

alpha-MSH and IL-8-induced biological responses with

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What is known about α -MSH?

Sunil Kumar Manna: The melanocortins are a group of small protein hormones derived by post-translational cleavage of the proopiomelanocortin (POMC) gene product. The known melanocortin hormones include alpha-melanocyte stimulating hormone (α -MSH), beta-MSH, gamma-MSH and adrenocorticotrophic hormone (ACTH). Five melanocortin receptors (MC1R through to MC5R) have been identified and most of these show tissue-specific expression patterns, as well as different binding affinities for each of the melanocortin hormones. The central melanocortin system consists of α -MSH, agouti-related protein (AGRP), MC3R and MC4R. AGRP and α -MSH are believed to be the natural antagonist and agonist respectively of MC3R and MC4R. This central melanocortin system is thought to play a fundamental role in the control of feeding and body weight. Knockout mice models and genetic studies have

pointed to the importance of the melanocortins in complex human pathways such as pigmentation, lipolysis, food intake, thermogenesis, sexual behavior, memory and inflammatory response. Recently the melanocortins and their receptors have been the target for drug-based treatment of human physiological processes. MC1R may be used in the treatment of inflammation and MC2R for the treatment of glucocorticoid deficiency. MC3R and MC4R are likely targets for controlling body weight. Of the different forms of MSH, the α -MSH is known to have pleiotropic functions including pigmentary, anti-inflammatory, antipyretic, and immunoregulatory roles in the mammalian body. It is a highly evolutionarily conserved molecule, which acts through G-protein coupled receptors. Most of the immunological functions are exhibited via cAMP generation. However the complete molecular mechanism still remains to be elucidated.

Does α -MSH have an effect on Neutrophils?

Sunil Kumar Manna: Neutrophils represent 50-60% of the total circulating leukocytes and constitute the first 'line of defense' against infectious agents or non-self substances that penetrate body's physical barriers. Once any agent initiates inflammatory response, neutrophils are the first cells to be recruited to sites of infection. Cytokines are the basic regulators of neutrophil functions. The pyrogenic cytokines, IL-1, TNF- α , and IL-6 all prime various pathways that contribute to the activation of NADPH oxi-

dase. Pro-inflammatory cytokine IL-8, which is also known as neutrophil-activating factor, is also a potent chemoattractant; it synergizes with IFN- γ , TNF- α , GM-CSF, and G-CSF to amplify various neutrophil cytotoxic functions. α -MSH regulates neutrophil functions by interfering at various levels. α -MSH is reported to inhibit the production of superoxide radicals by activated rat peritoneal neutrophils (1). The ability of either IL-1 or TNF to cause fever, enhance plasma levels of acute phase proteins,

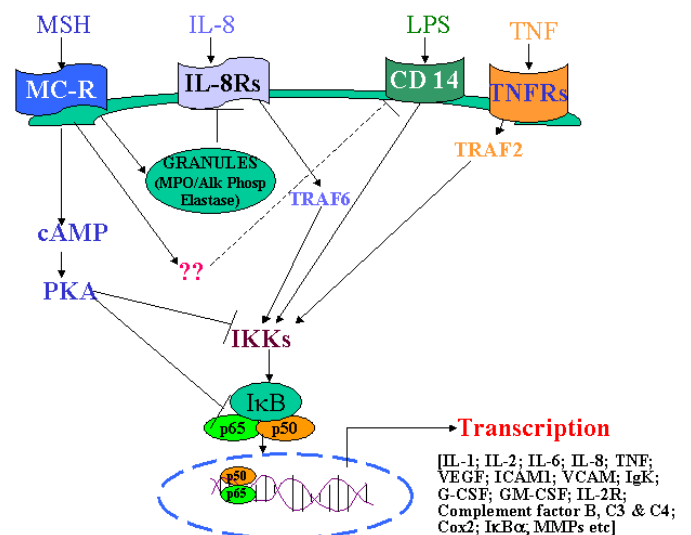
and increase the numbers of peripheral blood neutrophils was inhibited by the simultaneous peripheral administration of this neuropeptide (2). Accumulation of mRNA for melanocortin receptor 1 (MC1) in RT-PCR product was noticed in neutrophils stimulated with interferon and LPS. In subsequent studies showed that α -MSH inhibited migration of neutrophils from most normal

volunteers when the cells were placed in FMLP or IL-8 gradients. The inhibition by α -MSH could be traced to alterations in cAMP in neutrophils. Thus α -MSH contributes to its anti-inflammatory effect by inhibiting neutrophil migration (3). α -MSH also inhibited increases in endotoxin induced IL-8, TNF and iNOS in all contributing to its anti-inflammatory effects (4).

How does α -MSH selectively inhibit IL-8 mediated biological activities?

Sunil Kumar Manna: Interleukin-8 (IL-8) is a potent neutrophil chemotactic agent (5), which attracts the neutrophils to the site of tissue damage. It triggers respiratory burst response, degranulation, and stimulates neutrophil adhesion to the endothelial cells (6). IL-8 interacts with its receptor, IL-8Rs also known as CXCRs. The IL-8Rs are of two types (type I and II). α -MSH was found to inhibit IL-8-induced nuclear transcription factor kappa B (NF- κ B) DNA binding and its responsive gene expression such as ICAM-1, it inhibits IL-8 induced neutrophil migration, oxidative burst response, NO production, enzyme release such as myeloperoxidase, β -D-glucuronidase and alkaline phosphatase, in HL-60 derived neutrophils. We found that α -MSH acts predominantly via its receptor MC1R and down regulates both the IL-8 receptors -CXCR1 and -CXCR2 from neutrophil cell surface thereby inhibiting IL-8

mediated biological responses. Neutrophils bear granules that contain mediators of inflammation. A class of such mediators stored in azurophil granules of neutrophils, are serine proteases - cathepsin G, neutrophil elastase and proteinase 3. We observed that inhibitors of serine proteases -PMSF and -CMK, significantly protected α -MSH-mediated inhibition of IL-8-induced biological responses. Involved serine protease was found to be neutrophil elastase (7). Thus α -MSH seems to lead to activation of the elastase bearing granules leading to its release, which mediated the cleavage of CXCRs thereby inhibiting IL-8 driven neutrophil responses. The α -MSH with its ability to down regulate the CXCRs from neutrophil cell surface specifically inhibits IL-8 triggered migration of neutrophils towards the inflamed site and might help to reduce the suffering of patients in neutrophil driven diseases.



Do you believe that α -MSH is potent immunomodulator in inflammation?

Sunil Kumar Manna: Chronic inflammatory diseases such as rheumatoid arthritis, gout, asthma, or inflammatory bowel disease are characterized with uncontrolled accumulation of monocytes, mast cells, macrophages and neutrophils at the site of infection that liberate inflammatory molecules such as cytokines, reactive oxygen intermediates, and proteolytic enzymes which become major contributors to tissue damage. Thus, regulation of recruitment of mast cells, macrophages or neutrophils into the inflammatory sites and their clearance are critical processes assuring effective host defense without tissue injury.

Monocytes form one of the first lines of defense. They are reported to exhibit receptors of α -MSH and hence our group investigated the role of α -MSH in modulation of monocyte function. As most types of inflammation require activation of NF- κ B, we investigated the effect of α -MSH on the activation of this transcription factor by a wide variety of inflammatory stimuli. Electrophoretic mobility shift assay showed that α -MSH completely abolished TNF-mediated NF- κ B activation in a dose- and time-dependent manner (8). It also suppressed NF- κ B activation induced by LPS, okadaic acid, and ceramide. The effect was specific, as the activation of the transcription factor, activating protein-1 (AP-1) by TNF was unaffected. Western blot analysis revealed that TNF-dependent degradation of the inhibitory subunit of NF- κ B, I κ B α , and nuclear translocation of the p65 subunit of NF- κ B were also inhibited. This correlated with suppression of NF- κ B-dependent reporter gene expression induced by TNF. The inhibitory effect of α -MSH appeared to be mediated through generation of cAMP, as inhibitors of adenylate cyclase and of protein kinase A reversed its inhibitory effect. Similarly, addition

of membrane-permeable dibutyryl cAMP, like α -MSH, suppressed TNF- and endotoxin (serum activated-lipopolysaccharide, SA-LPS)-induced NF- κ B activation (8,9). Overall, these results suggest that α -MSH suppresses NF- κ B activated by various inflammatory agents and that this mechanism probably contributes to its anti-inflammatory effects.

Mast cells play a central role in inflammatory and immediate allergic reactions. These cells release preformed mediators, newly synthesized mediators, as well as cytokines upon antigen activation thereby forming the genesis of inflammation. Hence, the regulation of mast cell activity becomes important. α -MSH was found to inhibit SA-LPS mediated NF- κ B activation. It also inhibited NF- κ B dependent reporter gene (heat stable secretory alkaline phosphatase, SEAP) and ICAM1 expression. Since NF- κ B is also an important cell survival factor, we were interested if prolonged treatment of α -MSH affected the cell viability of MC-9. Treatment of α -MSH beyond 36 h resulted in decrease in cell viability as measured from MTT assay. α -MSH showed cell death with increase in ROI generation and lipid peroxidation. Cell death was further confirmed with markers of apoptosis, caspase-8 activation and PARP cleavage. α -MSH mediated NF- κ B down regulation occurred via activation of adenylate cyclase, followed by generation of cAMP and activation of PKA since inhibitors of these led to reversal of α -MSH mediated actions (9). The results were reproducible in human mast cell line HMC-1 also. As mast cells are key responders of allergic and inflammatory reactions in Asthma, arthritis, and so regulation of their number by α -MSH might prove to be beneficial.

Macrophages constitute the first line of body's defense system. They respond

to microbial pathogen by recognizing bacterial LPS via the CD14 expressed on their surface. Pretreatment of macrophage cells with α -MSH led to significant reduction in the enzymes released upon stimulation with endotoxin (SA-LPS) such as myeloperoxidase and β -D-glucuronidase. α -MSH also inhibited oxidative burst response, NO and ROI generated in response to endotoxin. It also inhibited SA-LPS activated NF- κ B and its responsive gene ICAM1 in presence of α -MSH. To understand the mechanism involved in all the above observations, we measured the level of CD14, which is the major receptor, documented in SA-LPS mediated actions. α -MSH treated cells showed disappearance of CD14 from cell surface and its appearance in the culture supernatant. Pretreatment with anti-MC1R antibody reversed the effect of α -MSH. The results thus indicate that α -MSH acts via its receptor MC1R to probably stimulate the release of certain proteases from the macrophages, which cleave the CD14 and thereby down regulate the SA-LPS mediated biological activities (10). Our data highlights the potential of α -MSH in treatment of diseases like sepsis where endotoxin mediated activation of macrophages is an important cause. In macrophages cardiac glycoside, oleandrin a plant derivative decreases IL-8Rs from cell surface by altering membrane fluidity and thereby downregulates IL-8-mediated biological responses (11).

Neutrophils, as described earlier, play a very significant role in inflammation. They are attracted to the site of tissue damage by chemokines, among with IL-8, which is the most important one. Thus, regulation of recruitment of neutrophils into the inflammatory sites and their clearance are critical processes assuring effective host defense without tissue injury. α -MSH with its ability to prevent migration of neutrophils to the inflamed site might

contribute to melioration of neutrophil driven distress. This it does so, by downregulating IL-8 Receptors from neutrophil cell surface, thereby inhibiting IL-8 mediated biological responses like NF- κ B activation, NF- κ B driven gene expression, NO production, reactive oxygen species generation, proteolytic enzyme release, all leading to inhibition of IL-8 mediated inflammatory responses in neutrophils (7).

Hence, α -MSH, by virtue of its ability to modulate responses in various cells of the immune system (see the cartoon figure), could emerge as a potent anti-inflammatory endogenous agent produced by our body.

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