Systemic α-MSH suppresses LPS fever via central melanocortin receptors independently of its suppression of corticosterone and IL-6 release

Huang, Qin-Heng, Victor J. Hruby, and Jeffrey B. Tatro. Systemic α-MSH suppresses LPS fever via central melanocortin receptors independently of its suppression of corticosterone and IL-6 release. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R524–R530, 1998.—Systemically administered α-melanocyte-stimulating hormone (α-MSH) inhibits endotoxin (lipopolysaccharide; LPS)-or interleukin (IL)-1-induced fever and adrenocortical activation, but the sites of these actions and the mechanisms involved are unknown. The aims of this study were, first, to determine whether melanocortin receptors (MCR) located within the central nervous system mediate the suppressive effects of peripherally administered α-MSH on LPS-induced fever and activation of the pituitary-adrenal axis and, second, to determine whether systemic α-MSH suppresses the LPS-induced rise in plasma IL-6 levels, potentially contributing to its antipyretic effect. Male rats received Escherichia coli LPS (25 µg/kg ip). Core body temperatures (Tb) were determined hourly by radiotelemetry (0–8 h), and blood was withdrawn via venous catheters for plasma hormone immunoassays (0–2 h) and IL-6 bioassay (0–8 h). α-MSH (100 µg/kg ip) completely prevented the onset of LPS-induced fever during the first 3–4 h after LPS and suppressed fever throughout the next 4 h but did not affect Tb in afebrile rats treated with intraperitoneal saline rather than LPS. Intraperitoneal α-MSH also suppressed the LPS-induced rise in plasma IL-6, ACTH, and corticosterone (CS) levels. Intracerebroventricular injection of SHU-9119, a potent melanocortin-4 receptor (MC4-R)/MC3-R antagonist, completely blocked the antipyretic effect of intraperitoneal α-MSH during the first 4 h after LPS but had no effect on α-MSH-induced suppression of LPS-stimulated plasma IL-6 and CS levels. Taken together, the results indicate that the antipyretic effect of peripherally administered α-MSH during the early phase of fever is mediated by MCR within the brain. In contrast, the inhibition of LPS-induced increases in plasma CS and IL-6 levels by intraperitoneal α-MSH appears to be mediated by a different mechanism(s), and these effects do not contribute to its antipyretic action.

IN VERTEBRATES, BACTERIAL infection activates an array of stereotypical adaptive responses, including fever and increased adrenal glucocorticoid secretion, that are believed to be mediated in large part by host-derived proinflammatory cytokines (5, 13). Exogenous melanocortins [α-melanocyte-stimulating hormone (α-MSH) and ACTH-related peptides] have been widely observed to suppress these responses. Administration of α-MSH either centrally or peripherally, at doses that do not affect body temperatures (Tb) of afebrile animals, inhibits its fevers induced by endotoxin, interleukin (IL)-1, IL-6, or tumor necrosis factor-α (3). Similarly, exogenous α-MSH suppresses lipopolysaccharide (LPS)- or IL-1-induced adrenocortical activation (17, 20).

The mechanisms involved in these effects and the precise sites of action of α-MSH are unknown, but certain evidence points to a role of melanocortin receptors (MCR) within the brain in mediating the antipyretic action of α-MSH. Melanocortins have greater antipyretic potency when administered centrally than peripherally (30), and intraparenchymal administration of melanocortins in preoptic and septal central nervous system (CNS) sites suppresses fever in animal models (2, 6). In addition, MCR proteins and mRNA encoding the MCR subtypes MC3-R and MC4-R are present in animals and humans in a number of hypothalamic and other brain regions believed to be involved in fever and regulation of the hypothalamic-pituitary-adrenal (HPA) axis (15, 18, 22, 25). We recently showed that the antipyretic effect of centrally administered α-MSH in rats is mediated by central MCR, because it was prevented by blockade of central MCR using intracerebroventricular injection of an MC4-R/MC3-R antagonist (9)[Ac-Nle<sup>4</sup>,-D-Val<sup>7</sup>,-Lys<sup>11</sup>,-NH<sub>2</sub>; SHU-9119 (8)]. Furthermore, SHU-9119 given intracerebroventricularly, but not intravenously, exacerbated Escherichia coli LPS-induced fever, indicating that endogenous melanocortin peptides act via central MCR during LPS-induced fever to exert an antipyretic effect (9).

Despite this evidence that α-MSH can act within the CNS to modulate fever, it is not clear whether systemic α-MSH may exert its antipyretic or HPA axis-inhibitory effects by acting on target sites within the CNS, because the ability of blood-borne α-MSH to traverse the blood-brain barrier (BBB) is very limited (28). Therefore, one objective of the present study was to determine whether MCR located within the CNS mediate the suppressive effects of peripherally administered α-MSH on LPS-induced fever and HPA responses. Although the postreceptor effectors involved in mediating the antipyretic action of α-MSH are unknown,
one potential mechanism for modulation of fever is via altered cytokine production. Therefore, our second objective was to determine whether systemically administered α-MSH suppresses the LPS-induced rise in blood levels of the endogenous pyrogenic cytokine IL-6 (13, 14), potentially contributing to α-MSH-induced suppression of LPS-induced fever and adrenocortical responses.

MATERIALS AND METHODS

Drugs

LPS (E. coli serotype 055:B5, no. L-4055, Sigma Chemical) was dissolved in sterile pyrogen-free 0.9% saline solution (saline). SHU-9119 was synthesized by Dr. Wei Yuan using methods previously reported (8). It was dissolved in saline containing 0.1% low-endotoxin BSA (Sigma, A-3675) at a concentration of 1 mg/ml and stored at −70°C. Immediately before each experiment, a stock aliquot of SHU-9119 was further diluted with saline to a final concentration of 50 ng/ml. Stock aliquots of synthetic α-MSH (Peninsula Laboratories, Belmont, CA) were diluted in saline immediately before use. Recombinant human IL-6 (specific activity, 10⁷ U/mg protein) was purchased from Collaborative Research (Bedford, MA).

Animals and Surgical Procedures

Adult male Sprague-Dawley rats (Taconic Farms or Harlan Sprague Dawley) initially weighing 270–300 g were used. Before surgery the rats were housed three per cage in a temperature (22 ± 1°C)- and light (12:12-h dark-light cycle, lights on at 0600)-controlled room, with standard rat chow and water available ad libitum. All procedures described were approved by the Animal Research Committee of Tufts University, School of Medicine and New England Medical Center. Each rat was anesthetized with pentobarbital sodium (50 mg/kg ip) and implanted intraperitoneally with a miniature radio transmitter (Mini-Mitter, Sunriver, OR). For experiments involving intracerebroventricular injections, rats were then implanted with a permanent 22-gauge stainless steel intracerebroventricular cannula (Plastics One, Roanoke, VA) in the right lateral ventricle, as described in detail previously (9). After surgery, the animals were kept in individual plastic cages and maintained in a separate room with temperature controlled at 25 ± 1°C, a near-thermoneutral ambient temperature for rats, by means of a convection heater with remote thermostat. Correct placement of intracerebroventricular cannulas was verified postmortem by injecting 10 µl of 0.4% trypan blue at the termination of the experiment. Only rats exhibiting staining throughout the ventricles were included in the analysis. For experiments involving blood sampling for hormone measurements, each rat was implanted with an indwelling jugular catheter under pentobarbital sodium anesthesia 2 days before the experiment. Intra jugular catheters consisted of a 5.5-in. length of silicone tubing (Silastic; Dow Corning, Midland, MI), exteriorized at the nape of the neck, filled with sterile heparinized saline solution, and sealed.

Animal Handling

To minimize the influence of nonspecific manipulative stress during experiments, each rat was conditioned by gentle handling daily for 5 consecutive days preceding the experiment. For rats bearing intracerebroventricular cannulas, the handling included a simulated intracerebroventricular injection performed by removing the dummy cannula and connecting the injection device.

Intracerebroventricular Infusion

For intracerebroventricular injections, the dummy cannula was removed and replaced with a 28-gauge internal cannula connected by a flexible connector tubing (C313CS, Plastic One) to a 100-µl Hamilton syringe driven by a microinfusion pump (BeeSyringe Pump MF-9090; Bioanalytical Systems, West Lafayette, IN), allowing each rat to move about freely in its home cage during infusion. Intracerebroventricular injectates were infused in a volume of 4 µl at a rate of 2 µl/min. After intracerebroventricular infusion, the internal cannula was left in place for 2 min to prevent backflow of injectate through the guide cannula.

Tb Measurements

Tb was measured hourly using a model RTA-500 receiver and a model SM-2372 frequency counter (Mini-Mitter). Emitted frequencies were converted to Tb by interpolating from the calibration curves of individual transmitters, and the transmitters were calibrated before and after each experiment according to the manufacturer’s instructions.

Experimental Protocols

Rats were randomly assigned to treatment groups, and each rat was used only once. Drugs for intraperitoneal injection (200-µl injection volume) or intracerebroventricular infusion were dissolved in saline. For each parameter tested, the total number of rats studied in each treatment group is indicated in the corresponding figure (Figs. 1–4). On the day of the experiment, immediately after baseline Tb data and hormone levels, this intraperitoneal LPS or saline injection was performed between 0900 and 1000 in all experiments.

Dose-response for antipyretic action of intraperitoneal α-MSH. Thirty minutes after LPS, rats were injected intraperitoneally with saline or with α-MSH at the indicated dose (25–100 µg/kg). Tb values were determined hourly for 8 h after LPS treatment by briefly placing each rat’s home cage on the receiver to record the emitted frequency (Fig. 1).

Effects of intraperitoneal α-MSH and central MCR blockade on LPS fever and IL-6 responses. Thirty minutes after LPS or saline treatment, rats received intracerebroventricular infusion of SHU-9119 or saline, followed immediately by intraperitoneal injection of saline or α-MSH (100 µg/kg) (Figs. 2 and 3). Blood samples (0.7 ml) were collected 2, 4, 6, and 8 h after the injection of intraperitoneal LPS or saline for the measurement of plasma IL-6 levels. To prevent loss of blood volume, immediately after each blood collection, 0.7 ml of sterile saline was infused via the intravenous catheter. No correction was applied for the minor dilution of total blood volume by the infused saline. Blood samples were placed in ice-chilled sterile test tubes containing Trasylol (500 kalikrein inhibitory units) and EDTA (1 mg). After centrifugation at 2,000 rpm for 10 min at 4°C, the plasma was aliquoted and kept at −20°C until assayed.

Effects of intraperitoneal α-MSH and central MCR blockade on LPS-induced ACTH and corticosterone secretion. In this experiment series, rats were prepared and experiments were performed exactly as described above for the studies of Figs. 2 and 3, except as follows: intraperitoneal implantation of radio transmitters and Tb determinations were omitted, and blood samples were collected immediately before and 30, 60, and 120 min after intraperitoneal administration of LPS or saline (Fig. 4).
Sal and Sal/LPS/Sal/Sal and LPS/comparisons at individual time points (ses. Symbols represent change in body temperature (Tb) from baseline (arrow). Numbers of animals in each group are indicated in parentheses and received intraperitoneal antipyretic effect of intraperitoneal ACTH and Corticosterone Assays

Plasma ACTH levels were determined using a two-site immunoradiometric assay kit (Nichols Institute DIAGNOSTICS, San Juan Capistrano, CA) according to the manufacturer’s instructions. Samples were assayed in duplicate. The assay limit of detection was 5 pg/ml. The assay is highly specific for ACTH and does not cross-react with α-MSH, β-MSH, β-lipotropin or β-endorphin. Plasma corticosterone (CS) levels were determined as described earlier (9). Briefly, samples were diluted to 1:100 in assay buffer (0.1 M PBS containing 0.1% gelatin and 0.04% sodium azide, pH 7.4) and heat denatured (70°C for 30 min). Samples were assayed in duplicate. The diluted plasma samples or CS reference standard (Sigma) was incubated overnight at 4°C with 125I-CS and rabbit anti-CS serum (ICN Pharmaceuticals, Costa Mesa, CA), and bound tracer was separated from unbound using a sheep anti-rabbit second antibody method on the following day. The inter- and intra-assay coefficients of variation were 9.2 and 10.3%, respectively.

Plasma IL-6 Assay

Plasma IL-6 levels were determined by bioassay using the IL-6-dependent B9 hybridoma cell line as described previously (10). Briefly, B9 cells were plated in 96-well flat-bottom tissue culture plates (Costar, Cambridge, MA) at a density of 7,000 cells per well in 200 µl RPMI 1640 media (Life Technologies, Gaithersburg, MD) containing 10% fetal bovine serum (HyClone Laboratories, Loral, UT) and 1% antibiotic-antimycotic (Life Technologies). The cells were incubated for 72 h in the presence of serial dilutions of samples, each assayed in duplicate, and the cells were pulsed with 10 ml of 0.5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) during the last 4 h of incubation. At the end of the incubation, conversion of MTT to formazan was determined colorimetrically as an index of B9 cell proliferation. Samples were read at 570 nm using a Bio-Rad model 3550 microplate reader, and the data were analyzed using microplate manager software (Bio-Rad Laboratories, Hercules, CA). IL-6 activity in the diluted plasma samples was interpolated from a reference standard curve based on recombinant human IL-6 (concentration range 0.028 to 1,666 pg/ml), run in the same
assay and expressed in nanograms per milliliter. The inter- and intra-assay variations were 8 and 12%, respectively.

Statistics

All data are presented as group means ± SE. T_b and plasma hormone data were first analyzed by two-way ANOVA for repeated measures. In experiments producing significant main effects, the data for each time point were then analyzed by one-way ANOVA, and significance of differences between treatment groups was determined by t-tests corrected for multiple comparisons by the method of Scheffe (19). Values of P < 0.05 were considered significant.

RESULTS

Potency and Time Course of Intraperitoneal α-MSH-Induced Suppression of LPS Fever

The antipyretic potency of intraperitoneal α-MSH in the rat was first determined. As shown in Fig. 1, LPS (25 µg/kg ip) produced a gradual rise in T_b, which reached maximal levels at 6–7 h after injection. α-MSH (100 µg/kg ip) injected 30 min after LPS administration produced a marked suppression of LPS-induced fever beginning at its onset and continuing throughout the 8-h observation period, whereas α-MSH at doses of 50 µg/kg (Fig. 1) and 25 µg/kg (data not shown) were ineffective. In contrast, α-MSH (100 µg/kg) had no effect on T_b in afebrile rats as compared with saline-injected controls (data not shown).

Effect of Central MCR Blockade on Antipyretic Action of Intraperitoneal α-MSH

To assess the role of central MCR in mediating the antipyretic action of intraperitoneal α-MSH, SHU-9119 (200 ng) was injected intracerebroventricularly 30 min after intraperitoneal LPS in α-MSH-treated and -untreated rats. Intracerebroventricular SHU-9119 completely prevented the antipyretic effect of intraperitoneal α-MSH during the first 4 h after LPS treatment (Fig. 2). During the period 4–8 h after LPS, T_b values in α-MSH-treated rats began to rise in parallel with, but still remained lower than, those in rats receiving LPS but not α-MSH (Fig. 2). SHU-9119 treatment had no effect on the sustained antipyretic effect of α-MSH during this latter period. In control rats receiving intraperitoneal saline rather than LPS, effects of comparable treatment with intraperitoneal α-MSH and intracerebroventricular SHU-9119 on T_b were negligible (Fig. 2).

Effect of α-MSH and of Central MCR Blockade on LPS-Induced Plasma IL-6 Elevation

The effects of intraperitoneal α-MSH treatment on LPS-induced plasma IL-6 levels were determined in the presence and absence of intracerebroventricular SHU-9119 injection. Plasma IL-6 levels increased markedly after LPS treatment, attaining maximal values 2 h after LPS and remaining elevated for several hours (Fig. 3). Treatment of rats with intraperitoneal α-MSH (100 µg/kg, 30 min after LPS) significantly reduced the LPS-induced elevation in plasma IL-6 levels (Fig. 3). In contrast to its blockade of the antipyretic effect of intraperitoneal α-MSH presented in Fig. 2, intracerebroventricular administration of SHU-9119 was completely without effect on α-MSH-induced suppression of plasma IL-6 levels in response to LPS (Fig. 3). The combined administration of intraperitoneal α-MSH and intracerebroventricular SHU-9119 had no significant effect on plasma IL-6 levels in control rats receiving intraperitoneal saline rather than LPS (Fig. 3).

Effect of Intraperitoneal α-MSH and of Central MCR Blockade on Pituitary-Adrenal Response to LPS

Baseline plasma ACTH (Fig. 4A) and CS levels (Fig. 4B) were <50 pg/ml and <50 ng/ml, respectively. LPS treatment markedly increased plasma ACTH and CS levels, which reached maximal values at 60 min. Thereafter, the levels of both ACTH and CS declined gradually. α-MSH (100 µg/kg ip) significantly inhibited the
LPS-induced rise in both ACTH and CS levels (Fig. 4). Intracerebroventricular administration of the α-MSH antagonist SHU-9119 reversed the α-MSH-induced suppression of ACTH levels evident at 120 min after LPS treatment (Fig. 4A) but had no effect on α-MSH suppression of LPS-induced plasma CS responses (Fig. 4B). In control rats receiving intraperitoneal saline rather than LPS, followed by intraperitoneal α-MSH and intracerebroventricular SHU-9119, plasma ACTH and CS levels were significantly lower than in LPS-treated rats, exhibiting little change from baseline levels (Fig. 4).

DISCUSSION

The first major finding of these studies is that the antipyretic action of peripherally administered α-MSH is mediated by activation of MCR located within the CNS. Central MCR blockade, induced by intracerebroventricular administration of the MC4-R/MC3-R antagonist SHU-9119, completely prevented the antipyretic effect of intraperitoneal α-MSH for at least 3.5 h. The ability of intracerebroventricular SHU-9119 to block the antipyretic effect of intraperitoneal α-MSH is not attributable to any potential nonspecific hyperthermic effects, nor to peripheral actions, of the antagonist, because we showed previously that the same dose of SHU-9119 had no effect on \( T_b \) when administered intracerebroventricularly in afebrile rats and had no effect on LPS fever when injected intravenously (9). Taken together, these findings indicate that the major and earliest component of the antipyretic effect of peripherally administered α-MSH is mediated by central MCR.

It has long been recognized that peripherally administered melanocortins can affect various aspects of CNS function (3, 4, 16). However, because the ability of circulating α-MSH to cross the BBB is very limited (28), previously it has been unclear whether α-MSH in systemic blood exerts these effects by acting directly on target sites within the CNS. The present results support the hypothesis that α-MSH administered peripherally does gain biologically effective access to central MCR, because central MCR blockade inhibited the antipyretic action of intraperitoneal α-MSH. Furthermore, intraperitoneal α-MSH (100 µg/kg) inhibited fever in a manner that was remarkably similar, in qualitative and temporal respects, to that observed after its intracerebroventricular injection (300 ng) in rats receiving an identical LPS challenge in our previous study (9). Together, these observations suggest that the BBB does not interfere qualitatively with the ability of α-MSH injected intraperitoneally to activate central MCR.

Theoretically, there are several routes by which systemic α-MSH may gain access to central MCR. For example, α-MSH could cross the BBB directly to activate MCR expressed within the CNS parenchyma, but the very low permeability of the BBB to α-MSH (28) does not overtly favor this hypothesis. One subset of central MCR that is perhaps more likely to contribute to the antipyretic effect of systemic α-MSH is that expressed in certain circumventricular organs (CVOs), which lack a tight BBB. MCR proteins are present in CVOs, including the median eminence (22, 23, 27) and several centrally projecting CVOs (J. Tatro and M. Entwistle, unpublished data). They are present at a low level in another CVO, the organum vasculosum of the lamina terminalis (OVLT), and at a much higher density in the adjacent ventromedial preoptic nucleus (VMPO) (24; Tatro and Entwistle, unpublished data). Extensive evidence supports a critical role of the OVLT and the VMPO in transducing LPS- and cytokine-induced pyrogenic signals leading to the increase in hypothalamic \( T_b \) set point of fever (5). Hence it is possible that blood-borne α-MSH could suppress LPS-induced fever by acting on MCR within the OVLT. Alternatively, because the VMPO receives a dense network of α-MSH-containing neuron projections (24), α-MSH acting within the OVLT could generate local signals that cause the release of α-MSH from nerve terminals in the neighboring VMPO, then activating MCR within the VMPO to suppress the febrile response. Therefore, MCR located within the OVLT and its immediate vicinity may play a role in mediating the antipyretic action of blood-borne α-MSH. Similarly, the SFO contains neurons projecting centrally to key autonomic regulatory sites (21), and the median eminence is a potential target of pluri potent neuroendocrine modulation by blood-borne α-MSH; hence MCR within these CVOs could also potentially be involved in the antipyretic and neuroendocrine actions of systemic α-MSH. Discriminating between the potential roles of MCR localized in CVOs and those requiring trans-BBB transport for effective access by blood-borne melanocortins is a complex problem that will require further study.

The second major issue addressed in this study was the potential role of altered IL-6 secretion in mediating the antipyretic action of α-MSH. Because IL-6 is an endogenous pyrogen (13, 14) believed to participate in mediating LPS-induced fever (11, 12), we tested whether systemically administered α-MSH suppresses LPS-induced IL-6 production. Indeed, α-MSH markedly attenuated LPS-induced elevation of plasma IL-6 levels. However, data from the same experiment also ruled out any significant contribution of the α-MSH-induced suppression of plasma IL-6 responses to the observed antipyretic effect, because intracerebroventricular administration of the MCR antagonist SHU-9119 blocked the antipyretic effect of α-MSH but had no effect on its suppression of LPS-induced plasma IL-6 responses. Furthermore, the same data also indicate that the suppression of plasma IL-6 responses to LPS caused by intraperitoneal α-MSH is not mediated by central MCR, at least not by the same MCR population that mediates its antipyretic effect.

The present results also provide some insight into the mechanism(s) by which intraperitoneal α-MSH suppresses LPS-induced CS secretion. Intraperitoneal α-MSH given at an antipyretic dose suppressed the LPS-induced rise in plasma ACTH and CS levels, consistent with previous findings by others (17, 20).
Intracerebroventricular SHU-9119 treatment reversed the α-MSH-induced suppression of ACTH levels observed 2 h after LPS, suggesting that α-MSH acts at least in part via central MCR to suppress LPS-induced ACTH release. However, despite its partial restorative effect on ACTH secretion, central MCR blockade by intracerebroventricular SHU-9119, at a dose that completely blocked the first 3–4 h of the antipyretic action of intraperitoneal α-MSH, had no effect whatsoever on α-MSH-induced inhibition of LPS-stimulated CS secretion. Therefore, additional mechanisms besides modulation of ACTH secretion may be involved in the inhibition of LPS-stimulated adrenal CS release by intraperitoneal α-MSH, such as direct actions of α-MSH at the adrenal cortical level. In this connection, an α-MSH-responsive MCR subtype known as MC5-R is expressed within the rat adrenal cortex (7, 26), but whether this receptor is involved in the regulation of glucocorticoid secretion is not yet known. In sum, these results suggest that systemic α-MSH suppresses LPS-induced ACTH release by acting at the central and/or adrenal cortical levels.

Our direct measurements of stress hormone levels also permit an assessment of the potential impact of nonspecific stress as a confounding or contributing factor in these studies. Basal (time zero) CS and ACTH levels in all groups were low, and in the non-LPS-treated rats only small increases in CS and ACTH occurred. These findings clearly indicate that the rats were relatively unstressed by their surgical implants and the experimental procedures, probably owing to extensive preconditioning of the rats to the procedures. Furthermore, in our previous study, treatment with intracerebroventricular SHU-9119 alone (9) had no significant effects on basal or LPS-stimulated ACTH or CS levels, indicating that intracerebroventricular treatment with SHU-9119 per se did not exert nonspecific effects on HPA responses. Taken together, these results suggest that nonspecific stress did not contribute significantly to the observed antipyretic and HPA-modulating effects of exogenous α-MSH.

The specific brain MCR subtype(s) that mediate the antipyretic effects of α-MSH in rats cannot be determined from this study, because SHU-9119 has similar antagonist potencies on the rat MC3-R and rat MC4-R (9). Nevertheless, it is likely that one or both of these MCR subtypes contribute to the antipyretic central action of peripherally injected α-MSH, because the mRNAs encoding rat MC3-R and MC4-R are distributed among ventral forebrain structures involved in thermoregulation (15, 18) and are the predominant MCR mRNA subtypes known to be expressed in rat brain (24). In contrast, mRNA encoding the other principal MCR subtype reportedly expressed in the rat brain, MC5-R, is of very low abundance, as it is not detectable by sensitive RNase protection assay or in situ hybridization, but only by the ultrasensitive polymerase chain reaction (1, 7). A very restricted presence of MC1-R in a few cells in the midbrain has also been reported (29). However, SHU-9119 is a full agonist of the MC5-R and MC1-R subtypes (8), whereas it blocked the antipyretic effect of α-MSH administered either intraperitoneally (present study) or intracerebroventricularly (9). Therefore, considered together with the low abundance and/or restricted distribution of the putative CNS-associated MC5-R and MC1-R, the data appear to rule out any role of these MCR subtypes in mediating the antipyretic action of α-MSH.

In summary, the present study demonstrates that, in the rat, peripherally administered α-MSH inhibits LPS-induced fever and suppresses LPS-induced plasma IL-6 and CS responses via different mechanisms. The antipyretic effect of α-MSH is mediated by central MCR, whereas α-MSH-induced suppression of IL-6 and CS responses to LPS are not mediated centrally, or if so must involve a different central MCR population, and the effects of α-MSH on IL-6 and CS levels do not contribute to its antipyretic action. These findings carry further significance beyond their direct implications concerning fever and HPA regulation, because they suggest that metabolic or other CNS-associated effects of systemic α-MSH (4, 16) may be mediated by MCR located within the brain.

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