Antimicrobial effects of α-MSH peptides

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Abstract: The presence of the ancient anti-inflammatory peptide α-melanocyte-stimulating hormone [α-MSH (1–13), SYSMEHFRWGKPV] in barrier organs such as gut and skin suggests a role in the nonspecific (innate) host defense. α-MSH and and its carboxy-terminal tripeptide (11–13, KPV) were determined to have antimicrobial influences against two major and representative pathogens: *Staphylococcus aureus* and *Candida albicans*. α-MSH peptides significantly inhibited *S. aureus* colony formation and reversed the enhancing effect of urokinase on colony formation. Antimicrobial effects occurred over a broad range of concentrations including the physiological (picomolar) range. Small concentrations of α-MSH peptides likewise reduced viability and germ tube formation of the yeast *C. albicans*. Antimicrobial influences of α-MSH peptides could be mediated by their capacity to increase cellular cAMP. Indeed, this messenger was significantly augmented in peptide-treated yeast and the potent adenylyl cyclase inhibitor dideoxyadenosine (ddAdo) partly reversed the killing activity of α-MSH peptides. Reduced killing of pathogens is a detrimental consequence of therapy with anti-inflammatory drugs. Because α-MSH has potent anti-inflammatory effects we determined influences of α-MSH on *C. albicans* and *S. aureus* killing by human neutrophils. α-MSH peptides did not reduce killing but rather enhanced it, likely as a consequence of the direct antimicrobial activity. α-MSH peptides that combine antipyretic, anti-inflammatory, and antimicrobial effects could be useful in treatment of disorders in which infection and inflammation coexist. *J. Leukoc. Biol.* 67: 233–239; 2000.

Key Words: *melanocortin peptides · antimicrobial peptides · natural immunity · Candida albicans · Staphylococcus aureus · flow cytometric assay · cAMP*

INTRODUCTION

Production of natural antimicrobial agents by phagocytes has been recognized for a long time [1, 2]. Endogenous antimicrobial peptides are likewise significant in epithelia, the barrier to environmental challenge that provides the first line of defense against pathogens [1, 2]. Mucosal secretions, phagocytes, and other components of the innate host defense system initiate the response to microbial penetration before time-consuming adaptive immunity starts. These natural antimicrobial peptides are generally effective against bacteria, fungi, and viruses [1, 2]. The existence of homologs of vertebrate antimicrobial peptides in invertebrates suggests that such agents are ancestral components of the host defense system. Survival of plants and invertebrates, which lack adaptive immunity, illustrates the effectiveness of host defense based on such innate mechanisms. Some of these peptides might be used as therapeutic agents to provide microbicidal activity against a broad spectrum of pathogens and/or to enhance the effects of the current antimicrobial agents. Indeed, one of them, a synthetic homolog of bactericidal/permeability-increasing protein (BPI), has been used successfully to treat children with severe meningococcal sepsis [3].

α-Melanocyte-stimulating hormone (α-MSH) is a 13-amino acid peptide with potent anti-inflammatory properties [4, 5]. It is produced by posttranslational processing of the larger precursor molecule pro-opiomelanocortin (POMC) and shares the 1–13 amino acid sequence with adrenocorticotropic hormone (ACTH) [6]. It is secreted by many cell types, including pituitary cells, monocytes, melanocytes, and keratinocytes [5]. α-MSH reduces production of proinflammatory mediators by host cells *in vitro* [7, 8] and local and systemic reactions in animal models of inflammation [9, 10]. The active message sequence resides in the carboxy-terminal tripeptide α-MSH (11–13, KPV), which has anti-inflammatory influences *in vivo* and *in vitro* that parallel those of the parent molecule [11, 12]. α-MSH occurs in the skin of rats [13] and in human epidermis [13]. Immunoreactive α-MSH is also found in the mucosal barrier of the gastrointestinal tract in intact and hypophysectomized rats [14] and in humans [15]. We recently found that human duodenal cells produce α-MSH in culture [Catania et al., unpublished]. The presence in barrier organs of this ancient peptide, relatively invariant in amino acid sequence over approximately 300 million years, suggests that it has a role in host defense. To test this possibility, we determined whether α-MSH and other melanocortin peptides have antimicrobial influences against two major and representative pathogens, the gram-positive bacterium *Staphylococcus aureus* and the yeast *Candida albicans*. α-MSH has potent anti-inflammatory influences, including reduction of neutrophil chemotaxis [16] and inhibition of nitric oxide production by macrophages [17]. Because reduced killing of pathogens is a detrimental consequence of therapies with corticosteroids and nonsteroidal anti-inflammatory drugs [18,19], it is important to learn whether

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α-MSH peptides also cause this dire effect. We determined, therefore, effects of α-MSH on *C. albicans* and *S. aureus* killing by human neutrophils.

MATERIALS AND METHODS

Peptides

The peptides used in this research: α-MSH (1–13), (4–10), (6–13), and (11–13), all N-acetylated and C-amidated, and ACTH (1–39) and (18–39) (CLIP), were kindly provided by Dr. Renato Longhi, CNR, Milan. Peptides were prepared by solid-phase peptide synthesis and purified by reversed-phase high-performance liquid chromatography.

Organism and culture conditions

C. albicans and *S. aureus* (clinical isolates) were obtained from the collection of the Laboratory of Microbiology, Ospedale Maggiore di Milano. *C. albicans* were maintained on Sabouraud's agar slants and periodically transferred to Sabouraud's agar plates and incubated for 48 h at 28°C. To prepare stationary growth phase yeast, a colony was taken from the agar plate and transferred into 30 mL Sabouraud-dextrose broth and incubated for 72 h at 32°C. Cells were centrifuged at 1,000 *g* for 10 min and the pellet was washed twice with distilled water. Cells were counted and suspended in Hanks' balanced salt solution (HBSS) to the desired concentration. Viability, determined by the exclusion of 0.01% methylene blue, remained >98%.

S. aureus (methicillin-resistant strain) colonies were transferred from blood agar plate into a suspension of HBSS. Bacterial suspension was then adjusted to 10^8 cells/mL based on corresponding transmittance at 0.5 McFarland in a Vitek Special DR700 Colorimeter (Hach Company, Loveland, CO).

C. albicans and *S. aureus* colony formation

C. albicans and *S. aureus* (both 1×10^6/mL in HBSS) were incubated in the presence or absence of α-MSH (1–13) or (11–13), 10^{-15} to 10^{-4} M, for 2 h at 37°C. Cells were then washed in saline solution and diluted with HBSS to a concentration of approximately 100 organisms/mL. One-milliliter aliquots were dispensed on blood agar plates and incubated for 24 (*S. aureus*) or 48 h (*C. albicans*) at 37°C. Organism viability was estimated from the number of colony-forming units (CFU). All the tests were run in triplicate. We made serial dilutions of bacteria to obtain 50–150 CFU per plate. In control plates there were approximately 100 CFU. Experiments with smaller or larger numbers of colonies were considered technically inappropriate. Furthermore, only experiments in which CFU in all three control plates were between 50 and 150 with a variability in counts of less than 10% were used to evaluate effects of α-MSH peptides.

In subsequent experiments using similar procedures, we compared activity of α-MSH (4–10), (6–13), (11–13), ACTH (1–39), (18–39), and fluconazole, an established antifungal agent, in reducing *C. albicans* viability. Melanocortin peptides and fluconazole were tested in concentration of 10^{-6} M. There were at least six replicates for each peptide.

In experiments on *S. aureus* we determined the influence of α-MSH on urokinase-induced growth enhancement [20]. *S. aureus* (10^6/100 μL) were incubated for 4 h at 37°C with recombinant human urokinase 500 U (Lepetit, Milan, Italy) in a shaking water bath, in the presence or absence of 10^{-6} M α-MSH (1–13) or (11–13). Appropriate dilutions of *S. aureus* were dispensed on agar plates and CFU counted after a 24-h incubation at 37°C.

Flow cytometric assay

The yeast isolates were grown on Sabouraud dextrose agar for 24 h at 35°C. Individual yeast suspensions of 5×10^6 cells/mL were prepared in sterile 0.9% saline solution. α-MSH (1–13) and (11–13) 10^{-4} M (final concentration) were dissolved in 0.1 mL of RPMI-1640 without sodium bicarbonate, buffered to pH 7.0 with MOPS [3-(*N*-morpholino) propanesulfonic acid] 0.165 M, and placed in 12- by 75-mm tubes (Falcon 2058). Yeast inocula (0.1 mL) were added, bringing the drug dilutions to the final test volume of 0.2 mL. The growth control received 0.1 mL of saline. Tubes were incubated without agitation at 35°C for 2, 4, and 6 h. After incubation, each sample was washed twice by centrifugation at 250 *g* at 4°C for 10 min in phosphate-buffered saline (PBS) and resuspended in 300 μL of PBS. Propidium iodide (PI) was added to each sample at the final concentration of 1 μg/mL and the tubes were gently mixed by tapping. Because addition of deoxycholate was suggested in a previous study [21] to enable prompt and optimal penetration of saturating amounts of PI into the damaged cells, a separate series of samples were prepared following the same procedures described above to perform parallel experiments in the presence of deoxycholate. After final washing, these samples were resuspended in 300 μL of sodium deoxycholate (25 mM, dissolved in PBS) and PI (1 μg/mL) was added as described above. Each tube was analyzed via flow cytometry with a FACScan (Becton Dickinson, Mountain View, CA). Each flow cytometric test analyzed 10,000 events.

C. albicans germ tube formation

C. albicans from stationary phase cultures were washed twice with distilled water and suspended in HBSS to a final concentration of 2×10^6/mL. Hyphal growth was induced by addition of 10% inactivated horse serum (GIBCO-BRL, Paisley, United Kingdom) to yeast incubated for 45 min at 37°C with continuous shaking. Horse serum was removed by washing cells twice with HBSS and incubation was continued for 60 min at 37°C in the presence of α-MSH (1–13), (6–13), or (11–13) 10^{-6} M, with continuous shaking. The percentage of filamentous cells was evaluated under a light microscope with the aid of a hemocytometer. Experiments were run in triplicate and at least 200 cells were scored. Photomicrographs were taken with a MC100 camera attached to an Axioskop Zeiss microscope.

cAMP accumulation and inhibition

C. albicans (5×10^8) were suspended in 1.4 mL of permeabilization buffer consisting of 20 mM Tris-HCl, pH 7.3, 5 mM β-mercaptoethanol, 1 mM EDTA, 1 mM Na_3N, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and 1 mg/mL bovine serum albumin (BSA) [22]. Seventy microliters of a mixture of toluene/ethanol (1:4 vol/vol) were added to a cell suspension that was shaken continuously for 5 min. Cells were centrifuged, resuspended, and used immediately for the cAMP accumulation test. *C. albicans* (10^6/mL) were incubated at 37°C with continuous shaking in the presence of 10^{-6} M α-MSH (1–13), (11–13), forskolin, an agent known to increase intracellular cAMP, or in medium alone. The reaction was stopped after 3 min by the addition of ice-cold ethanol. cAMP was measured in duplicate using a commercial enzyme immunoassay (EIA) kit (Amersham, Buckinghamshire, UK) after extraction via the liquid-phase method according to the manufacturer's instructions. The effect of forskolin (10^{-6} M) on *C. albicans* CFU was determined using the same procedures as for α-MSH peptides.

In related experiments, to examine the role of cAMP in inhibition of colony formation, we used dideoxyadenosine (ddAdo, Sigma) a potent inhibitor of adenylyl cyclase [23]. *C. albicans* (1×10^6 cells) were exposed to different concentrations of ddAdo (25, 50, and 100×10^{-5} M) for 2 h and to α-MSH 10^{-5} M or HBSS for another 2 h. At the end of the incubation period, cells were washed in saline solution and diluted with HBSS to a concentration of approximately 100 organisms/mL. One milliliter was dispensed on agar plates and CFU were counted at 48 h.

C. albicans killing by human neutrophils

Venous blood (20 mL) from healthy volunteers was anticoagulated with heparin. Neutrophils were isolated using dextran sedimentation and Ficoll-Hypaque (Sigma Chemical Co., St. Louis, MO) centrifugation. Erythrocytes were lysed via hypotonic shock. Neutrophils represented at least 97% of the cell suspension. Cell viability, estimated by trypan blue exclusion, was >98%. Neutrophils were suspended at final concentration in HBSS.

C. albicans (1×10^6) were opsonized with human AB serum in a shaking water bath for 30 min at 37°C. Organisms were then incubated with neutrophils (1×10^5; 1:10 ratio) in the presence of HBSS, α-MSH (1–13), or α-MSH (11–13) in concentrations of 10^{-15} to 10^{-4} M in a shaking water bath for 2 h at 37°C. After incubation, the culture tubes were placed on ice to stop growth and extracellular organisms were washed twice with centrifugation at 1000 *g* at 4°C. Sodium desoxycholate solution (2.5%) was added to the suspension and the tubes were shaken for 5 min. Cold distilled water was added to obtain a suspension of 10^6 *C. albicans*/mL. Two 1/100 serial dilutions in HBSS were made to obtain a final suspension of approximately 100 cells/mL. Aliquots of

1 mL were dispensed on blood agar plates and incubated for 48 h at 37°C. CFU were counted at the end of the incubation period. Experiments were run in triplicate and repeated using blood from five different donors. Experiments on killing of *S. aureus* were performed using similar procedures. *S. aureus* (1×10^7) were opsonized with human AB serum and incubated with neutrophils (1×10^6; 1:10 ratio) in the presence of HBSS, α-MSH (1–13), or α-MSH (11–13) in concentrations of 10^{-15} to 10^{-4} M in a shaking water bath for 2 h at 37°C. After incubation, the culture tubes were placed on ice to stop growth and extracellular organisms were washed twice with centrifugation at 1000 *g* at 4°C. Cold distilled water was added to obtain a suspension of 10^6 *S. aureus*/mL. Two serial dilutions of 1:100 in HBSS were made to obtain a final suspension of approximately 100 cells/mL. Aliquots of 1 mL were dispensed on blood agar plates and incubated for 24 h at 37°C. CFU were counted at the end of the incubation period.

Statistical analysis

One-way analysis of variance and Student's *t* test were used to analyze the data. Probability values <0.05 were considered significant.

RESULTS

S. aureus colony formation

α-MSH peptides (1–13) and (11–13) inhibited *S. aureus* colony formation (**Fig. 1**). The effect occurred over a wide range of concentrations and was significant ($P < 0.01$) with peptide concentrations of 10^{-12} to 10^{-4} M. Therefore, there was a statistically significant effect even with picomolar concentrations, i.e. the quantity of α-MSH found in human plasma [24]. Treatment with urokinase increased *S. aureus* colony formation and addition of α-MSH (1–13) or (11–13) 10^{-6} M significantly inhibited the enhancing effect of urokinase (**Fig. 2**).

C. albicans colony and germ tube formation

C. albicans CFU were greatly reduced by α-MSH (1–13) and (11–13) (**Fig. 3**). Concentrations of both peptides from 10^{-12} to 10^{-4} M had significant inhibitory influences on CFU ($P < 0.01$ vs. control). In experiments comparing the relative potency of 10^{-6} M melanocortin peptides in reducing *C. albicans* viability, α-MSH (11–13), (6–13), and (1–13) were the most effective (**Fig. 4**). Their inhibitory activity was similar to that of equimolar fluconazole. The

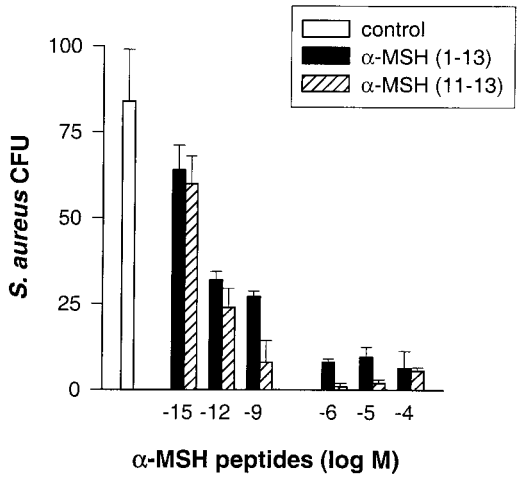

Fig. 1. Influence of α-MSH (1–13) and (11–13) on *S. aureus* colony-forming units. Scores are means ± SEM.

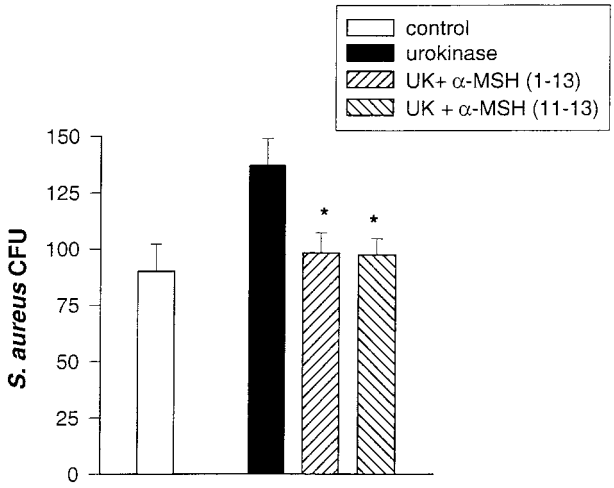

Fig. 2. Treatment with urokinase increased *S. aureus* colony formation and addition of α-MSH (1–13) or (11–13) significantly inhibited this urokinase-enhancing effect. *$P < 0.001$ vs. urokinase alone.

"core" α-MSH sequence (4–10), which has behavioral effects but little anti-inflammatory activity [5, 6], caused approximately 50% inhibition of CFU. Although this inhibitory effect was substantial ($P < 0.01$ vs. control), it was significantly less than that caused by α-MSH fragments bearing the KPV signal sequence, i.e. α-MSH (6–13) and (11–13) ($P < 0.01$), or the parent molecule α-MSH (1–13) ($P < 0.05$). ACTH (1–39) and its fragment (18–39) did not reduce *C. albicans* viability (Fig. 4). Coincubation of *C. albicans* with α-MSH (1–13) and (11–13) inhibited germ tube formation induced by horse serum (**Fig. 5**). α-MSH (1–13) caused 28–32% reduction in the number of filamentous cells; the tripeptide inhibited germ tube formation by 54–58%. The octapeptide α-MSH (6–13) had similar activity (approximately 50% inhibition; not shown).

Flow cytometric assay

Previous research has shown that flow cytometry can be used to provide rapid and reproducible antifungal susceptibility testing [21]. The effect of incubation of *C. albicans* with α-MSH (1–13) or (11–13) was assessed by measuring the red fluorescence that resulted from PI binding to DNA after uptake through damaged

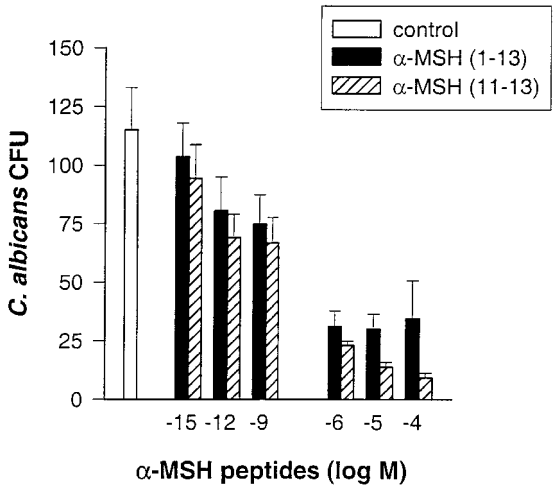

Fig. 3. Influence of α-MSH (1–13) and (11–13) on *C. albicans* colony-forming units.

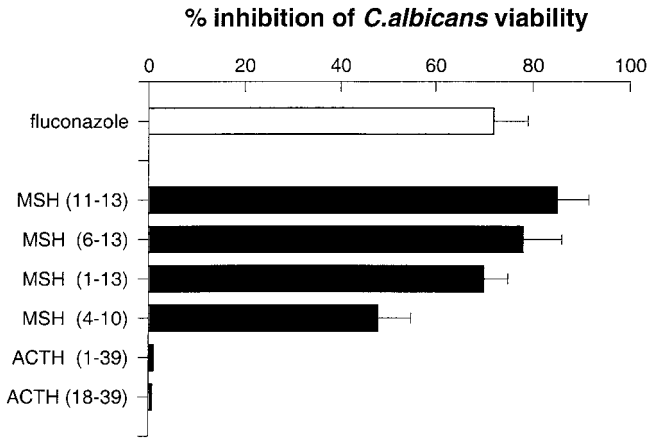

Fig. 4. Comparison of candidacidal activity of melanocortin peptides and fluconazole (all 10^{-6} M).

membranes. **Figure 6** shows changes in flow cytometric parameters of *C. albicans* induced by α-MSH (11–13) at 2, 4, and 6 h. When *C. albicans* was incubated for 2 h with the tripeptide there was no substantial change in PI incorporation relative to the control sample (8.8 vs. 6.5%). However, at 4 and 6 h α-MSH (11–13) significantly increased PI-positive cells to 72 and 58% relative to 7 and 14% in the respective controls. Significant increases in PI incorporation were likewise observed after incubation of *C. albicans* with α-MSH (1–13). In samples treated with the larger peptide, PI-positive cells at 2, 4, and 6 h were 10, 67.5, and 53.2%, respectively. When *C. albicans* were permeabilized with sodium deoxycholate to facilitate PI incorporation according to a previous report [21] there was an overall increase of PI-positive cells in both control and α-MSH-treated samples; however, the difference in proportion of PI-positive cells was similar.

cAMP accumulation in *C. albicans*

Because many of the effects of α-MSH are known to be mediated by induction of cAMP [6], we measured effects of α-MSH peptides on cAMP accumulation in *C. albicans*. α-MSH (1–13) and (11–13) enhanced cAMP content in the yeast (**Fig. 7**). The increase was of the same order of magnitude as that induced by equimolar forskolin, an adenylyl cyclase activator (Fig. 7). To determine whether increases in cAMP could be responsible for reduction in CFU, we tested effects of forskolin on *C. albicans* viability. Results showed that 10^{-6} M forskolin markedly inhibited *C. albicans* CFU relative to control ($<$ 0.01). The inhibitory effect was similar to that exerted by α-MSH peptides (Fig. 7). To further examine the role of cAMP in α-MSH inhibition of colony formation, we coincubated *C. albicans* with α-MSH in the presence or absence of ddAdo, a potent inhibitor of adenylyl cyclase [23]. There was a small reduction in CFU of *C. albicans* exposed to different concentrations of ddAdo (25, 50, and 100 $\times$ 10^{-5} M) alone (**Fig. 8**).

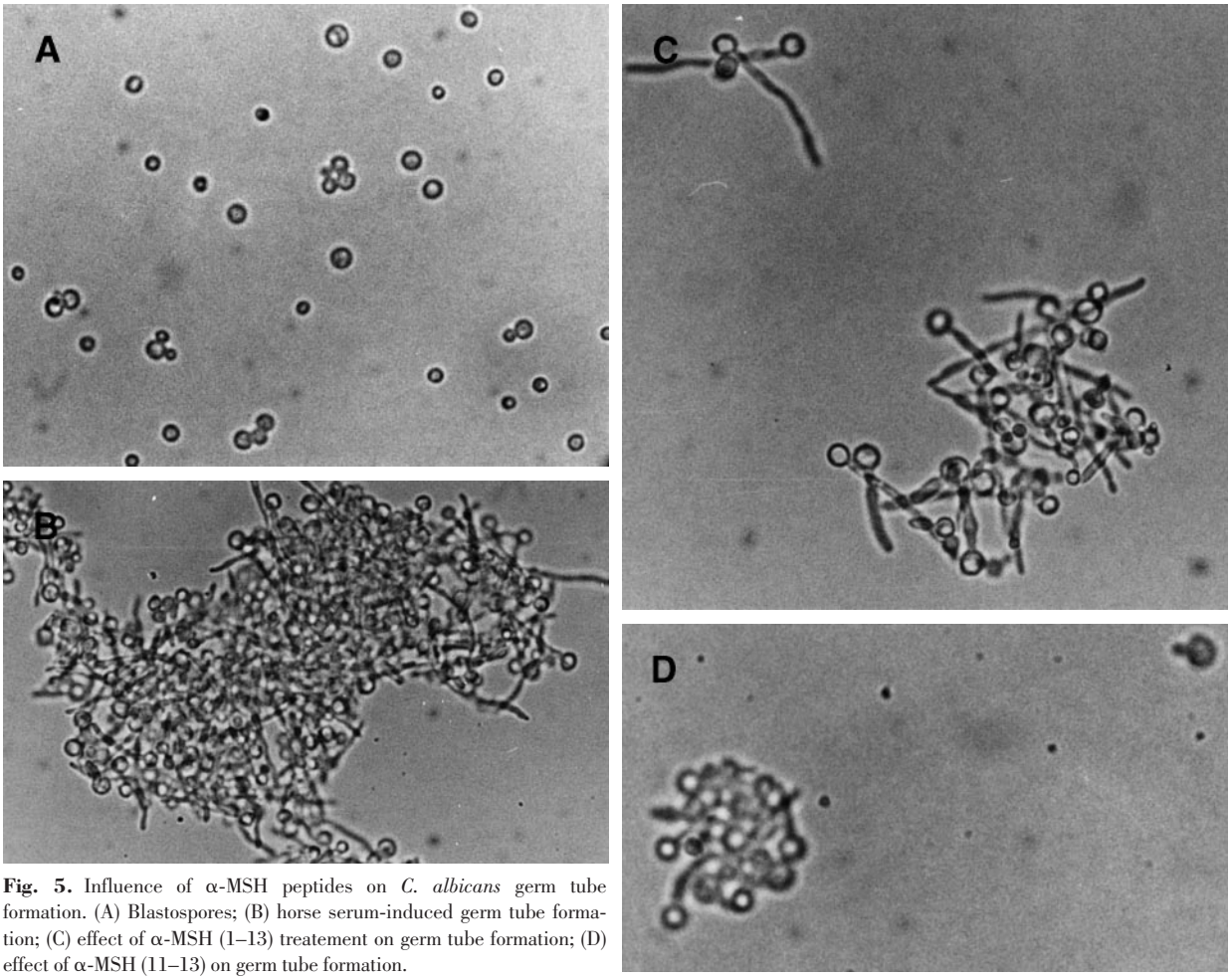

Fig. 5. Influence of α-MSH peptides on *C. albicans* germ tube formation. (A) Blastospores; (B) horse serum-induced germ tube formation; (C) effect of α-MSH (1–13) treatement on germ tube formation; (D) effect of α-MSH (11–13) on germ tube formation.

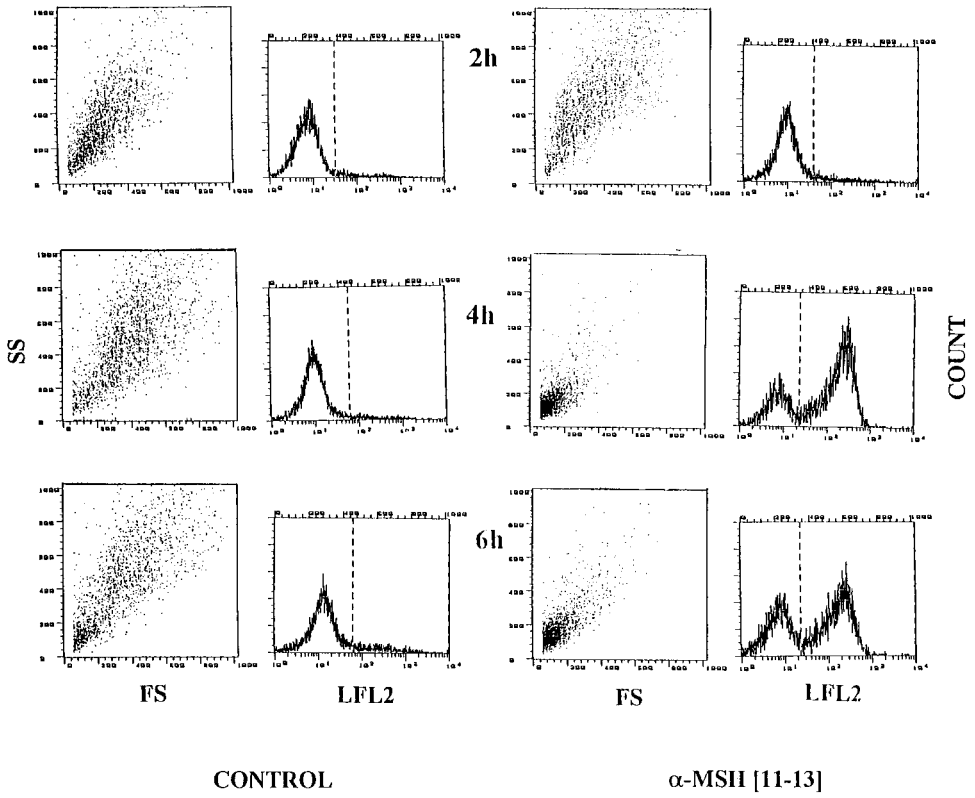

Fig. 6. Flow cytometry analysis of *C. albicans* stained with the fluorescent dye PI. *C. albicans* were incubated with medium (left panels) or α-MSH (11–13) (right panels) for 2, 4, and 6 h. Dot plots are shown for forward scatter (FS) and side scatter (SC). Cell count and log fluorescence (LFL2) values show distribution of cells according to the relative fluorescence intensity. Cells treated with α-MSH (11–13) show a marked increase in fluorescence at 4 and 6 h, indicating permeabilization.

However, when ddAdo was added to α-MSH (1–13) or (11–13), it partly reversed the killing activity of the peptides.

C. albicans killing by human neutrophils

α-MSH (1–13) and (11–13) enhanced killing of *C. albicans* by human neutrophils when administered in concentrations of 10^{-12} to 10^{-4} M ($P < 0.01$; **Fig. 9**). Concentrations as low as 10^{-15} still caused a small (approximately 20%) enhancement in killing that was not statistically significant. Therefore, enhanced killing occurred over a very broad range of concentrations as for direct killing of pathogens. α-MSH peptides likewise increased killing of *S. aureus* by normal neutrophils

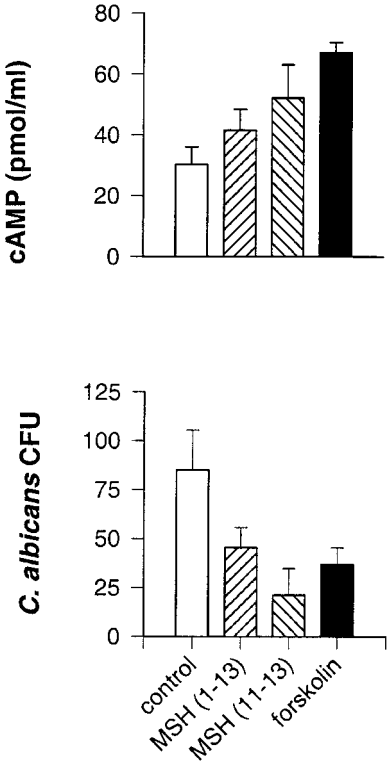

Fig. 7. (A) Effect of α-MSH (1–13), (11–13), and forskolin (all 10^{-6} M) on cAMP accumulation in *C. albicans*. (B) Inhibitory effect of α-MSH (1–13), (11–13), and forskolin on *C. albicans* CFU.

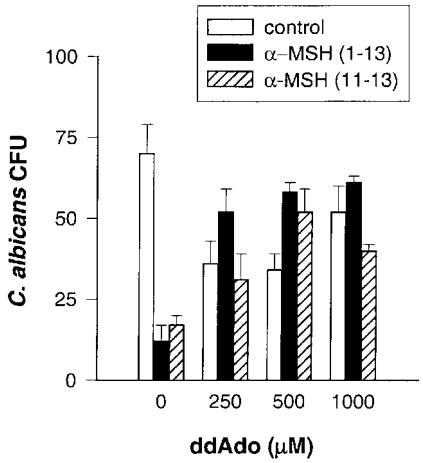

Fig. 8. Effect of the adenylyl cyclase inhibitor ddAdo on the candidacidal effect of α-MSH (1–13) and (11–13). Coincubation with ddAdo partly reversed the candidacidal effect of α-MSH peptides.

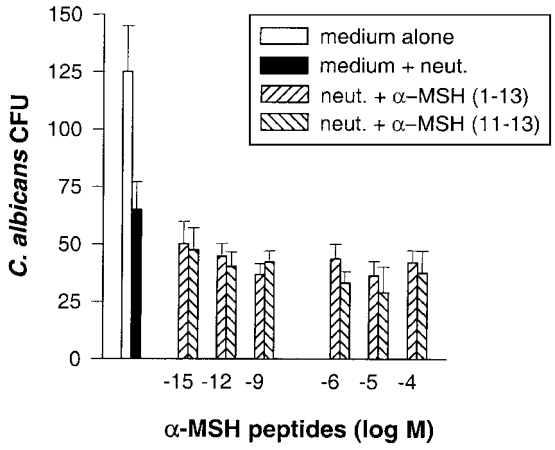

Fig. 9. Effect of α-MSH (1–13) and (11–13) on *C. albicans* killing by human neutrophils.

(**Fig. 10**). The enhancing effect was less pronounced than in *C. albicans* experiments because neutrophils alone killed a large proportion of *S. aureus*.

DISCUSSION

The results show that α-MSH (1–13), its carboxy-terminal tripeptide (11–13), and other α-MSH fragments have antimicrobial effects against two major pathogens: *S. aureus* and *C. albicans*. The most effective peptides were the entire α-MSH amino acid sequence (1–13) and the two α-MSH fragments (6–13) and (11–13). Therefore, the carboxy-terminal tripeptide KPV, which is essential for the antipyretic and anti-inflammatory effects of α-MSH, is likewise important for the antimicrobial activity. The α-MSH core sequence (4–10), which is known to influence melanogenesis, learning, and memory [4, 6], but lacks an antipyretic influence [4], was significantly less effective. Two other melanocortin peptides, ACTH (1–39) precursor of α-MSH and the ACTH fragment (18–39), which does not include the α-MSH amino acid sequence, did not have significant candidacidal effects. These observations indicate that antimicrobial activity is not common to all melanocortin peptides, but rather that it is specific to certain α-MSH amino acid sequences.

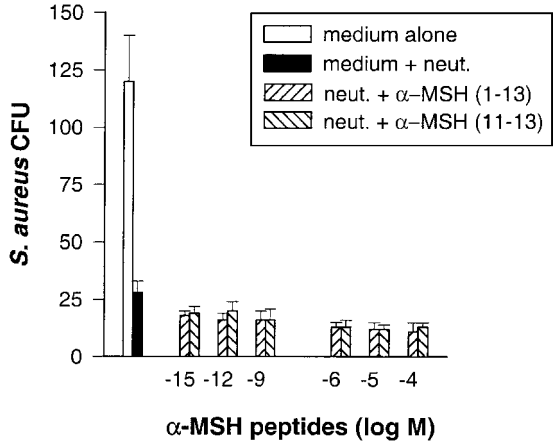

Fig. 10. Effect of α-MSH (1–13) and (11–13) on *S. aureus* killing by human neutrophils.

Antimicrobial effects of α-MSH occurred over a very broad range of concentrations, including picomolar concentrations that normally occur in human plasma [24]. This suggests that endogenous α-MSH has a physiological role in natural immunity.

α-MSH shares a number of similarities with other natural antimicrobial peptides: (1) it is produced in mammals but also in primitive organisms that lack adaptive immunity [6]; (2) like known antimicrobial peptides, its precursor molecule POMC is expressed in phagocytes [7] and epithelia [25] and posttranslational proteolytic processing is required to convert it to active α-MSH; (3) it is a cationic peptide; (4) it has antimicrobial influences against at least two disparate pathogens, a yeast and a bacterium. This observation, combined with our recent finding that α-MSH inhibits HIV-1 replication in acutely and chronically infected monocytes [26], indicates that α-MSH has the broad spectrum of activity of other innate antimicrobial substances.

An important question concerns how α-MSH peptides exert their antimicrobial effects and whether they operate like other natural antimicrobial agents. The mechanism of action of natural antimicrobial agents is only partially understood [1, 2]. Most of these peptides, including the defensins, alter membrane permeability and impair internal homeostasis of the organism. The first contact is made between the cationic groups of the peptide and the negatively charged head of the target membrane. Then, the tertiary structure determines the mode of insertion of the peptide into membranes where they form ion channels or pores that disrupt cell integrity. There is no evidence that this is the way α-MSH peptides operate. Two-hour incubation of *C. albicans* with α-MSH greatly reduced CFU but did not increase PI incorporation, which was elevated at 4 h. This suggests that membrane disruption is a late phenomenon, perhaps a consequence rather than a cause of cell damage. Histatins that cause membrane damage enhanced PI incorporation in *C. albicans* at 10 min [27].

Evidence presented here suggests that the candidacidal effect of α-MSH is mediated through induction of cAMP. α-MSH-induced cAMP in *C. albicans* and the adenylyl cyclase inhibitor ddAdo partly reversed the candidacidal effect of α-MSH. It is likely, therefore, that the antimicrobial effect was caused by enhancement of this mediator. Indeed, previous observations showed that cAMP-enhancing agents inhibit mRNA and protein synthesis in *C. albicans* [28]. It is interesting that in our research both the cAMP inducer forskolin and the adenylyl cyclase inhibitor ddAdo alone caused some inhibition of CFU. This suggests that cAMP is crucial in *C. albicans* viability and both increases and reductions in this mediator can reduce *C. albicans* viability.

Whether antimicrobial effects of α-MSH peptides depend on recognition of specific receptors on microorganisms or they occur through peptide penetration into microbial cells is not known. Homologs of mammal melanocortin receptors are expressed in invertebrates [29] but so far there is no evidence that they also occur in yeast or bacteria. On the other hand, the increase in cAMP induced by α-MSH in *C. albicans* suggests a receptor-mediated mechanism much as in higher species. Indeed, melanocortin receptors are G-protein-linked receptors whose signal transduction occurs through induction of adenylyl cyclase and increase in cAMP. It may be, therefore, that microorganisms express one or more so far unrecognized

melanocortin receptors that, like those present in higher species, bind α-MSH fragments with different affinity.

C. albicans is the leading cause of invasive fungal disease in premature infants, diabetics, surgical patients, and patients with human immunodeficiency virus infection or other immunosuppressed conditions. Despite appropriate therapy, mortality resulting from systemic *C. albicans* infection in immunocompromised patients is substantial [30, 31]. The pathogenesis of *C. albicans* infection involves adhesion to host epithelial and endothelial cells and morphological switching of yeast cells from the ellipsoid blastospore to various filamentous forms: germ tubes, pseudohyphae, and hyphae [32]. It is therefore important that α-MSH not only reduces *C. albicans* viability but also germ tube formation.

α-MSH has potent anti-inflammatory influences in models of acute, chronic, and systemic inflammation. Its wide spectrum of activity and low toxicity suggest that α-MSH may be useful for treatment of inflammation in human and veterinary disorders. Reduced killing of pathogens is a dire consequence of corticosteroids and nonsteroidal anti-inflammatory drugs [18,19]; such effect could be particularly dangerous in the immunocompromised host. It was, therefore, important to learn whether α-MSH peptides also reduce pathogen killing by phagocytes. Indeed, because α-MSH inhibits neutrophil chemotaxis [16] and nitric oxide production by macrophages [17], it could likewise reduce killing, despite the direct antimicrobial effect. The results of this research indicate that α-MSH peptides do not reduce killing but rather enhance it, likely as a consequence of the antimicrobial activity. Increased killing by neutrophils is in agreement with similar observations on separate keratinocytes [33]. α-MSH peptides that combine antimicrobial, antipyretic, and anti-inflammatory effects could be very useful in the treatment of infections.

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