α-Melanocyte–stimulating Hormone Reduces Endotoxin-induced Liver Inflammation

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Abstract

α-Melanocyte–stimulating hormone (MSH) is a potent anti-inflammatory agent in many models of inflammation, suggesting that it inhibits a critical step common to different forms of inflammation. We showed previously that α-MSH inhibits nitric oxide (NO) production in cultured macrophages. To determine how α-MSH acts in vivo, we induced acute hepatic inflammation by administering endotoxin (LPS) to mice pretreated with *Corynebacterium parvum*. α-MSH prevented liver inflammation even when given 30 min after LPS administration. To determine the mechanisms of action of α-MSH, we tested its influence on NO, infiltrating inflammatory cells, cytokines, and chemokines. α-MSH inhibited systemic NO production, hepatic neutrophil infiltration, and increased hepatic mRNA abundance for TNFα, and the neutrophil and monocyte chemokines (KC/IL-8 and MCP-1). We conclude that α-MSH prevents LPS-induced hepatic inflammation by inhibiting production of chemoattractant chemokines which then modulate infiltration of inflammatory cells. Thus, α-MSH has an effect very early in the inflammatory cascade. (*J. Clin. Invest.* 1996. 97:2038–2044.) Key words: neutrophils • KC/IL-8 • MCP-1 • TNFα • nitric oxide

Introduction

Both functional and morphological signs of hepatic inflammation occur early in multiorgan failure syndrome in endotoxemia and are associated with poor prognosis. The hepatic damage induced by LPS is associated with hepatic macrophage activation and neutrophil infiltration which precedes the parenchymal cell damage (1–4). LPS-activated hepatic neutrophils and macrophages release cytokines and cytotoxic mediators (reactive oxygen species) (1, 5, 6). Several proinflammatory cytokines and chemokines, particularly TNFα, IL-8, and monocyte chemoattractant protein-1 (MCP-1)[1] are believed to be important in LPS-induced injury. TNFα release is transient, peaking ~ 1–3 h after LPS administration (7). TNFα promotes neutrophil adhesion to hepatic sinusoidal endothelium which promotes neutrophil infiltration and liver damage (2, 8–10). MCP-1 and IL-8 are specific chemoattractant chemokines for monocytes and neutrophils, respectively (11–13). MCP-1 is produced by Ito cells, and its expression is increased in toxic liver injury (14). IL-8 is increased in ethanol-treated liver and in cultured hepatocytes exposed to LPS (15, 16). However, the roles of these chemokines in LPS-induced liver injury in vivo is unknown.

α-Melanocyte stimulating hormone (α-MSH), a tridecapeptide found in the pituitary, central nervous system, and in peripheral tissues, has potent antiinflammatory action in several experimental models of inflammation (17). α-MSH prevents local inflammation in a rat model of arthritis (18) and increases survival in experimental endotoxemia/peritonitis (19). Cultured mouse and human macrophages contain mRNA for a specific α-MSH receptor (melanocortin-1, or MC-1) (20, 21). α-MSH stimulates cAMP production and inhibits LPS/cytokine-stimulated nitric oxide (NO) production in mouse cells, and neopterin in human cells (20). In addition, α-MSH inhibits TNFα production by human PBMCs (22). α-MSH likewise inhibits IFN-γ production by murine lymph node cells and peripheral blood monocytes (23, 24). Because α-MSH inhibits cellular infiltration and both the production and action of proinflammatory cytokines, it should prevent LPS-induced hepatic inflammation.

In this study, we examined the effects of α-MSH on LPS-induced changes in hepatic enzyme release, morphology, cytokines, and chemokines, to further understand the antiinflammatory mechanisms of α-MSH in vivo. Because of the wide spectrum of antiinflammatory properties of α-MSH, we sought to determine which actions of α-MSH predominate very early in inflammation. Since α-MSH inhibits NO production in cultured macrophages, we also measured the effects of α-MSH on systemic NO production.

Methods

Animals. Female BALB/c mice aged 7–8 wk (20–22 g) were purchased from The Jackson Laboratories (Bar Harbor, ME). All mice had free access to food (4% mouse–rat diet; Harlan Sprague Dawley Inc., Indianapolis, IN and Teklad Premier Laboratory Diets, Madison, WI) and water. Animal care followed the criteria of the Univer-

Portions of this paper were presented at the annual meeting of the American Society of Nephrology, 5–8 November 1995 and published in abstract form (*J. Am. Soc. Nephrol.* 1995. 6:824).

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Received for publication 20 November 1995 and accepted in revised form 8 February 1996.

J. Clin. Invest.

0021-9738/96/05/2038/07 $2.00
Volume 97, Number 9, May 1996, 2038–2044

1. *Abbreviations used in this paper:* ALT, alanine aminotransferase; AST, aspartate aminotransferase; KC/IL-8, mouse KC homologue of IL-8; MC, melanocortin; MCP-1, monocyte/macrophage chemoattractant peptide-1; MPO, myeloperoxidase; MSH, melanocyte stimulating hormone; RT, reverse transcriptase.

sity of Texas Southwestern Medical Center for the care and use of laboratory animals in research.

Materials. Corynebacterium parvum was purchased from Ribi Immunochemical Research (Hamilton, MT). LPS from *Escherichia coli* 08:B was purchased from Difco Laboratories Inc. (Detroit, MI). α-MSH (1-13) was purchased from Sigma Chemical Co. (St. Louis, MO). PBS was purchased from GIBCO BRL (Gaithersburg, MD).

Experimental protocol. Heat-killed *C. parvum*, LPS, and α-MSH were dissolved in sterile PBS and injected intravenously via a tail vein. Endotoxemia was induced with a single injection of LPS (1 μg/mouse, i.v.). Mice were pretreated with either *C. parvum* or PBS 1 wk before the experiments. Each of the these two groups was further subdivided into four groups and challenged with vehicle, LPS, or LPS + 50 μg α-MSH, or α-MSH alone.

Mice were anesthetized with ether and killed at 30 min, 1 h, or 6 h after treatment to evaluate liver damage. Blood was collected via cardiac puncture, centrifuged to separate plasma, and assayed for hepatocellular enzymes and nitrite/nitrate. A sample of liver was frozen for analysis of myeloperoxidase (MPO) activity, a specific indicator of neutrophil accumulation, and also processed for total RNA.

Hepatocellular enzymes and nitric oxide assays. Liver damage was estimated from the plasma concentrations of the hepatocellular enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) measured with an Ektachem DT60 analyzer (Eastman Kodak Co., Rochester, NY). Plasma nitric oxide was assayed by measuring the stable end products of nitric oxide metabolism, nitrite + nitrate. Plasma was first incubated with *Pseudomonas oleovorans* nitrate reductase to convert the nitrate into nitrite. The nitrite/nitrate concentration was measured by Griess reaction (25) in a micro plate reader at 570 nm. A standard curve of nitrate was made for each assay.

Histological examination. Formalin (10%)-fixed and paraffin-embedded specimens of the liver were cut at 6 μm and stained with hematoxylin and eosin. Changes in hepatic histology were measured by semiquantitative scales for the extent of inflammation and neutrophil accumulation on 10–20 aggregates per slide (four to six animals per treatment) performed on coded slides in a blinded fashion. The extent of inflammation was scored from 1 to 4 as: 0, no lesions; 1, compact aggregation of cells within liver sinusoids; 2, minimal; 3, moderate; or 4, severe extension of inflammation into surrounding liver parachyma. Neutrophil infiltration was scored semiquantiatively from 0 to 3 as: 0, 0; 1, 1–3; 2, 4–10; and 3, > 10 neutrophils per aggregate.

MPO assay. Liver was homogenized in 1% HTAB buffer, sonicated, and centrifuged at 15,000 *g*. The supernatant (5 μL) was incubated with 1.5 ml of 1.6 mM tetramethylbenzidine (dissolved in DMSO), 3 mM H_2O_2 diluted in 80 mM phosphate buffer, pH 5.4 (26, 27). Results were calibrated with neutrophil MPO (Sigma Chemical Co.) and normalized for protein content (28). In preliminary studies, we found that 5 μl of supernatant from LPS-treated mice liver was within the linear range of the assay. Larger volumes (100–200 μl) resulted in no signal, presumably because of destruction of H_20_2 by hepatic catalase. To further evaluate the presence of catalase or other MPO-inhibitors, we performed known addition studies. Addition of a known amount of MPO, equivalent to the amount of MPO contained in LPS-treated liver supernatant, resulted in complete recovery of MPO activity.

RNA extraction and Northern blot hybridization. Total RNA was extracted from liver using guanidinium thiocyanate-phenol-chloroform extraction (29). Samples of RNA (10 μg) were denatured and fractionated via electrophoresis on 0.9% agarose-formaldehyde gel and transferred onto a nylon membrane (Gene Screen, Boston, MA). The equality of RNA samples after transfer to the membrane was substantiated by ultraviolet illumination of ethidium bromide. After fixing the membrane by baking at 80°C, the membrane was prehybridized at 42°C in 50% formamide, 10% SDS and 10% dextran sulfate. The membrane was hybridized with [^{32}P]dCTP-labeled cDNA clones. 0.5–1-kb portions of mouse TNFα, KC/IL-8, and MCP-1 were used as DNA probes (12, 30). The mouse KC probe was generated by PCR (31), and product was confirmed by automated sequencing. The hybridized membranes were washed two times in 0.1 SSC and 0.1% SDS at 50°C. To normalize for the amount of RNA that was loaded, the blot was rehybridized with human β-cyclophilin.

Detection of melanocortin-1 (MC-1) receptor by reverse transcriptase (RT)-PCR. RT-PCR was performed as described earlier (20). Briefly, cDNA was generated from RNA derived from liver samples by MMLV RT. Portions of the cDNA were used for PCR with primer pairs and PCR conditions specific for mouse MC-1 isoform (20). The PCR products were electrophoresed, transferred to nylon filters, and probed with a [^{32}P]-labeled internal primer specific for MC-1 receptor (20).

Statistical analysis. All data were described as mean±SEM. Different treatments were compared using ANOVA techniques (completely randomized design) for omnibus comparisons followed by Dunnett's test for individual comparisons between means. The null hypothesis was rejected when $P < 0.05$.

Results

Effects of α-MSH on hepatocellular enzymes. Fig. 1 shows the effects of α-MSH administration on serum ALT level in two models of liver injury. 6 h after LPS administration, serum ALT significantly increased from 54±9 to 226±48 IU/ml in the LPS group and 86±12 to 399±54 IU/ml in the *C. parvum*/LPS group (both $P < 0.01$ by ANOVA). α-MSH reduced LPS-induced ALT release by 86% in the LPS model and completely prevented the ALT increase in the *C. parvum*/LPS model (both $P < 0.01$ by ANOVA). The pattern of AST change in the *C. parvum* model was similar (control: 342±51; LPS 1,025±108; LPS + α-MSH 447±65 [$P < 0.01$ by ANOVA]). Vehicle (PBS) treatment did not change basal level of serum ALT or AST in these groups (data not shown). α-MSH administration alone had no effect in control or *C. parvum* pretreated mice (42 and 66 U/ml, respectively).

Because we wanted to determine the early actions of

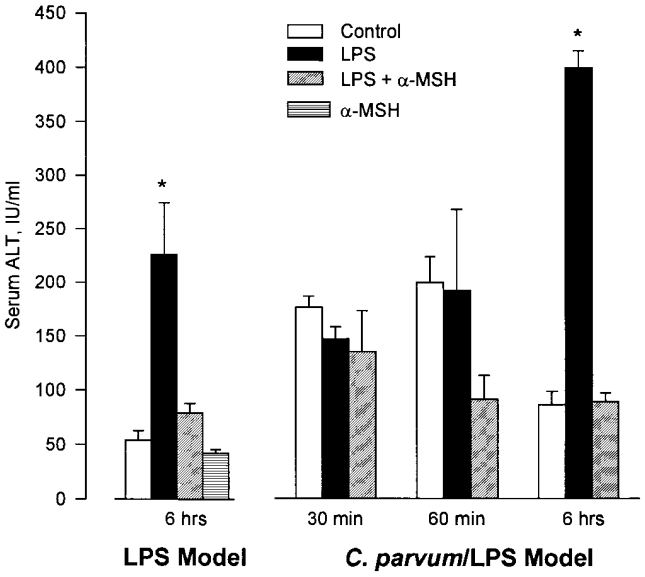

Figure 1. Effect of α-MSH on plasma ALT in two models of liver injury. (*A*) Effect on LPS model. (*B*) Effect on *C. parvum*/LPS model of liver injury. In this and following figures, data is presented as mean (±SEM). Where indicated, animals received 50 μg α-MSH i.v. *$P <$ 0.05 vs *C. parvum* by ANOVA with multiple comparisons (n = 5–8 mice/group).

α-MSH, we measured the effect of LPS and α-MSH at 30 and 60 min after LPS injection. Plasma ALT was not altered at 30 min or 60 min after LPS injection in *C. parvum*-treated mice.

Liver histology. The effect of α-MSH on liver morphologic changes at 6 h is shown in Fig. 2. As described previously by others (7, 32), *C. parvum*-treated mice had focal accumulations of macrophages and neutrophils (subsequently called macrophage aggregations), separated by distinct margins from normal liver parenchyma (Fig. 2 *A*). Liver histology was normal 30 min after LPS. At 60 min after LPS, there was a slight increase in neutrophil infiltration, primarily in the region of the macrophage aggregates. After 6 h, addition of LPS caused an intense inflammatory infiltrate consisting of neutrophils and macrophages which spilled out into the surrounding liver parenchyma (Fig. 2 *B*). In LPS-treated animals, there was evidence of hepatocyte damage, with unmistakable Councilman bodies. In contrast, mice treated with both LPS and α-MSH appeared similar to those treated with *C. parvum* alone, with no evidence of hepatocyte injury (Fig. 2 *C*). The macrophage aggregates appeared tightly compacted, with minimal numbers of neutrophils. Thus, α-MSH decreased both neutrophil and monocyte/macrophage cell infiltration and diminished hepatocyte damage.

Semiquantitative histology. The effects of α-MSH on neutrophil infiltration and hepatic inflammation were measured using a semiquantitative scoring system (Table I). LPS significantly increased both the extent of inflammation and neutrophil infiltration (both $P < 0.05$ by ANOVA). In contrast, α-MSH prevented the LPS-induced increase in extent of inflammation and neutrophil infiltration. These semiquantitative measurements confirmed the qualitative observations described above.

Table I. Effect of α-MSH on Hepatic Inflammation

Treatment group	Extent of inflammation	Neutrophil infiltration
C. Parvum	1.2±0.1	1.2±0.1
+ LPS	3.4±0.2*	2.5±0.2*
+ LPS +α-MSH	1.0±0.3‡	0.7±0.2‡

Hepatic inflammation was measured using semiquantitative scores as described in Methods. *$P < 0.05$ vs. *C. parvum* by ANOVA with multiple comparisons (n = 4–6/group). ‡$P < 0.05$ vs. *C. parvum* + LPS.

Liver MPO activity. The histological evidence–described neutrophil infiltration was confirmed by measures of liver MPO activity, a quantitative meter of neutrophil infiltration. Fig. 3 shows the assay results at 30 min, 60 min, and 6 h after LPS administration. LPS significantly increased liver MPO activity only at 6 h. α-MSH significantly inhibited the LPS-induced increase of hepatic MPO activity at 6 h ($P < 0.05$ by ANOVA) and had no effect at 30 or 60 min after LPS infusion.

Effects of α-MSH on nitrate/nitrite. We previously showed that α-MSH decreases LPS/IFN-γ stimulated NO production by ~ 45% in mouse-cultured macrophages (RAW 267.4). In the current study, we measured serum nitrate/nitrite, as an index of body NO generation, to determine if this effect also occurs in vivo. Fig. 4 shows the changes in serum nitrate/nitrite at 6 h by different treatments. In both groups, LPS caused an increase in serum nitrate/nitrite and α-MSH prevented these increases. The changes in nitrate/nitrite (Fig. 4) paralleled those of serum ALT (Fig. 1).

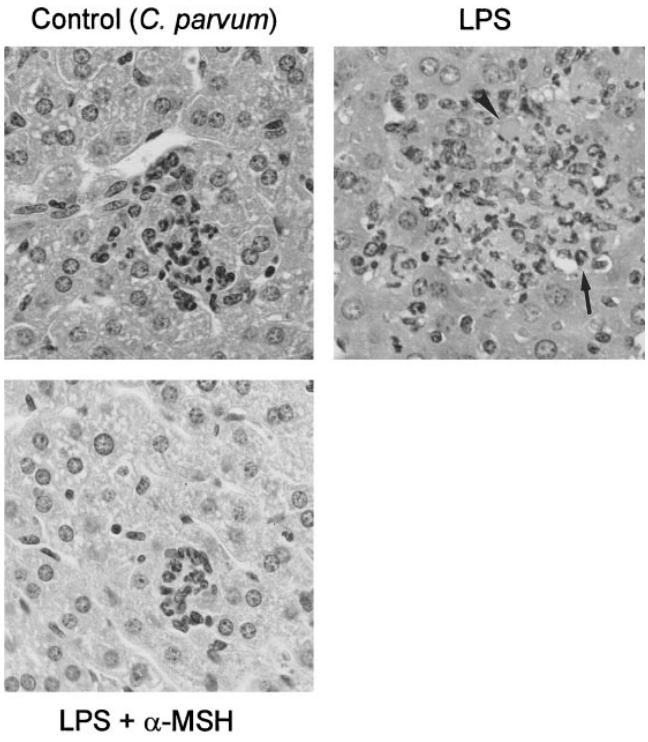

Figure 2. Effect of α-MSH on liver histology in mice pretreated with *C. parvum* for 1 wk. Comparison of typical histology from animals treated with vehicle, LPS, or LPS + α-MSH. Animals were killed 6 h after treatment.

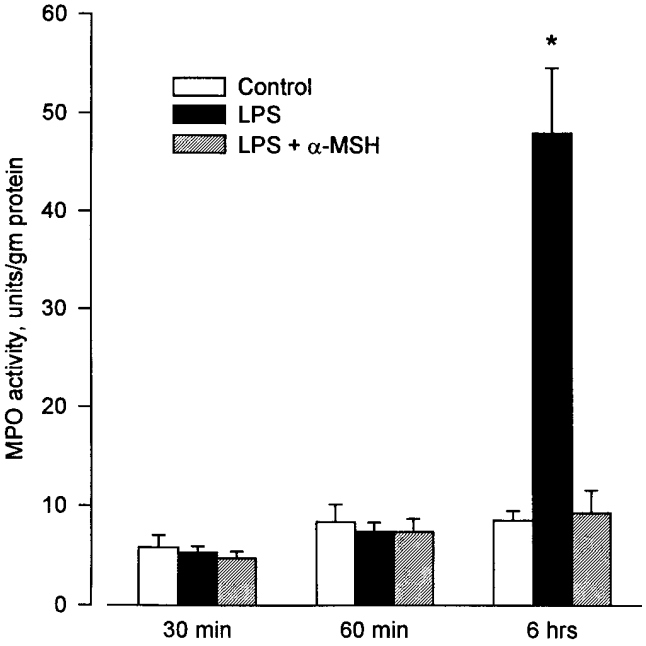

Figure 3. Effect of α-MSH on liver MPO activity. Animals were treated as in Fig. 2 and killed after 30 min or 60 min or after 6 h. MPO activity was measured in liver homogenate. *$P < 0.05$ vs control by ANOVA with multiple comparisons (n = 3–4/group).

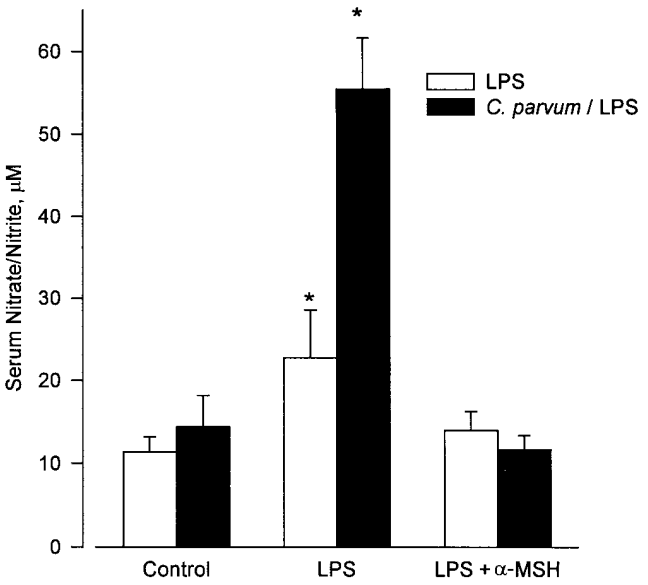

Figure 4. Effect of α-MSH on serum nitrate/nitrate. Animals were treated as in Fig. 2 and killed at 6 h. $*P < 0.05$ vs control by ANOVA with multiple comparisons (n = 5–8 animals/group).

Dose response of α-MSH treatment. A dose-response study was performed to determine the minimal effective dose of α-MSH. Fig. 5 shows that infusion of 25 μg and 50 μg α-MSH completely prevented liver injury and the LPS-induced rise in nitrate/nitrite. However, 10 μg α-MSH did not alter serum ALT, although serum nitrate/nitrite was decreased by ~ 50%. Lesser doses of α-MSH had no effect on either serum ALT or nitrate/nitrite.

Effect of delaying α-MSH treatment. Most agents used to treat experimental sepsis must be given before or at the same time as the septic insult to be effective, suggesting that the agents alter events early in the inflammatory cascade. The effectiveness of delaying α-MSH infusion in the *C. parvum*/LPS model is shown in Fig. 6. α-MSH prevented the serum ALT elevation induced by LPS if given 30 min after LPS administration. However, if α-MSH was delayed 60 min, it was only partially effective.

Expression of MC-1 receptor in mouse liver. α-MSH binds to a family of five melanocortin receptor isoforms (MC-1 through MC-5). We found using RT/PCR that liver from both normal and *C. parvum*-treated mice contain mRNA for MC-1 receptor (Fig. 7). Although we did not specifically attempt to quantitate differences between these two groups, we detected more hepatic MC-1 PCR product in mice treated with *C. parvum* than in control mice.

RNA blot analysis of cytokine and chemokine mRNA. We next sought to determine how α-MSH acts in vivo. To determine whether α-MSH alters liver cytokine or chemokine mRNA during inflammation, we measured the changes of mRNA by Northern blotting (Figs. 8 and 9). As expected, LPS significantly increased mRNA for KC/IL-8 and MCP-1 at 30 min, 60 min, and 6 h. α-MSH prevented the LPS-induced increase of KC/IL-8 and MCP-1 at 30 min (before cellular infiltration), 60 min, and at 6 h (after cellular infiltration in the LPS-treated animals). Finally, α-MSH prevented the LPS-induced increase of mRNA for TNFα at 60 min (Fig. 9).

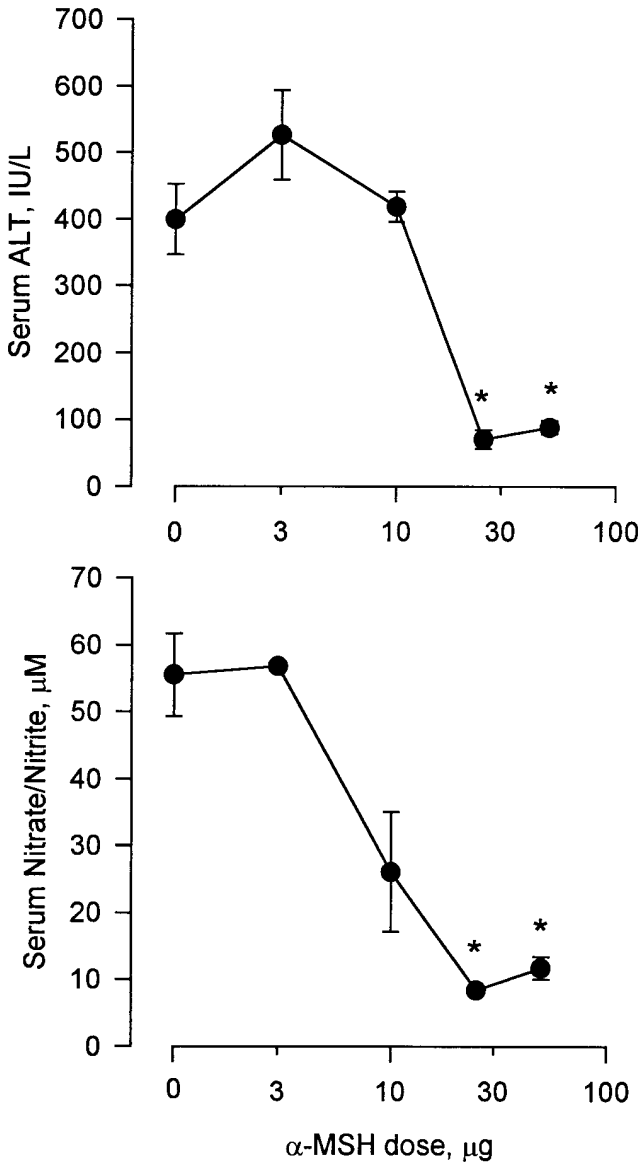

Figure 5. Dose-response relationship between serum ALT (*top*) and serum nitrate/nitrite (*bottom*) and dose of α-MSH. Animals were killed at 6 h. $*P < 0.05$ vs no α-MSH by ANOVA with multiple comparisons (n = 3–4 animals/group).

Discussion

These results characterize the protective action of α-MSH in liver inflammation during endotoxemia and the mechanism underlying its action. There are three main findings: (*a*) α-MSH prevents LPS-induced liver damage even when it is administered 30 min after LPS, (*b*) α-MSH decreases LPS-induced hepatic leukocyte infiltration, and (*c*) α-MSH decreases LPS-induced cytokine and chemokine mRNA accumulation.

α-MSH prevents LPS-induced liver damage. α-MSH protects against liver damage in two different models of endotoxemia (i.e., LPS alone and *C. parvum*/LPS, Fig. 1). It inhibits LPS-induced elevation of serum ALT in a dose-related pattern (Fig. 5) and prevents histological evidence of liver cell damage

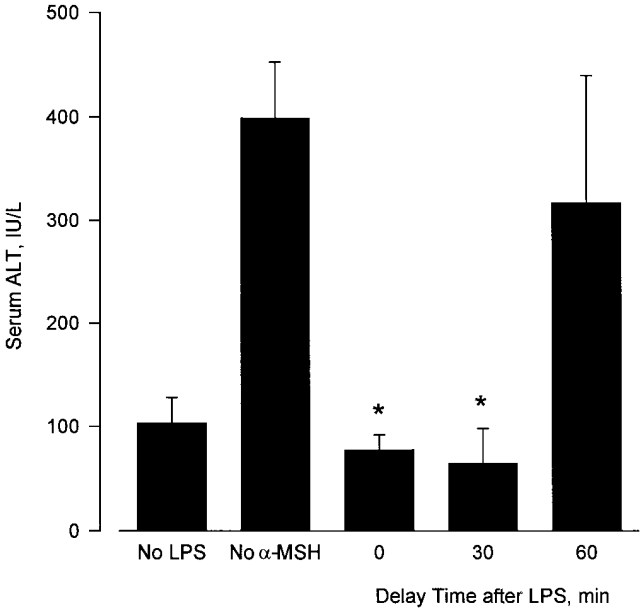

Figure 6. Effect of delaying α-MSH treatment on serum ALT. Animals were treated as shown and sacrificed at 6 h. $*P < 0.05$ vs No α-MSH by ANOVA with multiple comparisons (n = 3–4 animals/group).

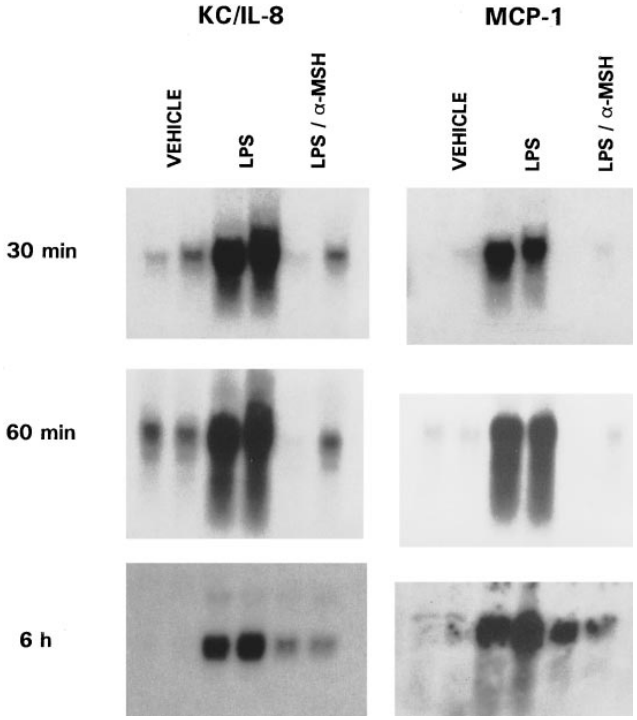

Figure 8. RNA blot of chemokine mRNA abundance for KC/IL-8 and MCP-1. *C. parvum*-treated animals were injected with vehicle, LPS, or LPS plus α-MSH. Animals were killed at 30 min or 60 min or at 6 h. Liver total RNA was isolated and electrophoresed (10 μg/lane). Results from two animals are shown for each condition.

(Fig. 2). α-MSH is protective even when administered 30 min after LPS (Fig. 6). α-MSH increases survival in a peritonitis model of septic shock induced by cecal ligation and puncture (19). α-MSH is as effective as gentamicin, and its effect is additive with that of gentamicin, indicating that α-MSH and gentamicin work by independent pathways. Taken together, these findings indicate that α-MSH has a wide spectrum of action in sepsis, and protects against the liver damage and mortality of systemic endotoxemia.

The prevention of liver damage was striking, especially since α-MSH also prevented the LPS-stimulated increase of plasma nitrate/nitrite, a marker of systemic NO synthesis. We previously found that α-MSH inhibits NO synthesis by 45–50% in murine macrophages stimulated with endotoxin (20). However, α-MSH is more effective in vivo since α-MSH inhibits LPS-induced increases in serum nitrate/nitrite in both LPS and *C. parvum*/LPS-induced liver injury models by 85–100% (Fig. 4). Previous studies by Billiar and colleagues found that inhibition of NO synthesis promotes hepatic damage, probably by preventing oxygen radical toxicity and maintaining organ blood flow during sepsis (32–34). In addition, NOS inhibitors increase neutrophil adherence and reduce sinusoidal blood flow (35). That α-MSH can prevent liver injury despite an inhibition of inducible NO synthesis suggests that it has other more important actions to inhibit inflammation.

α-MSH prevents LPS-induced hepatic leukocyte infiltration. The histologic and MPO data obtained in this study indicate that cellular infiltration/proliferation occurs during 1–6 h after LPS administration, similar to that described by Schlayer et al. (2). At 6 h after LPS treatment, α-MSH significantly inhibited LPS-induced hepatic neutrophil and macrophage infiltration (Fig. 2, Table I) and inhibited MPO accumulation, a quantitative marker of neutrophil infiltration (Fig. 3). We could not detect if α-MSH was active sooner (at 30 or 60 min),

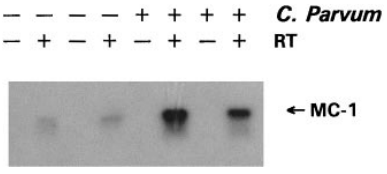

Figure 7. Expression of MC-1 receptor mRNA detected by RT-PCR. Two animals in each group were treated with *C. parvum* or saline for 1 wk, then liver mRNA was extracted as described in Methods.

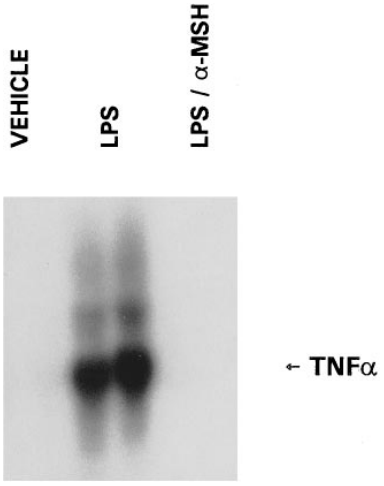

Figure 9. RNA blot of proinflammatory cytokine TNFα. Data shown as in Fig. 8.

because there was no detectable influx of cells at these early times. α-MSH also appeared to inhibit hepatic macrophage activation, based on the histologic appearance of the macrophage nucleoli (Fig. 2). Previous studies have shown that α-MSH prevents both acute inflammation after intradermal injection of LPS, cytokines, or irritants. In addition, α-MSH inhibits neutrophil infiltration after IL-1, TNFα, or C5a instillation into air-pouches (36). The current study extends these findings to a model of inflammation in a solid organ, where more detailed studies can be easily performed.

α-MSH decreases LPS-induced cytokine and chemokine mRNA accumulation. Since α-MSH prevents neutrophil and macrophage infiltration, we focused on factors that cause immune cell infiltration early in inflammation. Examination of this period was also supported by the ability of α-MSH to protect against liver damage at 30 min, but not 60 min, after LPS administration. This period coincides with the period before neutrophil and macrophage infiltration, suggesting that α-MSH inhibits the production of chemoattractant agents. Two families of chemokines have recently been identified; α chemokines (e.g., KC or IL-8) attract neutrophils whereas β chemokines (e.g., MCP-1 or colony stimulating factor-1) attract monocytes. However, the role of IL-8 and MCP-1 in LPS-induced hepatic inflammation is not well characterized. We found that stimulation of KC/IL-8 and MCP-1 mRNA temporally precedes cellular infiltration (Fig. 8), suggesting that they may be important to attract neutrophils and monocytes to the liver. A similar temporal expression of KC/IL-8 has been found in a liver injury by ethanol feeding (15).

α-MSH may prevent immune cell infiltration by inhibiting the release or action of chemokines. We found that α-MSH significantly inhibits both KC/IL-8 and MCP-1 mRNA accumulation induced by LPS at 30 and 60 min, and that these effects persist for up to 6 h (Fig. 8). Chemokine mRNA accumulation was inhibited both before cell infiltration/activation (30–60 min) and after infiltration (6 h). These results suggest that α-MSH inhibits neutrophil and monocyte infiltration via inhibition of KC/IL-8 and MCP-1 production, respectively. These results do not rule out a direct effect of α-MSH on either neutrophils or monocytes, which prevent them from responding to these chemokines. Indeed, α-MSH receptors are present on monocytes and macrophages (20, 21); it is currently unknown if neutrophils have α-MSH receptors. α-MSH inhibits acute inflammation caused by intradermal IL-8 injection (19), and hence, a direct effect on neutrophils is likely.

TNFα is an important mediator in the pathogenesis of septic shock (37). We found that α-MSH significantly inhibits the LPS-stimulated increase in TNFα mRNA abundance at 60 min after LPS infusion (Fig. 9), which is at the time of maximal TNFα production (38). TNFα stimulates neutrophil infiltration by alteration of adhesion molecules or by stimulation of IL-8 production (9, 11, 39). α-MSH interferes with the TNFα pathway at many locations: it inhibits TNFα mRNA induction in liver (Fig. 9), inhibits TNFα production in human peripheral blood monocytes, and prevents TNFα-induced neutrophil migration (22). Therefore, α-MSH inhibits both the synthesis and action of TNFα, two actions which may contribute to the anti-inflammatory actions of α-MSH.

Evidence for direct action of α-MSH on hepatic cells. Since α-MSH inhibits chemokine and cytokine mRNA accumulation before exogenous inflammatory cells have infiltrated into the liver, α-MSH is likely to affect at least one type of liver cell. We detected mRNA for MC-1 receptors in liver tissue by RT-PCR (Fig. 7). The precise location of its receptor has not been defined. We hypothesize that hepatic macrophages respond to α-MSH because both human and murine macrophages express MC-1 receptors (20, 21). In addition, hepatic macrophages respond to LPS by producing IL-8 and MCP-1, two agents which are inhibited by α-MSH (40). Other potential targets for α-MSH include hepatocytes (because LPS stimulates KC/IL-8), and Ito cells (because LPS stimulates MCP-1) (14, 16, 41).

Physiological significance. Taken together, the evidence in this study indicates that α-MSH exerts its protective action in liver damage by occupying MC receptors (MC-1) presumably on hepatic macrophages. Stimulation of MC receptors inhibits cytokine and chemokine mRNA accumulation (including TNFα, IL-8, and MCP-1) which results in a reduction in the release of chemokines. The reduction in these chemokines results in a decrease of neutrophil and macrophage host cell infiltration into the liver and a subsequent inhibition of liver injury. A wide spectrum of agents has been found to lessen liver damage during endotoxemia, including dexamethasone, anti-TNFα antibodies, PGE_2, pentoxifylline, depletion of neutrophils with antineutrophil antisera, and depletion of hepatic macrophages with gadolinium chloride (4, 7, 10, 42–44). Among these, dexamethasone, pentoxifylline, or neutrophil depletion are the most efficacious, decreasing liver injury by 75–90%. However, these agents were always given before LPS, which is impractical in a clinical setting. For example, dexamethasone and pentoxifylline were administered 30–60 min before LPS, and the antineutrophil antisera had to be administered 12–18 h before LPS. In contrast, α-MSH protects against hepatic damage even when given 30–60 min after LPS administration (Fig. 6). The clinical utility of PGE_2 and its analogues is limited by dose-limiting diarrhea, while deletion of leukocytes is impractical because of the risk of infection. The use of antibodies to single cytokines, chemokines, or adhesion molecules is theoretically constrained by the multiple parallel pathways in the inflammatory cascade; inhibition of a single pathway may cause a shift to an alternative pathway. In contrast, α-MSH has been well tolerated in human studies (45, 46). Our study shows that α-MSH acts to inhibit multiple cytokine and chemokines and may therefore be a good candidate for protecting against hepatic damage induced by endotoxin.

Acknowledgments

We thank Drs. Paul Bankey and Richard Turnage for their careful review of this manuscript, and Dr. Bruce Beutler for the probe for mouse TNFα, and Dr. J.S. Warren for rat MCP-1.

This study was supported by the National Institutes of Health Grant DK 45923. This work was done during the tenure of an Established Investigatorship from the American Heart Association. H. Chiao was supported by funds from an NIH training grant.

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