistical analysis. Times of ip and icv injections are indicated (arrows). Baseline Tc in respective groups were as follows (°C): NaCl/aCSF, 37.5 ± 0.05 (n = 5); NaCl/MRLOB-0001, 37.5 ± 0.03 (n = 5). A: time course of Tc responses. B: ∆Tc AUC during phase 1 (0.5–3.5 h) and phase 2 (4.0–8.0 h). *Significant vs. NaCl/aCSF (P = 0.003; phase 1 only). C: time course of Tsk responses. D: ∆Tsk AUC during phases 1 and 2. No significant differences occurred between groups. E: time course of gross motor activity responses. Note brief rise in gross motor activity associated with injections in all treatment groups. F: summation of gross motor activity during phases 1 and 2. No significant differences occurred between groups.
MC4R, we used the MC4R-selective antagonist HS014. Central administration of HS014 had no significant effects on Tc in either febrile or afebrile rats, indicating that it is not intrinsically hyperthermic. The intracerebroventricular dose of HS014 used was predicted to be adequate to block the action of coadministered MRLOB-0001 at the ~3:1 molar dose ratio used, based on the in vitro potencies reported in binding and biological assays (3, 25). For example, centrally injected (ivc) HS014 blocked the anorectic and several other behavioral effects of coadministered α-MSH when used at approximate molar dose ratios of (2–3):1 (32). Furthermore, HS014 is particularly selective for the MC4R of the rat, for which it has been shown to have 85–100 times greater binding affinity than for the MC3R (19, 25). Together, these lines of evidence strongly support the conclusion that the blockade of the antipyretic effect of MRLOB-0001 by HS014 is caused by its selective antagonism at MC4R.

Previous studies indicated that endogenous central melanocortins exert antipyretic effects during experimental fever (12, 14, 27), probably by acting at MC3R or MC4R since central MCR blockade using the MC3R/MC4R antagonist SHU-9119 (12, 14) exacerbated LPS-induced fevers in rats. The present finding that the selective MC4R antagonist HS014 failed to exacerbate LPS-induced fever suggests that endogenous melanocortins may not exert these physiological antipyretic effects by acting at the MC4R, but it does not rule out a physiological antipyretic role of the MC4R. The dose of HS014 used was found to be sufficient to block the antipyretic effects of coadministered MRLOB-0001 at its site(s) of action. However, it is conceivable that the antipyretic actions of endogenous neuronal melanocortins are exerted at multiple CNS sites, in which case intraparenchymal concentrations of HS014 in certain critical regions could potentially have been insufficient to antagonize such actions, e.g., at CNS sites more distant from the ventricles. Alternatively, it is possible that the antipyretic actions of endogenous melanocortins are mediated at least in part via the MC3R, at which HS014 is a less potent antagonist than SHU-9119 (26), or that an antipyretic influence of the MC4R is exerted in a time frame that exceeds that of the biological activity of HS014 in the present study. A recent study found that febrile responses to intracerebroventricular injection of interleukin-1β were unaffected by a coinjected MC3R/MC4R antagonist. This may indicate that MC3R and MC4R do not modulate the thermoregulatory response to intracerebral interleukin-1β, but whether that model system is directly relevant to fever induced by systemic microbial pyrogens such as LPS is not clear, and it is uncertain whether intraparenchymal antagonist concentrations would have been adequate to block antipyretic actions of endogenous melanocortins at their target sites (18). Further studies will be required to elucidate the nature and physiological significance of the regulation of fever by the central melanocortin system.

The mechanisms involved and CNS sites at which MC4R activation produces antipyretic effects are unknown. The effects are almost certainly mediated, because the effective systemic dose of melanocortins required for antipyretic effects is ~100-fold greater than that required by intracerebroventricular administration, and the antipyretic effect of centrally injected α-MSH was blocked by coadministered MC3R/MC4R antagonist at a dose that was ineffective when administered intravenously (12, 13). Furthermore, MC4R is believed to be predominantly or exclusively expressed in the CNS, where it is distributed in numerous autonomic centers, including hypothalamic and preoptic regions containing circuitry believed to be involved in establishing body temperature set point, integration of afferent temperature sensory inputs, and descending control of thermogenesis (5, 30, 31). These areas also receive substantial innervation by endogenous propiomelanocortin-producing neurons (9, 30). Therefore, it is likely that activation of MC4R expressed in thermoregulatory neurons either inhibits the elevation of body temperature set point, or acts more distally and in a physiological state-dependent manner to inhibit descending thermoeffecter pathways. These possibilities remain to be tested.

In terms of effector systems, the present studies demonstrate that selective MC4R activation results in the inhibition of at least one thermoeffecter system involved in the febrile response, the peripheral vaso-motor response. This is consistent with a previous study in rabbits (10) that demonstrated antipyretic effects of α-MSH and ACTH, both of which are nonselective MCR agonists. Based on thermodynamic considerations alone, this effect, which inhibits retention of body heat, must contribute to the suppression of Tc elevation by MC4R activation. Whether this effect alone is sufficient to account for the net suppression of febrile Tc elevation by centrally administered melanocortins, or whether additional thermoeffecter systems are involved, remains to be determined.

In this connection, the present study provides no direct evidence that MC4R activation during the febrile state modulates general motor activity, one potential source of thermogenesis, since no significant effects of MRLOB-0001 or HS014 treatments on LPS-induced suppression of motor activity were observed. This contrasts somewhat with the moderate exacerbation of the LPS-induced suppression of motor activity we observed in response to intracerebroventricular α-MSH treatment in a related study (14). This difference could potentially be attributable to activation by the nonselective agonist α-MSH of MCR subtypes other than MC4R, which influence locomotor activity during fever. Alternatively, these differences in melanocortin influences on motor activity may be attributable to other major differences in design in the two studies, including the facts that the rats were fasted and received a higher LPS dose in our earlier study (14). One additional consideration in the present study is that all rats exhibited a robust increase in motor activity during the first 1.5 h after intraperitoneal injections, which may have obscured subtle treatment-associated changes in motor activity during that period. The ques-
tion of whether the antipyretic action of MC4R agonists could potentially involve alterations in thermogenesis via altered motor activity merits further study.

The suppressive effects of MRLOB-0001 on LPS-induced $T_c$ elevation and peripheral vasomotor responses persisted during both phases of fever, whereas the blockade of antipyretic effects by coadministration of HS014 subsided partially during the second phase (4–8 h) of fever. The relatively greater persistence of MRLOB-0001 effects compared with those of HS014 may reflect pharmacokinetic differences, such as greater in vivo stability or decreased receptor dissociation rates of MRLOB-0001 compared with HS014, but available data are insufficient to address these possibilities directly. Nevertheless, the results provide additional evidence of the in vivo potency of MRLOB-0001, as indicated by its brisk induction of grooming activity in afebrile rats. Induction of grooming behavior is a classic response to centrally administered melanocortins, including α-MSH, and is thought to be mediated by MC4R within the CNS (1). By comparison, induction of grooming behavior by α-MSH requires at least a 10-fold higher molar dose than the presently effective dose of MRLOB-0001 (8). In addition, treatment with MRLOB-0001 failed to elicit a grooming response in LPS-treated rats, indicating that the induction of grooming behavior by MC4R activation, like its effects on body temperature, is highly dependent on physiological state.

An alternative interpretation of the MRLOB-0001-induced grooming response, seen exclusively in afebrile controls, concerns its thermoregulatory implications. The induction of grooming behavior in afebrile rats after intracerebroventricular MRLOB-0001 administration was accompanied by a coincident increase in $T_c$. Grooming is thought to be an adaptive response employed by rodents to lower body temperature during hyperthermic states, i.e., when $T_c$ exceeds body temperature set point, and is characterized by use of the tongue and paws to spread saliva over the body starting at the head, progressing to the torso, and ending with the limbs (11). Because the presently observed MRLOB-0001-induced grooming response followed this pattern, it is possible that activation of central MC4R by MRLOB-0001 induces a neurally mediated hyperthermic effect in afebrile rats, leading in turn to induction of grooming behavior, a secondary homeostatic response to the hyperthermia. This is consistent with reports that pharmacological activation of central MC4R and/or MC3R stimulates oxygen consumption and thermogenic activity in brown fat in normal rats and that MC4R-deficient mice exhibit decreased melanocortin- and leptin-induced thermogenesis (7, 24, 28).

In contrast, LPS-treated rats receiving MRLOB-0001 treatment exhibited little or no rise in $T_c$ during phase 1, and the MRLOB-0001-induced grooming response was absent in those animals. Because the results of MRLOB-0001 treatment were qualitatively opposite in febrile and afebrile rats ($T_c$ lowering vs. $T_c$ elevating, respectively), these findings underscore the exquisite specificity of the antipyretic effect of centrally administered MRLOB-0001 and further indicate that the effects of MC4R activation on $T_c$ are highly dependent on physiological state.

In summary, these results indicate that central MC4R activation suppresses fever and that prevention of fever-associated peripheral vasconstriction contributes to the antipyretic response. This study also demonstrates that the thermoregulatory effects of MC4R activation are markedly dependent on physiological state, suppressing the elevation of $T_c$ associated with an inflammatory state while elevating $T_c$ in afebrile rats. Determination of the neural mechanisms involved in these effects, and the potential role of the MC4R in mediating other recognized melanocortin-induced suppressive effects on neuroinflammatory processes in the CNS (15), will be of substantial interest.

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