Central Neurotranspeptide, Alpha-Melanocyte-Stimulating Hormone (α-MSH) is Upregulated in Patients with Congestive Heart Failure

Minako Yamaoka-Tojo, Taiki Tojo, Tetsuo Shioi, Takashi Masuda, Takayuki Inomata and Tohru Izumi

Abstract

Background α-melanocyte-stimulating hormone (α-MSH), a pro-opiomelanocortin (POMC) derivative, is a neuropeptide with potent anti-inflammatory properties that inhibits tissue injury in a wide array of inflammation models.

Objective To determine if α-MSH is involved in the development of congestive heart failure (CHF) with the specific aim of examining its peripheral source and one of the mechanisms.

Methods The circulating levels of α-MSH were measured in 115 patients with CHF using a double-antibody radioimmunoassay. To determine one of the sources of circulating α-MSH, human peripheral blood mononuclear cells (PBMC) were stimulated with lipopolysaccharide (LPS) or tumor necrosis factor (TNF-α). Furthermore, to clarify one of the functions of α-MSH, PBMC were cultured in the presence or absence of α-MSH.

Results Plasma levels of α-MSH were significantly higher in NYHA class II patients with CHF than in control subjects (p<0.0001). A significant correlation was found between the levels of α-MSH and high-sensitivity testing for C-reactive protein in patients with CHF (r=0.41, p<0.0005). PBMC stimulated with LPS or TNF-α released α-MSH in a concentration-dependent manner. α-MSH inhibited LPS-induced TNF-α production, and α-MSH simultaneously augmented production of interleukin (IL)-10 by PBMC.

Conclusions Circulating α-MSH was increased in patients with CHF. Inflammatory response induced α-MSH production in cultured human PBMC. Treatment of α-MSH could modify the immunobalance between inflammatory and anti-inflammatory responses in cultured PBMC. These findings suggest that α-MSH may play an important role in the pathophysiology of CHF.

Key words: α-melanocyte-stimulating hormone, tumor necrosis factor-α, congestive heart failure

Introduction

A large amount of evidence suggests that inflammatory cytokines are involved in the pathophysiology of congestive heart failure (CHF) (1-4). Tumor necrosis factor (TNF-α) and other proinflammatory cytokines are elevated in patients with advanced CHF (5-10). On the other hand, anti-inflammatory cytokines and/or cytokine receptors are likely to regulate these proinflammatory cytokines in CHF (7, 11, 12). However, the precise mechanism of proinflammatory response in CHF remains unknown. Indeed, a number of clinical trials using immune-modulating agents have been tried, but none of them have proven to be useful. For instance, in a recent trial to define the effect of immunotherapy in mortality using a TNF antagonist, etanercept in patients with CHF appeared to be ineffective (13).

Production and actions of proinflammatory mediators are probably modulated by endogenous molecules that counteract their effects on the host. α-Melanocyte-stimulating hormone (α-MSH) is a potent anti-inflammatory peptide with prominent effects in reducing production and actions of me-
diators of inflammation, including cytokines (14, 15). α-MSH is a 13 amino acid pro-opiomelanocortin (POMC) derivative expressed in the central nervous system and in peripheral cells, including phagocytes and keratinocytes (14, 16, 17). The anti-inflammatory effects of α-MSH are mainly exerted through antagonism of proinflammatory mediators, including TNF-α, interleukin (IL)-6, and NO (15). Importantly, α-MSH also induced IL-10, a potent anti-inflammatory cytokine, production in monocytes (18). However, the role of α-MSH in patients with CHF has not been clarified.

To examine if α-MSH is involved in CHF, we measured the circulating levels of α-MSH in patients with CHF. In addition, to study one of the inducers of α-MSH production in peripheral cells, α-MSH production in cultured human peripheral blood mononuclear cells (PBMC) stimulated with proinflammatory agents was measured by EIA kit. Furthermore, to examine the mechanism of circulating α-MSH regulating systemic inflammation, human PBMC were stimulated with lipopolysaccharide (LPS) or TNF-α under the treatment of α-MSH.

### Material and Methods

#### Patient population

The study population consisted of 115 patients with chronic CHF who were treated at the Department of Cardiology, Kitasato University Hospital for heart failure management. All the patients were free from infection, acute myocardial infarction, and unstable angina pectoris. The 81 men and 34 women ranged in age from 26 to 87 years (mean age 62.3 years). Thirty-nine patients were in New York Heart Association (NYHA) functional class II, 12 were in class III, and the remaining 64 were in class IV. The etiology of CHF was ischemic heart disease (IHD) for more than three months since myocardial infarction (MI) in 35 patients, dilated cardiomyopathy (DCM) in 29 patients, hypertrophic cardiomyopathy and hypertensive heart disease (HCM) in 21 patients, valvular heart disease (VHD) in 15 patients (predominantly in regurgitation of the aortic and/or mitral valves), and the others (right ventricular heart failure due to pulmonary hypertension, or tachyarrhythmia-induced heart failure) consisted of 15 patients. Patients who had clinical or laboratory evidence of malignancy, chronic inflammatory disease, liver or renal dysfunction, or acute myocardial infarction were excluded. All patients with DCM and IHD were evaluated by cardiac catheterization for their diagnoses at the present and/or in the past in our hospital. All patients gave their consent to participate in this study. The control subjects consisted of 40 healthy volunteers with normal left ventricular (LV) function and without LV hypertrophy (LVH, LV wall thickness >11 mm) in echocardiogram; 33 men and 7 women, ranging in age from 34 to 76 years of age (mean, 61 years). None of the control subjects had acute or chronic illness such as cardiovascular diseases, diabetes mellitus, hyperlipidemia, obesity, and/or hypertension on medication. Table 1 and Table 2 show the clinical characteristics of the population studied.

#### Blood processing

Peripheral venous blood was taken and immediately centrifuged for 15 min at 4°C to measure plasma brain natriuretic peptide (BNP), α-MSH, and serum high-sensitivity testing for C-reactive protein (hs-CRP). All samples were stored at -80°C before analysis.

#### Plasma α-MSH determination

α-MSH was measured with a double-antibody radioimmunoassay (Euro-Diagnostica AB, Malmo, Sweden). The sensitivity of the assay was 0.5 pg/ml and cross-reactivity with other POMC peptides [adrenocorticotropic hormone (ACTH) (1-24), ACTH (1-39), β-MSH, γ-MSH] was <

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**Table 1.** Demographic Characteristics of Contro subjects and Patients with Congestive Heart Failure

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Control subjects (n = 40)</th>
<th>CHF (n = 115)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/Women</td>
<td>55.4 (2.0)</td>
<td>62.3 (1.3)</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Table 2.** Demographic Characteristics of Patients with Congestive Heart Failure according to the Etiology

<table>
<thead>
<tr>
<th>Etiology of heart failure</th>
<th>IHD (n = 35)</th>
<th>DCM (n = 29)</th>
<th>HCM (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.2</td>
<td>62.4</td>
<td>63.4</td>
</tr>
<tr>
<td>Male/Female</td>
<td>30/5</td>
<td>24/5</td>
<td>9/12</td>
</tr>
<tr>
<td>NYHA class (mean)</td>
<td>2.6</td>
<td>3.2</td>
<td>2.1</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>36.4 (2.2)^*</td>
<td>26.9 (3.2)^*</td>
<td>61.0 (2.2)</td>
</tr>
<tr>
<td>BNP (pg/ml)</td>
<td>631 (117)^*</td>
<td>563 (64)^*</td>
<td>193 (49)^*</td>
</tr>
<tr>
<td>hs-CRP (μg/l)</td>
<td>2494 (789)</td>
<td>521 (563)</td>
<td>38 (28)</td>
</tr>
<tr>
<td>α-MSH (μg/ml)</td>
<td>20.2 (1.3)^*</td>
<td>16.5 (2.2)^*</td>
<td>21.6 (2.2)^*</td>
</tr>
</tbody>
</table>

*Data are presented as mean value (SE). IHD = ischemic heart disease; DCM = dilated cardiomyopathy; HCM = hypertrophic cardiomyopathy and hypertensive heart disease; LVF = left ventricular ejection fraction; BNP = brain natriuretic peptide; hs-CRP = high sensitivity test for C-reactive protein; α-MSH = alpha-melanocyte-stimulating hormone.*

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Plasma levels of alpha-melanocyte-stimulating hormone (α-MSH) in 115 patients with congestive heart failure (CHF) and 40 control subjects. Thirty-two patients were in New York Heart Association (NYHA) functional class II, 12 were in class III, and the remaining 64 were in class IV. Data are expressed as means (SEM).

Figure 1. Plasma levels of alpha-melanocyte-stimulating hormone (α-MSH) in 115 patients with congestive heart failure (CHF) and 40 control subjects. Thirty-two patients were in New York Heart Association (NYHA) functional class II, 12 were in class III, and the remaining 64 were in class IV. Data are expressed as means (SEM).

0.002%.

Plasma BNP and hs-CRP determinations

Circulating levels of plasma BNP were measured by radioimmunoassay (Shionoria BNP kit, Osaka, Japan; sensitivity, 18.4 pg/ml). The concentration of hs-CRP was determined from serum samples in patients with CHF (LPIA-CRP, Iatron Laboratories, Inc., Tokyo, Japan; sensitivity, 20 μg/dl).

PBMC experiments

PBMC were isolated from heparinized blood obtained from a normal volunteer by density centrifugation through Lymphoprep™ (AXIS-SHIELD PoC AS, Oslo, Norway). Cells were washed twice in sterile PBS and suspended in RPMI 1640 (Gibco BRL, Paisley, UK), supplemented with 10% FBS (Gibco) at a density of 4×10^6 cells/ml. To examine the production of α-MSH in PBMC, cells were treated with human recombinant TNF-α (Chemicon International, Inc., Temecula, CA) or LPS from *Escherichia Coli* 055:B5 (List Biological Laboratories, Inc., Campbell, CA) for 24 hours in 5% CO₂ atmosphere at 37°C in 24-well flat-bottomed plates (2×10^6 per well). On the other hand, PBMC were incubated in 24-well flat-bottomed with medium or 1 ng/ml of LPS for 3 hours after pretreatment with several doses of α-MSH (Peninsula Laboratories, Inc., San Carlos, CA) for 1 hour. After the incubation, samples were centrifuged and supernatants separated and stored at -80°C for cytokine assays. The concentrations of α-MSH in culture media were measured using a commercially available immunoassay kit (alpha-melanocyte stimulating hormone enzyme immunoassay kit; Phoenix Pharmaceuticals, Inc., Belmont, CA) according to the manufacturer’s instructions. The sensitivity of the assay was 0.3 ng/ml. Concentrations of TNF-α and IL-10 in cell culture supernates were detected using commercial enzyme-linked immunosorbent assays (R&D Systems; Minneapolis, MN). The minimum detectable concentration was 4.4 pg/ml and 3.9 pg/ml, respectively.

Statistical analyses

All values are expressed as mean (SEM). If blood results were below the limit of detectability of a test, the lower limit of detection was recorded. The significance of the differences between 2 groups was evaluated by the Mann-Whitney U test. The values in more than 3 groups were tested by one-way analysis of variance (ANOVA), and were followed by Scheffe’s F test. Correlations of cytokine levels with demographic characteristics were performed using Spearman’s rank correlation test. Statistical significance was accepted at p<0.05.

Results

Circulating level of α-MSH in patients with CHF

As shown in Fig. 1, plasma levels of α-MSH in patients with CHF were significantly higher than in control subjects (mean (SEM) 18.5 (0.7) vs 12.7 (0.6) pg/ml; p<0.0001). Their demographic characteristics are shown in Table 1. When CHF patients were classified according to NYHA functional classes, the mean value of α-MSH was highest in NYHA class II. Concentration of α-MSH was 19.8 (1.3) pg/ml in class II, 18.4 (2.0) pg/ml in class III, and 17.7 (0.9) pg/ml in class IV. There was no significant difference between these 3 functional classes. Significant correlation was found between the levels of α-MSH and hs-CRP in patients with CHF (r=0.41, p<0.0005). Circulating α-MSH in patients with CHF had no significant correlation with the patient’s sex, age, plasma BNP concentration, or left ventricular ejection fraction (LVEF). There were no significant dif-
Figure 3. alpha-melanocyte-stimulating hormone (α-MSH) inhibited TNF-α production by LPS. PBMC were seeded in 24-well plates (2×10^6 cells/well), pre-treated with α-MSH for 1 hour, and stimulated with LPS (1 ng/ml) for 3 hours. The data represent the average percentage of the controls (LPS alone; 100%) from independent experiments (mean (SEM)). *p < 0.001 vs LPS alone.

Differences in circulating levels of α-MSH in patients treated with angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, β-blockers, calcium antagonists, diuretics, digitalis, statins, and/or nitrates.

The age of patients, LVEF, plasma level of BNP, and NYHA functional classes were significantly different in subsets of patients’ etiology (Table 2). As compared with IHD and DCM groups, plasma levels of α-MSH were increased in patients with IHD much more than in patients with DCM (20.3 (1.3) vs 16.6 (5.2) pg/ml; p=0.031). Although mean age of DCM patients was younger than that in IHD patients, there were no significant differences in concentrations of plasma BNP and serum hs-CRP between these groups. Moreover, though mean NYHA class in patients with HCM was lower than that in the other groups, the circulating level of α-MSH in HCM patients was more increased among these groups.

Production of α-MSH in peripheral cells

Production of α-MSH by circulating cells (lymphocytes, monocytes, etc.) is essential to an autocrine circuit. To determine the possibility of the production of α-MSH by peripheral cells, PBMC were stimulated with inflammatory agents. As shown in Fig. 2, α-MSH was produced by resting cells, and its concentration was increased when the cells were stimulated with LPS or inflammatory cytokine TNF-α. Stimulation with 10 ng/ml of LPS showed a 3.1-fold increase of α-MSH production compare to the unstimulated cells. Twenty ng/ml of TNF-α also revealed a 2.7-fold increase of α-MSH production in PBMC.

Effects of α-MSH on TNF-α production

To confirm the inhibitory effect of α-MSH as previously reported by others, PBMC were treated with various concentrations of α-MSH (0.1 fg/ml to 10 ng/ml) (14, 15). Rather, the addition of α-MSH to PBMC stimulated with 1 ng/ml of LPS for 3 hours caused significant, dose related inhibition of TNF-α production (Fig. 3). Very small concentrations of α-MSH, even in the 100 fg/ml of α-MSH, markedly inhibited production of TNF-α (p<0.0001). These effective concentrations of α-MSH were lower than plasma concentrations in patients with CHF.

As shown in Fig. 4, treatment with α-MSH (0.01, 0.1, and 1 pg/ml) significantly augmented IL-10 production by PBMC. While α-MSH inhibited production of TNF-α in the short term, the induction of IL-10 production was detected at 12-24 hours after α-MSH stimulation. As previously reported (18), induction of IL-10 production was detected by treatment with 1 pg/ml of α-MSH for 24 hours.

Discussion

The present study demonstrates that the neuropeptide α-MSH, an endogenous modulator of inflammation, is increased in the peripheral blood of patients with CHF. Furthermore, α-MSH, even at the femto-gram range, inhibited production of TNF-α in cultured human PBMC. In human diseases, the relationship between concentrations of α-MSH and disease progression in HIV-infection was explored in a prospective study (19). Circulating α-MSH returned to normal levels in patients with sepsis syndrome who recovered and it remained low in those who died (20). This suggests that a low release of the peptide, or an insufficient release during sepsis, is linked to a poor prognosis. Furthermore, previous reports showed that during infections or inflammatory disorders (e.g. AIDS, hemodialysis patients with detectable endotoxia, administration of endotoxin to normal human subjects) there are substantial changes in the concentrations of circulating and/or local α-MSH (21). These observa-
tions suggest that, in the presence of inflammation, there is generally an increase in the α-MSH concentration as a compensatory reaction. It is reasonable to assume that, whenever such an increase does not occur or is insufficient to counteract the action of inflammation mediators, the disease process is more severe (20). The concentration of α-MSH in patients with CHF were higher than in control subjects. However, the circulating level of α-MSH had a tendency of reverse correlation to NYHA functional class and had no correlation with the LVEF or plasma level of BNP. To determine whether α-MSH concentrations are associated with mortality, we further analyzed 15 patients who died within a year after blood sampling. However, there appears to be no correlation between α-MSH concentration and the survival of patients. Future prospective study is necessary to confirm the possibility of the convalescence prediction of heart failure by circulating α-MSH.

Considering the reason of increased levels of α-MSH in patients with IHD, α-MSH levels may reflect the etiology of systemic inflammation. Because serum levels of hs-CRP were also markedly increased in patients with IHD compared to other groups. Actually, a rough but significant correlation was found between the levels of α-MSH and hs-CRP in patients with CHF. Elevated levels of α-MSH were observed in the HCM group compared with the DCM group. Although plasma levels of α-MSH were higher in patients with HCM than in those with DCM, patients with non-symptomatic LVH (n=15, EF>55%, LV wall thickness; mean 13.5 mm) had circulating α-MSH values similar to those of control subjects (data not shown). In a very interesting report, rat myocardium was found to release α-MSH (22). We also confirmed that the expression of POMC mRNA, a precursor of α-MSH, was increased in the myocardium of rats with cardiac hypertrophy and heart failure (data not shown). These data suggest that α-MSH may be related to cardiac hypertrophy and remodeling.

As previously reported (23, 24), the response to α-MSH was biphasic in inhibitory effects on LPS-induced TNF-α production. One of the possibilities of the reason may be that α-MSH could modulate both inflammatory and anti-inflammatory responses regulating intracellular peroxide levels and glutathione peroxidase activity, and an excess dose of α-MSH could break the immunobalance (23).

Inflammation is reduced by α-MSH via direct and indirect mechanisms (25). The direct action of the peptide are through its receptors, small seven-transmembrane G-protein-linked receptors (MC1-R to MC5-R), in peripheral inflammatory cells (inhibition of proinflammatory cytokine production and inhibition of iNOS). The indirect effects on peripheral inflammation are mediated through the activity of descending anti-inflammatory neural pathways induced by the stimulation of α-MSH receptors. All these effects of the peptide are exerted in some part through inhibition of the nuclear transcription factor NF-κB (27, 28). These observations suggest that α-MSH could be a candidate for the treatment of the large spectrum of pathological conditions in which NF-κB is activated.

α-MSH is necessary for monocyte/macrophage regulation of important inflammatory modulators: NO, neopterin, and TNF-α (29). In our PBMC experiments, a very low dose of α-MSH could inhibit LPS-induced TNF-α production. It is reported that the cardiac α-MSH protein concentration in the rat is relatively low (1-10 fmol/mg protein) (26), but such a small concentration of α-MSH is thought to be a sufficient dose for paracrine or autocrine function. As very small concentrations of α-MSH can inhibit TNF-α production effectively in vivo and in vitro studies (30, 31), α-MSH may be one of the therapeutic targets of CHF in the future (32, 33). IL-10 is also considered to be one of the therapeutic targets for acute myocarditis and heart failure (12, 32, 33). Interestingly, the induction of IL-10 was reported as a major effect of anti-inflammatory effects by α-MSH in monocytes (18). An imbalance exists between proinflammatory cytokines (eg, TNF-α, IL-1β, and IL-8) and antiinflammatory cytokine cyto-kines (eg, IL-10, transforming growth factor-β) in patients with CHF (34). As recently reported, targeted approaches to neutralize TNF have resulted in worsening heart failure (13). Targeting a single component of the inflammatory cascade may be not sufficient in a disease as complex as heart failure. α-MSH may become one of the strong candidates for immunomodulating therapy restoring the balance.

In conclusion, the present observations indicate that the potent anti-inflammatory peptide α-MSH is increased in the circulation of CHF patients, probably to modulate the immuno-response to CHF. α-MSH has a broad spectrum of anti-inflammatory properties and immunomodulatory strategies that activate anti-inflammatory pathways. Our findings suggest that α-MSH may play an important role for immuno-regulation in the pathophysiology of CHF, and provide a future direction for more specific immunomodulating therapy.

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