α-Melanocyte-stimulating Hormone Inhibits Lipopolysaccharide-induced Tumor Necrosis Factor-α Production in Leukocytes by Modulating Protein Kinase A, p38 Kinase, and Nuclear Factor κB Signaling Pathways*

Received for publication, March 10, 2003, and in revised form, May 27, 2003
Published, JBC Papers in Press, June 19, 2003, DOI 10.1074/jbc.M302444200

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The peptide α-melanocyte-stimulating hormone (α-MSH) inhibits inflammation by down-regulating the expression of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) in leukocytes via stimulation of α-MSH cell surface receptors. However, the signaling mechanism of α-MSH action has not yet been clearly elucidated. Here, we have investigated signaling pathways by which α-MSH inhibits lipopolysaccharide (LPS)-induced TNF-α production in leukocytes such as THP-1 cells. We focused on the possible roles of protein kinase A (PKA), p38 kinase, and nuclear factor κB (NFκB) signaling. In THP-1 cells, LPS is known to activate p38 kinase, which in turn activates NFκB to induce TNF-α production. We found that pretreatment of cells with α-MSH blocked LPS-induced p38 kinase and NFκB activation as well as TNF-α production. This response was proportional to α-MSH receptor expression levels, and addition of an α-MSH receptor antagonist abolished the inhibitory effects. In addition, α-MSH treatment activated PKA, and PKA inhibition abrogated the inhibitory effects of α-MSH on p38 kinase activation, NFκB activation, and TNF-α production. Taken together, our results indicate that stimulation of PKA by α-MSH causes inhibition of LPS-induced activation of p38 kinase and NFκB to block TNF-α production.

The α-melanocyte-stimulating hormone (α-MSH) is a 13-amino-acid-long neuropeptide produced by intracellular cleavage of the proopiomelanocortin hormone. α-MSH mediates the communication between the nervous and immune systems (1, 2) and is expressed in pituitary cells, neurons, keratinocytes, and macrophages, where it regulates neuroendocrine, and immune activities (1, 3–6). The anti-inflammatory activity of α-MSH has been demonstrated in various disease models including arthritis, septic shock induced by hepatic injury, and endotoxicemia/ischemia, suggesting that α-MSH is a promising candidate therapeutic drug for inflammatory diseases (7–10). The anti-inflammatory effects of α-MSH involve a reduction in expression of inflammatory cytokines, including tumor necrosis factor (TNF)-α, interferon-γ, and interleukin-1, -6, and -8, and inhibition of the inflammatory actions of leukocytes such as neutrophils and macrophages (9, 11–13). In addition, it has been shown that the anti-inflammatory action of α-MSH is due to its ability to block proinflammatory signaling such as activation of nuclear factor κB (NFκB) (13, 14). α-MSH exerts its cellular effects by binding to five different G protein-coupled receptors called melanocortin receptors (MC1R, MC5R) (15–18). Ligand binding to MCRs activates adenylyl cyclase, which leads to the production of cAMP and subsequent activation of protein kinase A (PKA) (15, 19, 20). MC1R, which is expressed on the surface of leukocytes, is thought to be the major receptor mediating the anti-inflammatory activity of α-MSH (19, 20). However, the molecular mechanism of intracellular signal transduction leading to the anti-inflammatory action of α-MSH is not yet clearly understood.

Lipopolysaccharide (LPS) is a major inflammatory molecule that triggers the production of proinflammatory cytokines such as TNF-α in various cell types (21, 22). In monocytes and macrophages, LPS is known to stimulate TNF-α production by activating mitogen-activated protein (MAP) kinase subtypes including extracellular signal-regulated kinase (ERK), p38 kinase, and c-Jun N-terminal kinase (23–25). Among the MAP kinase subtypes, specific inhibitors for p38 kinase have been shown to inhibit LPS-induced TNF-α production (26–28). In addition, α-MSH is known to block LPS-induced expression of TNF-α (19), and the inhibitory effects of α-MSH are mediated by the inhibition of NFκB, which stimulates TNF-α production at the transcriptional level (29, 30). Although the signaling pathway by which α-MSH blocks TNF-α production is not clearly understood, the above observations suggest the possibility that α-MSH blocks LPS-induced TNF-α production by modulating MAP kinase and NFκB activation. Accordingly, we have investigated the functional relationships among PKA, p38 kinase, and NFκB in the anti-inflammatory action of α-MSH within inflammatory leukocytes (i.e. macrophages and neutrophils). For this purpose, we treated THP-1 and HL-60 cells with phorbol myristate acetate (PMA) or Me2SO, which induces differentiation into macro-

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*This work was supported by Grants KGM1000111 and KGS1010212 from the Korea Research Institute of Bioscience and Biotechnology. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡§ The abbreviations used are: α-MSH, α-melanocyte-stimulating hormone; ERK, extracellular signal-regulated protein kinase; IkB, inhibitory κB; IkK, IkB kinase; LPS, lipopolysaccharide; MAP, mitogen-activated protein; MAPK, MAP kinase; MEK, MAP/ERK kinase; MEKK, MEK kinase; MCR, melanocortin receptors; NFκB, nuclear factor κB; PKA, protein kinase A; PMA, phorbol myristate acetate; TNF-α, tumor necrosis factor-α.
phages and neutrophils, respectively. Using these cells, we
found that activation of PKA by α-MSH inhibits LPS-induced 
TNF-α production in differentiated THP-1 cells by inhibiting 
LPS-induced activation of p38 kinase and subsequent NFκB 
activation to block TNF-α production. However, the differenti-
ated HL-60 cells expressed lower expression of MC1R, so they
did not show the significant effect of α-MSH on the activation of p38 
kine and NFκB. To our knowledge, this would appear to be 
the first report to show that p38 kinase is a major signaling 
molecule that transduces α-MSH-mediated anti-inflammatory 
intracellular signal to the nucleus by inhibiting the NFκB 
activation and TNF-α production.

EXPERIMENTAL PROCEDURES

Reagents—The anti-MC1R antibody was obtained from Research Di-
agnostic Inc. (Flanders, NJ). The horseradish peroxidase-conjugated 
goat anti-mouse monoclonal antibody was purchased from Bio-Rad. 
Rabbit anti-phospho p38 kinase and anti-p38 kinase polyclonal anti-
body, mouse anti-phospho ERK-1/2 monoclonal antibody, rabbit anti-
phospho IκBα polyclonal antibody, and horseradish peroxidase-conju-
gated goat anti-rabbit polyclonal antibody were purchased from Cell 
Signaling Technology (Beverly, MA). Rabbit anti-actin polyclonal anti-
body and LPS (Escherichia coli serotype 055:B5) were purchased from 
Sigma. p38 kinase inhibitor, PD169316, and PKA inhibitor, H-89, 
were purchased from Calbiochem. Rabbit polyclonal antibodies against 
IκBα, IκB kinase (IKK)-α, and GST-IκB-α were purchased from Santa 
Cruz Biotechnology (Santa Cruz, CA). Protein A/G-Agarose, luciferase 
reporter gene assay kit, and SigmaTETC PKA assay kit were purchased 
from Promega (Madison, WI). The α-MSH antagonist, GHRP-9, and 
MC1R antagonist, SHU9119, were purchased from Bachem (Bubendorf, Switzerland).

Cell Culture—HL-60 cells cultured in RPMI 1640 medium with 10% 
heat-inactivated fetal bovine serum were treated with 1.25% Me2SO for 
45 s; and 72
° C for 45 s.

Experiment, and luciferase activity was determined by using a lucifer-
ase reporter gene assay kit from promega (Madison, WI). Luciferase 
activity was normalized against β-galactosidase activity.

Immunoprecipitation Assay—Total cell lysates were prepared in lysis 
buffer containing 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM 
EGTA, 150 mM NaCl, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 
1 mM β-glycerol phosphate, and protease inhibitors (1 mM leupeptin, 
1 mM pepstatin A, and 1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride) 
and phosphatase inhibitors (1 mM NaF and 1 mM Na3VO4). The cell 
lysates were precipitated with antibody against IKK-α. Immune 
complexes were collected using protein A/G-agarose beads.

Kinase Assay for IKK and PKA—Differentiated THP-1 cells were 
lysed, and IKK was immunoprecipitated as described above. IKK activity 
was determined by resuspending immune complexes in 20 μl of 
kine reaction buffer (50 mM HEPES, pH 7.4, 1 mM EDTA, 0.01% Brij 
35, 0.1 mg/ml, 0.1% β-mercaptoethanol, 0.15 μM NaCl) and conducting 
kinase reactions for 30 min at 30
° C by adding 12.5 μl of [γ-32P]ATP and 
1 μg of bacterially expressed GST-IκB-α as a substrate. The reaction 
mixtures were separated by SDS-PAGE, and radiolabeled proteins were 
visualized by autoradiography. PKA activity was determined by meas-
uring the transfer of [32P]-labeled phosphates to a phosphocellu-
lose filter-bound peptide substrate using the SigmaTETC PKA assay kit. 
Briefly, the kinase reaction was initiated by adding 25 μg of proteins with 
100 μM biotinylated Kemptide (LRARSGL) to 25 μl of reaction 
mixture. After incubation at 30
° C for 5 min, the reaction was termi-
nated by adding 12.5 μl of 7.5% guanidine hydrochloride. An aliquot 
of the reaction mixture was spotted to a phosphocellulose filter, and PKA 
activity was measured using an LS 6010TA liquid scintillation counter.

RESULTS

α-MSH Inhibits LPS-induced TNF-α Production in THP-1 Cells—We 
first investigated α-MSH receptor (MC1R) expression 
levels in Me2SO-treated HL-60 and PMA-treated THP-1 
cells, i.e. cells that had been caused to differentiate into macro-
phages and neutrophils, respectively. RT-PCR using primers 
specific to MC1R mRNA yielded the expected 495-bp product. 
The mRNA expression levels of MC1R in differentiated THP-1 
cells were significantly higher than those in differentiated 
HL-60 cells (Fig. 1A). The expression levels of MC1R protein
determined by Western blot analysis also indicated that PMA-treated THP-1 cells expressed significantly more MC1R than did Me2SO-treated HL-60 cells (Fig. 1B). Consistent with the observations by others (25, 33), LPS treatment caused TNF-α production in both PMA-treated THP-1 cells and Me2SO-treated HL-60 cells. To examine the role of α-MSH in LPS-induced TNF-α production, we incubated cells with α-MSH for 24 h prior to stimulation with LPS. As shown in Fig. 1C, α-MSH treatment significantly reduced LPS-induced TNF-α production in PMA-treated THP-1 cells. However, α-MSH did not significantly affect LPS-induced TNF-α production in Me2SO-treated HL-60 cells (Fig. 1C), suggesting that the ability of α-MSH to block LPS-induced TNF-α production is dependent on the levels of its receptor expression.

α-MSH Blocks LPS-induced TNF-α Production by Inhibiting p38 Kinase Signaling—LPS is known to stimulate TNF-α production in monocytes and macrophages by activating MAP kinase signaling (21, 27, 34). Therefore, we next examined whether α-MSH inhibits LPS-induced TNF-α production by modulating LPS-induced p38 kinase activation. As expected, Western blot analysis showed that LPS treatment activated p38 kinase in both differentiated THP-1 cells and HL-60 cells (Fig. 2A). Treatment of cells with α-MSH (50 nM) prior to LPS stimulation significantly reduced the amount of activated p38 kinase in PMA-treated THP-1 cells but not in Me2SO-treated HL-60 cells (Fig. 2B). This suggests that the ability of α-MSH to block LPS-induced p38 kinase activity is proportional to the levels of its receptor expression, which is consistent with the previous set of experimental results. We further confirmed the significance of the inhibition of LPS-induced p38 kinase activation by α-MSH in TNF-α production by a p38 inhibitor. As shown in Fig. 3, α-MSH inhibited LPS-induced p38 kinase activation (Fig. 3A) and TNF-α production (Fig. 3C) in PMA-treated THP-1 cells in a dose-dependent manner. In addition, direct inhibition of LPS-induced p38 kinase activation with specific inhibitor PD169316 also blocked LPS-induced p38 kinase activation (Fig. 3A) and TNF-α production (Fig. 3D) in a dose-dependent manner. The results from multiple Western blots were scanned and quantified. The densitometric analysis indicated that the phospho-p38 kinase levels were reduced to...
27–31% and 25–28% of the control level (treated with LPS only) by 50 nM α-MSH and 2 μM PD169316 (Fig. 3B). The results suggest that the inhibition of p38 kinase by α-MSH contributes to the inhibition of LPS-induced TNF-α production in PMA-treated THP-1 cells.

α-MSH Inhibits LPS-induced NFκB Activation via p38 Kinase Signaling—Because α-MSH is known to inhibit activation of NFκB (13, 14), we next investigated whether α-MSH inhibits LPS-induced NFκB activation and whether there is a functional relationship between p38 kinase and NFκB activation.

NFκB activation was determined by examining phosphorylation of IκB because degradation of IκB via its phosphorylation is necessary for nuclear translocation of NFκB and subsequent activation of target gene expression. In PMA-treated THP-1 cells, LPS treatment caused activation of NFκB as demonstrated by the measures of IκB phosphorylation (Fig. 4A, upper panel) and NFκB reporter gene assay (Fig. 4C). As expected, the level of IκBα protein decreased as the phosphorylation level of the IκBα protein increased (Fig. 4A, upper panel). Pretreatment of α-MSH that inhibits LPS-induced p38 kinase activation or direct inhibition of p38 kinase with PD169316 blocked LPS-induced IκB phosphorylation and LPS-induced IκB degradation (Fig. 4A, middle and lower panels) and transcriptional activity of NFκB (Fig. 4C), suggesting that inhibition of LPS-induced p38 kinase activation by α-MSH is responsible for the inhibition of NFκB. The phospho-IκBα levels were reduced to 22–28% of the control level (treated with LPS only) by 50 nM α-MSH and 2 μM PD169316, respectively (Fig. 4B). The ability of α-MSH to inhibit IκBα phosphorylation appears related to its ability to inhibit IKK as α-MSH inhibits the LPS-induced IKK activity (Fig. 4D). In contrast to the inhibition of NFκB by the blockade of p38 kinase activation, inhibition of NFκB activation by treatment with SN50 peptide, which blocks NFκB activation by inhibiting nuclear translocation of NFκB (35, 36), did not affect p38 kinase activation (Fig. 4C) but did inhibit LPS-induced TNF-α production (Fig. 4B). Taken together, these results suggest that LPS-induced p38 kinase activation is necessary for NFκB activation and that α-MSH inhibits NFκB production by blocking LPS-induced p38 kinase activation and subsequent NFκB activation.

It was found that the PMA-treated THP-1 cells also expressed MC₃R and MC₅R, in addition to the MC₁R receptor, whereas the Me₂SO-treated HL-60 cells only expressed the MC₁R receptor (Fig. 6A). As such, this finding is consistent with the recent report by Taherzadeh et al. (19), who found that THP-1 cells express MC₁R, MC₃R, and MC₅R. Since MC₁R and MC₃R, and yet not MC₅R, are known to be associated with the anti-inflammatory effect of α-MSH (15, 30), plus the expression level of MC₃R in THP-1 cells and MC₃R affinity to α-MSH are much lower than those for MC₁R and MC₅R (37), the receptor specificity was investigated using GHRP-6, which is a non-selective antagonist of α-MSH receptors, and SHU9119, which is a specific antagonist of MC₃R. When the differentiated THP-1 cells were pretreated with GHRP-6, the inhibitory effects of α-MSH on LPS-induced p38 kinase activation (Fig. 6B) and IκB-α phosphorylation (Fig. 6C) were completely abro-
 Activation of PKA Is Required for the Inhibitory Effects of α-MSH on p38 Kinase and NFκB—Because α-MSH binding to MC1R is known to activate the PKA signaling pathway (15, 19, 20), we also examined the functional relationship between PKA activation and α-MSH inhibition of p38 kinase and NFκB. As expected, α-MSH stimulated PKA activity in PMA-treated THP-1 cells. The addition of LPS alone did not significantly affect PKA activation, whereas addition of the PKA-specific inhibitor H-89 dramatically blocked α-MSH-induced PKA activation (Fig. 7A). The inhibition of α-MSH-induced PKA activation by H-89 treatment blocked the inhibition of LPS-induced activation of p38 kinase, IκB phosphorylation, and IκB degradation (Fig. 7B) as well as TNF-α production (Fig. 7C). These results clearly indicate that stimulation of PKA by α-MSH causes inhibition of LPS-induced activation of p38 kinase and subsequent NFκB activation to block TNF-α production.

**DISCUSSION**

α-MSH is known to suppress inflammation by inhibiting expression of inflammatory cytokines, including TNF-α in leukocytes by inhibiting NFκB activation (38). However, the molecular mechanisms of these α-MSH anti-inflammatory effects have not been defined previously. MC1R is constitutively expressed in monocytes and subpopulations of lymphocytes and plays an important role in the anti-inflammatory action of α-MSH (19, 39). Here, we found that PMA-treated THP-1 cells express significantly more MC1R when compared with Me2SO-treated HL-60 cells. By using differentiated THP-1 (high MC1R cells) and HL-60 (low MC1R cells), we demonstrated that α-MSH blocks LPS-induced TNF-α production by inhibiting LPS-induced activation of p38 kinase and subsequent NFκB activation in a manner dependent on MC1R expression. We also demonstrated that the inhibitory effects of α-MSH require MC1R-mediated activation of PKA. Since MC1R and MC2R are known to be associated with the anti-inflammatory effect of α-MSH (15, 30) and PMA-treated THP-1 cells express MC2R, we checked the effects of α-MSH antagonists, GHRP-6 (a non-selective antagonist of α-MSH receptors) and SHU9119 (an antagonist of MC1R), on the activation of p38 kinase and the phosphorylation of IκBα. We found that the anti-inflammatory effects of α-MSH were observed only in high MC1R cells and that these effects were completely abolished by the addition of GHRP-6 but not by that of SHU9119 (Fig. 6). These observations also suggest that MC1R expression is required for the inhibitory action of α-MSH in differentiated THP-1 cells. Studies using cultured human astrocytes, whole murine brain, and human monocyte/macrophages have indicated that a primary effect of α-MSH is modulation of activation of NFκB (38). Consistent with this is our observation that the ability of α-MSH to inhibit TNF-α production is due to the blockade of LPS-induced NFκB activation. The ability of α-MSH to inhibit NFκB activation appears to be indirect in that it involves inhibition of IKK activity. This is based on the observation that α-MSH inhibits LPS-induced IKK activity, leading to decreased IκB-α phosphorylation (Fig. 4). We also observed that α-MSH inhibits TNF-α-induced IκBα phosphorylation and NFκB activation (data not shown), as has already been reported by Manna et al. (40).

We also found that the inhibition of NFκB by α-MSH is due to inhibition of the upstream signaling molecule p38 kinase. This is consistent with observations by others indicating that LPS stimulates p38 kinase in various cell types (41, 42) and that p38 kinase activation by various extracellular stimuli...
leads to the activation of NFκB (43, 44). Experimentally, we found that the optimal α-MSH concentration for inhibition of p38 kinase activation and subsequent NFκB activation under our experimental conditions was 50 nM. Indeed, we found that higher concentrations of α-MSH were less effective in attenuating p38 kinase activation (Fig. 3A). This biphasic inhibitory effect of α-MSH on p38 kinase is consistent with the previous observation that α-MSH is most effective at a nanomolar concentration and that its anti-inflammatory effects are biphasic in terms of concentration (20, 45).

Stimulation of MC1R activates adenyl cyclase, leading to the production of cAMP and subsequent activation of PKA (15, 19, 20). Our results indicate that the inhibitory effects of α-MSH on LPS-induced activation of p38 kinase and NFκB are mediated by the activation of PKA via the stimulation of the MC1R receptor. This is based on the observation that inhibition of PKA with H-89 blocks the inhibitory effects of α-MSH on the inhibition of p38 kinase activation and TNF-α production (Fig. 7). Negative control of PKA on NFκB activation has been reported previously (46), and this result, in combination with our findings, suggests that it would be interesting to elucidate the mechanisms leading to the inhibition of p38 kinase by the activation of PKA. One possibility is that the inhibition is mediated through inhibition of Raf-1 by PKA (47) because Raf-1 is reported to induce the activation of NFκB through MAP kinase kinase kinase (MEKK)-1, which induces MAP kinase kinase (MEK)-3/6-5 and p38 kinase activation (44, 48).

Recently, Mandrika et al. (30) reported that inhibition of PKA by H-89 blocks the inhibitory effects of α-MSH on LPS/interferon-γ-induced nitric oxide production and NFκB activation measured by NFκB-dependent reporter assay but that it does not affect NFκB translocation to the nucleus in RAW 264.7 mouse macrophages. This group suggested that α-MSH acts via two mechanisms: one CAMP-independent and the other dependent on MC1R/cAMP activation. In this study, we demonstrated that PKA activity is required for the blockade of LPS-induced activation of p38 kinase and subsequent NFκB activation and TNF-α production. Thus the α-MSH inhibition of p38 kinase activation in LPS-stimulated THP-1 occurs through a MC1R/cAMP-dependent mechanism.

The current work demonstrated that the LPS-induced activation of p38 kinase was decreased by α-MSH treatment and that the IKK activity was subsequently down-regulated, thereby leading to a decrease in the phosphorylation and degradation of IκBα and the inhibition of NFκB activation. It was reported that p38 kinase inhibitors could be used for the therapeutic drug for cytokine-mediated diseases (26). Because our results showed that the down-regulated p38 kinase in LPS-induced monocytes treated with α-MSH or the p38 kinase inhibitor PD169316 induces the inhibition of IKK, NFκB activation, and TNF-α production, the application of α-MSH as a therapeutic drug for inflammatory diseases by acting as a p38 kinase inhibitor should be attempted.

Acknowledgments—We thank Michael Melinick (Cell Signaling Technology, Beverly, MA) for the anti-phospho MAPK antibodies and Hyunmi Pyo (Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea) for technical assistance.

REFERENCES
1. Catania, A., and Lipton, J. M. (1994) Neuroimmunomodulation 1, 93–99
2. Catania, A., Gerloni, V., Procaccia, S., Ariaghi, L., Manfredi, M. G., Lomater, C., Grossi, L., and Lipton, J. M. (1994) Neuroimmunomodulation 1, 321–328
3. Lipton, J. M., and Catania, A. (1998) Ann. N. Y. Acad. Sci. 840, 373–380
4. Ichiyama, T., Kim, J., Park, J., Park, H., and Lipton, J. M. (1999) J. Cell. Biol. 143, 171–179
5. Lipton, J. M., and Catania, A. (1999) J. Leukocyte Biol. 66, 704–709
6. Gantz, I., Konda, Y., Tashiro, T., Shimoto, Y., Miwa, H., Munzert, G., Watson, S. J., DelValle, J., and Yamada, T. (1993) J. Biol. Chem. 268, 8246–8250
7. Taherzadeh, S., Sharma, S., Chhablani, V., Gantzi, I., Rajora, N., Demtri, M. T., Kelly, L., Zhao, H., Ichiyama, T., Catania, A., and Lipton, J. M. (1999) Am. J. Physiol. 276, R1289–R1294
8. Catania, A., Rajora, N., Capsoni, F., Star, R. A., and Lipton, J. M. (1999) Peptides (Elmsford) 20, 1271–1277
9. Rajora, N., Boccoli, G., Catania, A., and Lipton, J. M. (1997) Peptides (Elmsford) 18, 381–385
10. Ariaghi, L., Lettino, M., Manfredi, M. G., Lipton, J. M., and Catania, A. (1995) J. Cell. Biol. 129, 204–211
11. Catania, A., Gerloni, V., Procaccia, S., Ariaghi, L., Manfredi, M. G., Lomater, C., Grossi, L., and Lipton, J. M. (1994) Neuroimmunomodulation 1, 321–328
12. Catania, A., Gerloni, V., Procaccia, S., Ariaghi, L., Manfredi, M. G., Lomater, C., Grossi, L., and Lipton, J. M. (1994) Neuroimmunomodulation 1, 321–328
13. Rajora, N., Boccoli, G., Burns, D., Sharma, S., Catania, A. P., and Lipton, J. M. (1999) J. Neuroimmunol. 99, 211–217
14. Ichiyama, T., Zhao, H., Catania, A., Furukawa, S., and Lipton, J. M. (1999) J. Neuroimmunol. 99, 211–217
15. Getting, S. J. (2002) Trends Pharmacol. Sci. 23, 447–449
16. Rajora, N., Ceriani, G., Catania, A., Star, R. A., Murphy, M. T., and Lipton, J. M. (1996) J. Leukocyte Biol. 59, 248–253
17. Mountjoy, K. G., Robbins, L. S., Morrud, M. T., and Cone, R. D. (1992) Science 257, 1248–1251
18. Gantzi, I., Kondo, Y., Tashiro, T., Shimoto, Y., Miwa, H., Munzert, G., Watson, S. J., DelValle, J., and Yamada, T. (1993) J. Biol. Chem. 268, 8246–8250
19. Taherzadeh, S., Sharma, S., Chhablani, V., Gantzi, I., Rajora, N., Demtri, M. T., Kelly, L., Zhao, H., Ichiyama, T., Catania, A., and Lipton, J. M. (1999) Am. J. Physiol. 276, R1289–R1294
20. Catania, A., Rajora, N., Capsoni, F., Minonzio, F., Star, R. A., and Lipton, J. M. (1999) Peptides (Elmsford) 20, 675–679
21. Guha, M., and Mackman, N. (2001) Cell. Signal. 13, 85–94
22. Hawiger, J. (2001) Immunol. Rev. 179, 99–109
23. White, J. E., Lin, H. Y., Davis, F. B., Davis, P. J., and Tsam, M. F. (2000) J. Cell. Physiol. 182, 383–389
24. Macunese, G., Midiri, A., Beninatti, C., Piraino, G., Valenti, A., Nicolai, G., Teti, D., Cook, J., and Teti, G. (2002) J. Immunol. 169, 1401–1409
25. MacKenzie, S., Fernandez-Trey, N., and Espel, E. (2002) J. Leukocyte Biol. 71, 1026–1032
26. Salturo, F. G., Germann, U. A., Wilson, K. P., Bemis, G. W., Fox, T., and Su,

Fig. 7. Activation of PKA is required for the inhibition p38 kinase and NFκB by α-MSH. PMA-treated THP-1 cells were pretreated with α-MSH (50 nM) with or without PKA inhibitor H-89 (10 μM) for 2 h, and then the cells were stimulated with LPS (10 ng/ml) for 1 h before PKA activity was determined. *p < 0.05; **p < 0.001 versus the value of control (A). Levels of p38, phosphorylated p38 kinase (p-p38), phosphorylated IκBα (pIκBα), and IκB were determined by Western blot analysis (B). Transcript levels of TNF-α and β-actin were determined by RT-PCR (C). The data represent mean values with standard deviation (A) and results of typical experiments from four independent experiments.
Anti-inflammatory Signaling of α-MSH

32920

M. S. (1999) Curr. Med. Chem. 6, 807–823
27. Rutault, K., Hazzalin, C. A., and Mahadevan, L. C. (2001) J. Biol. Chem. 276, 6666–6674
28. Nick, J., Young, S. K., Arndt, P. G., Lieber, J. G., Suratt, B. T., Foch, K. R., Avdi, N. J., Malcolm, K. C., Tausbe, C., Henson, P. M., and Worbsen, G. S. (2002) J. Immunol. 169, 5260–5269
29. Lager, T. A., Brzoska, T., Scholzen, T. E., Kalden, D. H., Sunderkotter, C., Armstrong, C., and Ansel, J. (2000) Ann. N. Y. Acad. Sci. 917, 232–238
30. Mandrika, I., Muceniece, R., and Wikberg, J. E. (2001) Biochem. Pharmacol. 61, 613–621
31. Collins, S. J., Bucsetti, F. W., Gallagher, R. E., and Gallo, R. C. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 2458–2462
32. Kurosaka, K., Watanabe, N., and Kobayashi, Y. (1998) J. Immunol. 161, 6245–6249
33. Nagahira, A., Nagahira, K., Murafi, H., Abe, K., Magota, K., Matsui, M., and Oikawa, S. (2001) Biochem. Biophys. Res. Commun. 281, 1030–1036
34. Haddad, E. B., Birrell, M., McCluskie, K., Ling, A., Webber, S. E., Foster, M. L., and Belvisi, M. G. (2001) Br. J. Pharmacol. 132, 1715–1724
35. Uzzo, R. G., Dulin, N., Bloom, T., Bukowski, R., Finke, J. H., and Kelenko, V. (2001) Biochem. Biophys. Res. Commun. 287, 885–899
36. Yan, X., Wu Xiao, C., Sun, M., Tsang, B. K., and Gibb, W. (2002) Biol. Reprod. 66, 1667–1671
37. Wikberg, J. E., Muceniece, R., Mandrika, I., Prusis, P., Lindblom, J., Post, C., and Skottner, A. (2000) Pharmacol. Res. 42, 393–420
38. Lipton, J. M., Zhao, H., Ichiyama, T., Barsl, G. S., and Catania, A. (1999) Ann. N. Y. Acad. Sci. 885, 173–182
39. Neumann Andersen, G., Nagaeva, O., Mandrika, I., Petrovka, R., Muceniece, R., Mincheva-Nilsson, L., and Wikberg, J. E. (2001) Clin. Exp. Immunol. 126, 441–446
40. Manna, S. R., and Aggarwal, B. B. (1998) J. Immunol. 161, 2873–2880
41. Wang, C., Deng, L., Hong, M., Akkaraju, G. R., Inoue, J., and Chen, Z. J. (2001) Nature 412, 346–351
42. Shuto, T., Xu, H., Wang, B., Han, J., Kai, H., Gu, X. X., Murphy, T. F., Lim, D. J., and Li, J. D. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 8774–8779
43. Chen, C. C., and Wang, J. K. (1999) Mol. Pharmacol. 55, 481–488
44. Wang, D., and Richmond, A. (2001) J. Biol. Chem. 276, 3650–3659
45. Haycock, J. W., Rowe, S. J., Cartbridge, S., Wyatt, A., Ghanem, G., Morandini, R., Rennie, I. G., and MacNeil, S. (2000) J. Biol. Chem. 275, 15629–15636
46. Zheng, H., SuYang, H., Erdjument-Bromage, H., Tempst, P., and Ghosh, S. (1997) Cell 89, 415–424
47. Dhillon, A. S., Pollock, C., Steen, H., Shaw, P. E., Mischak, H., and Kolch, W. (2002) Mol. Cell. Biol. 22, 3237–3246
48. Baumann, B., Weber, C. K., Toppmair, J., Whiteside, S., Israel, A., Rapp, U. R., and Wirth, T. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 4615–4620