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Binding of Melanotropic Hormones to the Melanocortin Receptor MC1R on Human Melanocytes Stimulates Proliferation and Melanogenesis*

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ABSTRACT

α-Melanocyte stimulating hormone (α-MSH) and ACTH increase the proliferation and melanogenesis of cultured human melanocytes. To further analyze how melanotropins produce these biological effects, we investigated the regulation of the melanocortin receptor MC1R expression by α-MSH and ACTH using Northern blot analysis and determined the relative affinity of the receptor for the structurally similar peptides α-MSH, ACTH, β-MSH, and γ-MSH. We also determined the relative potencies of these hormones to stimulate cAMP formation, tyrosinase activity, and melanocyte proliferation. The order of affinity and potency of the noted melanotropins in these assays were α-MSH = ACTH > β-MSH > γ-MSH. Because the binding affinity of each of these melanotropins for the MC1R correlated with its ability to stimulate human melanocyte proliferation and melanogenesis, we conclude that these effects are mediated specifically by binding to and activation of the MC1R. γ-MSH stimulated cAMP formation without affecting proliferation or melanogenesis. However, we found that relative to α-MSH, the effect of γ-MSH on cAMP formation was transient. Our results suggest that α-MSH, ACTH, and possibly β-MSH, but not γ-MSH, are capable of a physiological role in regulating human pigmentation, and that melanocytes in human skin are a specific target for these hormones. (*Endocrinology* **137:** 1627–1633, 1996)

MELANOTROPIC hormones are a family of structurally related peptides derived from one precursor protein, POMC (1, 2). Differential enzymatic cleavage of POMC results in the production of ACTH, α-melanocyte stimulating hormone (α-MSH), β-MSH, and γ-MSH, and other hormones that include β-endorphin and β-lipotropic hormone (β-LPH) (3). Melanotropins have many effects, the best described of which is stimulation of integumental pigmentation in many vertebrate species (4–6). Melanotropins also have neurotrophic, behavioral, and immunologic effects and influence a wide range of target cells in the brain and peripheral tissues (7–11). So far, five different melanocortin receptors (MC1–MC5) have been cloned and found to differ in their tissue distribution and relative affinities for the various melanotropic peptides (12–17). Melanocortin receptor MC1R is expressed on normal and malignant melanocytes and has been termed the α-MSH receptor because of its high affinity for this hormone (12, 13). The cloned human MC1R has been shown to have equal affinity for α-MSH and ACTH (13).

Although melanotropins are best known for their ability to increase pigmentation in many vertebrates including mammals, their effects on human melanocytes remained controversial until recently. Hunt *et al.* (18, 19) reported that α-MSH and ACTH were melanogenic for human melanocytes. On the other hand, De Luca *et al.* (20) found that α-MSH was mitogenic but not melanogenic for these cells. We have demonstrated that α-MSH and ACTH, in subnanomolar concentrations, were both mitogenic and melanogenic for human melanocytes (21). The expression of melanocortin receptors by human melanocytes and the ability of those cells to respond to physiological concentrations of α-MSH and ACTH suggest a physiological role for these hormones in regulating human pigmentation.

In this study, we have used human melanocytes as physiological target cells to investigate the expression of the MC1R and to compare the relative potencies of the different melanotropic peptides in binding to this receptor, activating adenylyl cyclase, and stimulating melanogenesis and proliferation. That melanotropins are synthesized by epidermal cells, particularly in response to UV light and during wound healing, suggests that these hormones might have a paracrine role in regulating human pigmentation and the response of human melanocytes to solar radiation and inflammation (22, 23).

Materials and Methods

Melanocyte culture conditions

Primary cultures of normal human melanocytes were established from neonatal foreskins representing different skin types as described previously (24). The complete growth medium used consisted of MCDB 153 supplemented with 5% FCS, 1 μg/ml human transferrin, 5 μg/ml insulin, 1 μg/ml α-tocopherol, and the melanocyte mitogens human recombinant basic fibroblast growth factor (0.6 ng/ml), 12–0-tetradeca-

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noylphorbol-13-acetate (8 nM) (all purchased from Sigma Chemical Co., St. Louis, MO), and bovine pituitary extract (BPE; 13 ng/ml) (Clonetics, San Diego, CA), as previously described (25). Because BPE contains high concentrations of melanotropic hormones, we found it essential to remove it from the culture medium in order for melanocytes to respond to melanotropins. The melanotropic peptides used are [Nle^4,D-Phe^7]-α-MSH (NDP-α-MSH), synthetic α-MSH, β-MSH, γ-MSH, and ACTH$_{1-39}$ (Sigma).

Northern blot analysis of the MC1R messenger RNA (mRNA)

Melanocytes in BPE-free medium were plated onto 100-mm dishes at a density of 5×10^5 cells/dish. Forty-eight hours later, melanocytes were treated for various time periods (3, 6, 8, 9, and 24 h) with 0.1 μM α-MSH or ACTH. At the end of each treatment, melanocytes were lysed using phenol and guanidine thiocyanate (Molecular Research Center, Cincinnati, OH). Total RNA was purified by phase separation followed by isopropanol precipitation. The concentration of RNA was determined spectrophotometrically. Fifteen to 20 μg of total RNA were separated by electrophoresis through agarose-formaldehyde gel. After electrophoresis, the RNA was blotted onto nylon membrane filters (Hybond-N$^+$; Amersham, Arlington Heights, IL). The amount and quality of RNA were detected using methylene blue staining. Filters were hybridized with ^{32}P-labeled human MC1R (12), human MC2R, rat MC3R, human MC4R, and mouse MC5R complementary DNA (cDNA) probes in a solution containing 50% formamide, 5 × saline-sodium phosphate-EDTA buffer (SSPE), 5 × Denhardt's solution (1 g Ficoll, 0.1 g polyvinylpyrrolidone, 0.1 g BSA in 100 ml H_2O), 0.1% SDS, and 100 μg/ml of Herring sperm DNA for 16 h at 42 C. All of the above reagents were purchased from Sigma Chemical Co. After hybridization, filters were washed in a solution of 2 × saline-sodium citrate buffer (SSC), 0.1% SDS at 42 C for 30 min, and 0.1 × SSC, 0.1% SDS at 42 C for 30 min. Filters were exposed to x-ray film for 3–16 h with intensifying screen. Filters were rehybridized with G3PDH cDNA probe (Clontech Laboratory, Inc., Palo Alto, CA) (26) to normalize for the amount of RNA.

Receptor binding studies: competition of ^{125}I-NDP-α-MSH binding to MC1R by α-, β-, γ-MSH or ACTH

Melanocytes were maintained in growth medium lacking BPE for 4 days before, and for the duration of, the experiment. Melanocytes were inoculated into 24-well plates at a concentration of 4×10^5 cells/well and allowed to attach for at least 2 days. Cells were then washed twice with 1 ml of MCDB medium containing 0.5% BSA (fraction V, Sigma) and incubated in 0.5 ml of MCDB medium containing 0.5% BSA, 0.3 mM 1,10-phenanthroline (proteinase inhibitor; Sigma) and 100,00–150,000 cpm of ^{125}I-NDP-MSH and various concentrations of cold ligand (10^{-12}–10^{-6} M) at room temperature for 2 h. After incubation, melanocytes were washed 3 times with 1 ml of ice-cold MCDB medium containing 0.5% BSA and lysed with 1 ml of 0.5 N NaOH, 0.4% deoxycholate. Radioactivity was counted in a γ-counter and the data were analyzed by linear regression using software from KaleidaGraph (Version 3.04, Abelbeck Software).

cAMP assays

Melanocytes were maintained in BPE-free growth medium for at least 2 days before, and for the duration of, the experiments. Cells were plated onto 24 well plates at a density of 3×10^5 cells/well. Forty-eight hours later, fresh medium was added to each well, and on the next day, the cAMP assays were performed as follows. For the dose-response to melanotropins, the medium was removed from each well and replaced with 450 μl growth medium containing 0.1 mM isobutyl methylxanthine (IBMX), a phosphodiesterase inhibitor. The cultures were incubated for 20–30 min with this medium at 37 C and then treated with 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, or 10^{-6} M of α-MSH, β-MSH, γ-MSH, ACTH, or NDP-α-MSH (Fig. 1) for 30 min at 37 C. For comparing the time-dependent effects of α-MSH and γ-MSH on cAMP formation, melanocytes in 24-well plates were treated with 10^{-7} M of either peptide in the absence of IBMX for 0.5, 1.5, 4, 8, or 24 h. The total volume in each well was 0.5 ml. The reaction was stopped by the addition of 50 μl 1 N HCl to each well. Duplicate 250-μl samples were removed from each well and a volume of 750 μl of 0.1 N HCl was added to each sample. Each sample was acetylated by the addition of 40 μl triethylamine and acetic anhydride (2.5:1 vol:vol), and the amount of cAMP was determined by RIA as previously described by Liggett *et al.* (27).

Determination of cellular proliferation and tyrosinase activity

Melanocytes maintained for 2–4 days in medium devoid of BPE were plated in 12.5-cm^2 flasks at a density of 1×10^5 cells/flask. Forty-eight hours later, cells were treated with α-MSH, β-MSH, γ-MSH, or NDP-α-MSH, at concentrations ranging between 10^{-11} and 10^{-7} M. Fresh medium and melanotropin were added every other day for a total of 6 days of treatment. On day 5 of treatment, each flask received 2.2 μCi ^{3}H-tyrosine (0.733 μCi/ml medium), and 16–18 h later, cell number and tyrosinase activity were determined. Cells from each flask were harvested and counted using a Coulter counter, and the tyrosine hydroxylase activity of tyrosinase was measured *in situ* using a modification of the charcoal absorption method of Pomerantz, as described previously (28, 29).

Results

Northern blot analysis of the MC1 receptor

We have confirmed by Northern blot analysis that primary human melanocytes normally express mRNA for the MC1R (Fig. 2). Treatment of melanocytes with 10^{-7} M α-MSH or ACTH resulted in increased expression of the MC1R mRNA (Fig. 2). This effect was evident within 4 h, reached a maximum level (about 2-fold increase) in 6 h, and declined to basal level after 24 h of treatment with either hormone. In these experiments we found that, unlike the melanocytes, human epidermal keratinocytes lacked MC1R mRNA (data not shown). Chakraborty and Pawelek (30) reported that melanotropin receptors are expressed on immortalized human keratinocytes. Our results suggest that human keratinocytes might express melanocortin receptor(s) other than MC1R. Using Northern blot analysis and cDNA probes for the human MC2 (ACTH), rat MC3, human MC4, and mouse MC5 receptors, we could not detect specific mRNA transcript

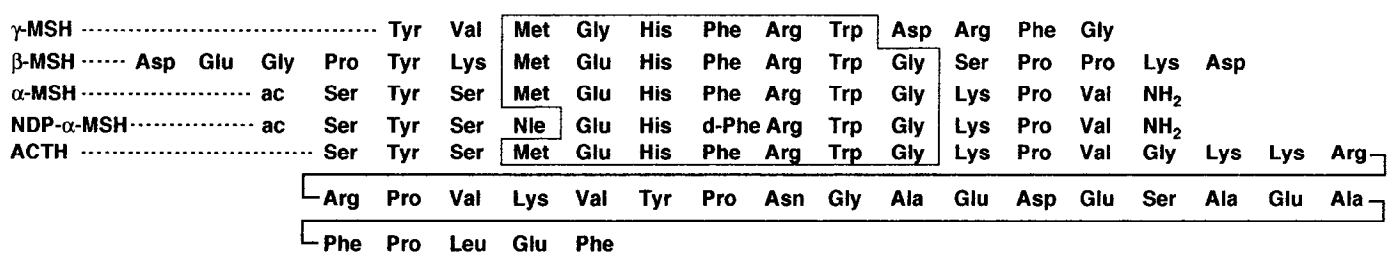

FIG. 1. Amino acid sequence of [Nle^4,D-Phe^7]-α-MSH, α-MSH, β-MSH, γ-MSH and ACTH.

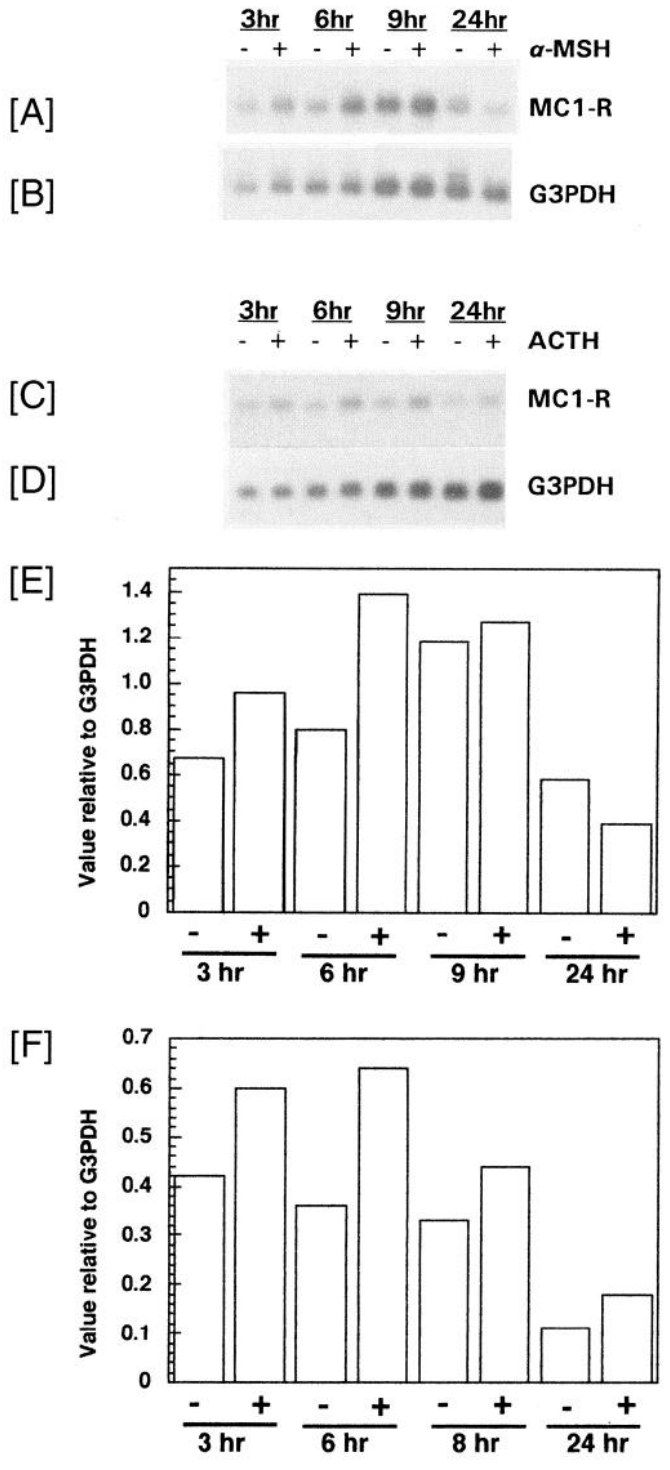

FIG. 2. Northern blot analysis of the MC1 receptor mRNA. Basal (-) and melanotropin induced (+) MC1R mRNA levels were determined after 3, 6, 8, 9 and 24 h of treatment with 10^{-7} M α-MSH or ACTH, as described in Materials and Methods. A, Basal and α-MSH induced MC1R mRNA expression; B, corresponding G3PDH control. The effect of α-MSH was investigated using four different cell strains with similar results obtained each time. C, Basal and ACTH-induced MC1R mRNA expression; D, corresponding G3PDH control. The effect of ACTH was observed in two independent experiments using two different cell strains. The observed increase in G3PDH mRNA over time seems to represent a response to addition of fresh culture medium to the cells before treatment with melanotropins. A and C, Two different melanocyte strains. E and F, Levels of MC1R mRNA relative to the G3PDH mRNA after treatment of melanocytes with α-MSH or ACTH, respectively, as determined by densitometry.

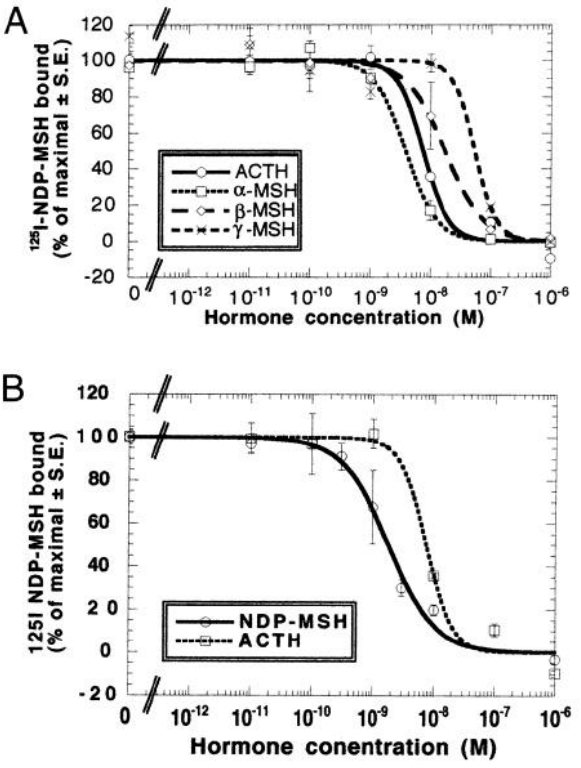

FIG. 3. Displacement of ^{125}I-NDP-α-MSH from the MC1R by α-, β-, γ-MSH or ACTH (A), or by cold NDP-α-MSH or ACTH (B). Melanocytes were incubated in the presence of 100,000–150,000 cpm of ^{125}I-NDP-α-MSH alone (for total binding) or in the presence of increasing concentrations (10^{-11}–10^{-6} M) of NDP-α-MSH, ACTH, α-, β-, or γ-MSH for 2 h at room temperature, as described in *Materials and Methods*. The amount of radioactivity was counted in a γ-counter, and the data were analyzed by Kaleidagraph. Each value represents the mean cpm of three determinations ± SD. Maximal binding was obtained in the absence of any cold ligand.

for these particular receptors in human melanocytes (data not shown).

Relative binding affinity of melanotropins for the MC1 receptor

To determine the relative binding affinities of α-MSH, ACTH, β-MSH, and γ-MSH to the MC1R, we compared their ability to compete with ^{125}I-NDP-α-MSH for binding. From the displacement curves that we have generated, we found approximate IC_{50} values ranging from 2 nM (NDP-MSH) to 40 nM (γ-MSH) with the following order of potency: NDP-α-MSH > α-MSH ≥ ACTH > β-MSH > γ-MSH in affinity for the MC1 receptor (Fig. 3). This order of affinity of the above melanotropins is consistent with that previously reported by Chhajlani *et al.* (31), using COS cells that were transfected with the human MC1R cDNA.

Dose-dependent effects of melanotropins on cAMP formation

Binding of ligands to G_s protein coupled receptors is known to activate adenylyl cyclase and increase cAMP formation. For this, we compared the relative potencies of the above melanotropic peptides to elevate cAMP levels in human melanocytes. In these experiments, melanocytes were treated with NDP-α-MSH, α-MSH, ACTH, β-MSH, or γ-MSH at concentrations of 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, and 10^{-7} M for 30

min. All of the above peptides resulted in a dose-dependent increase in cAMP levels. We found that a significant increase in cAMP levels was first evident at a concentration as low as 10^{-11} M NDP-α-MSH, compared with 10^{-10}–10^{-9} M α-MSH or ACTH, 10^{-8} M β-MSH, and 10^{-7} M γ-MSH (Fig. 4). At concentrations of 10^{-9}, 10^{-8}, or 10^{-7} M α-MSH and ACTH consistently caused a greater induction of cAMP than β-MSH, whereas γ-MSH was least effective in stimulating cAMP formation (Fig. 4A). Based on these results, it can be concluded that the order of potency of these melanotropic peptides in stimulating adenylyl cyclase activity is: NDP-α-MSH > α-MSH = ACTH > β-MSH > γ-MSH. This is in agreement with the previously reported results using the cloned human MC1R (12, 31) and correlates directly with our findings regarding the relative binding affinity of these peptides to the MC1R.

Comparison of the dose-dependent effects of α-MSH, β-MSH, γ-MSH, and NDP-α-MSH on tyrosinase activity and melanocyte proliferation

We have previously demonstrated that α-MSH and ACTH are equipotent in their ability to stimulate tyrosinase activity and the proliferation of human melanocytes. Using the same experimental procedure whereby melanocytes were treated for a total of 6 days with doses of melanotropins ranging between 10^{-11} M and 10^{-7} M, we found that a significant increase in melanocyte proliferation and tyrosinase activity was first observed in response to 10^{-10} M α-MSH and 10^{-9} M β-MSH, respectively (Figs. 5 and 6). While comparing NDP-α-MSH and α-MSH, we found that both peptides had significant stimulatory effects at a dose of 10^{-10} M. However, at concentrations of 10^{-10} and 10^{-9} M, NDP-α-MSH was more effective in inducing melanocyte proliferation and at 10^{-10} M concentration had a greater stimulatory effect on tyrosinase activity than α-MSH. Therefore, at doses ranging between 10^{-10} and 10^{-8} M, the order of potency of the melanotropic peptides tested in stimulating melanocyte proliferation and tyrosinase activity is NDP-α-MSH > α-MSH = ACTH > β-MSH > γ-MSH. Except for γ-MSH, which had a minimal or no effect in these experiments, we found that maximal mitogenic effect was achieved with 10^{-8} M α-MSH or β-MSH, compared with 10^{-9} M NDP-α-MSH. Maximal stimulation of tyrosinase activity was achieved with 10^{-9} M α-MSH or NDP α-MSH, compared with 10^{-8} M β-MSH. Except for γ-MSH, the relative potencies of the other melanotropins in eliciting mitogenic and melanogenic effects correlated directly with their binding affinity to the MC1R and their ability to stimulate cAMP formation.

Comparison of the time-dependent effects of α-MSH and γ-MSH on cAMP formation

It was puzzling to find that α-MSH and γ-MSH, both at 10^{-6} M, elicited equivalent effects on cAMP levels (Fig. 4A), yet only α-MSH, not γ-MSH, resulted in a profound

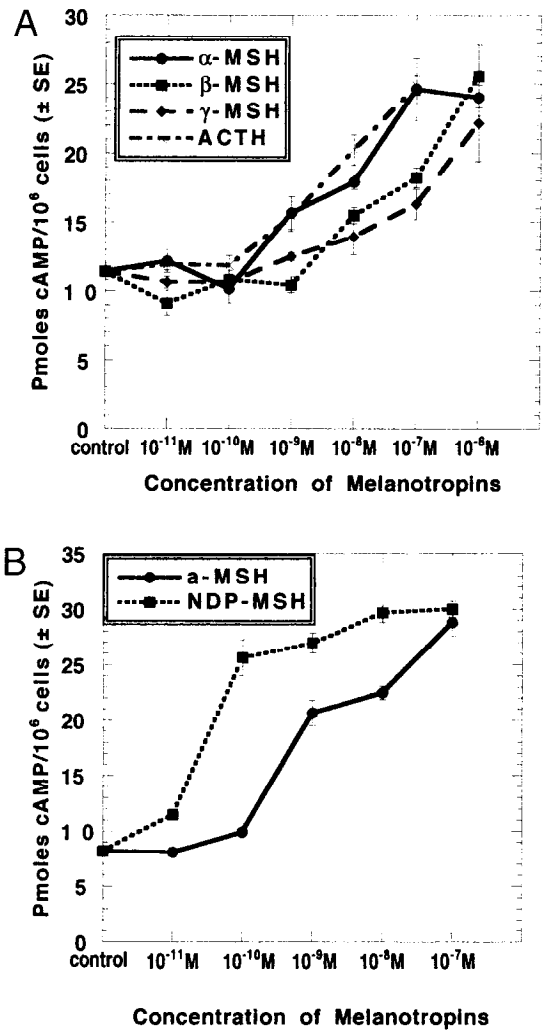

FIG. 4. Dose-dependent stimulation of cAMP formation by NDP-α-MSH, α-, β-, γ-MSH, and ACTH. Melanocytes were incubated in the presence of 0.1 mM IBMX and treated with increasing concentrations (10^{-11} - 10^{-6} M) of the above melanotropic hormones for 30 min at 37 C. cAMP levels were determined by RIA. Each value represents the mean of six determinations ± SE. The data were expressed as pmol cAMP/10^6 cells. A, Dose response to α-, β-, γ-MSH, and ACTH. B, Dose response to α-MSH and NDP-α-MSH. Similar results were obtained using two different melanocyte strains.

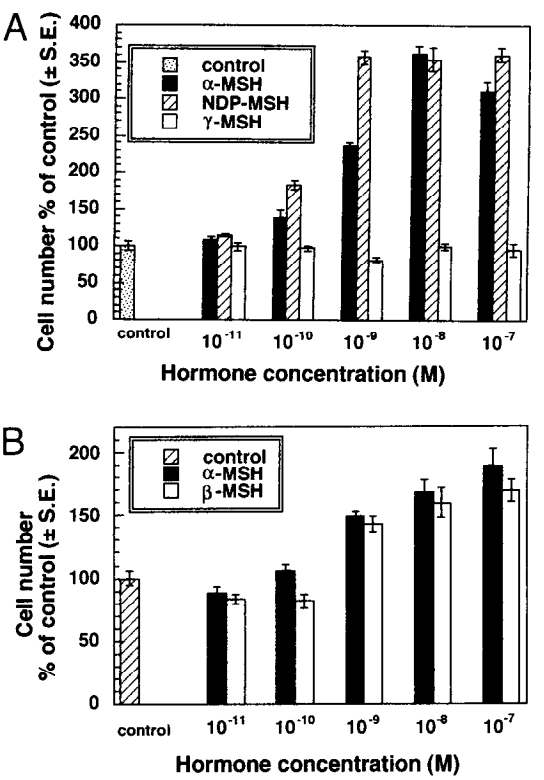

FIG. 5. Dose-dependent stimulation of melanocyte proliferation by α-, β-, γ-MSH, and NDP-α-MSH. Melanocytes were treated 48 h after plating and every other day thereafter with increasing doses (10^{-11}–10^{-7} M) of the above melanotropins, for a total of 6 days. Cell number in each flask (n = 3 per group) was determined using a Coulter counter. Each value represents the mean of three determinations ± SE. A, Dose dependent effects of α-, γ-MSH and NDP-α-MSH. B, Effects of α- and β-MSH. Similar findings were obtained in two separate experiments using two different melanocyte strains.

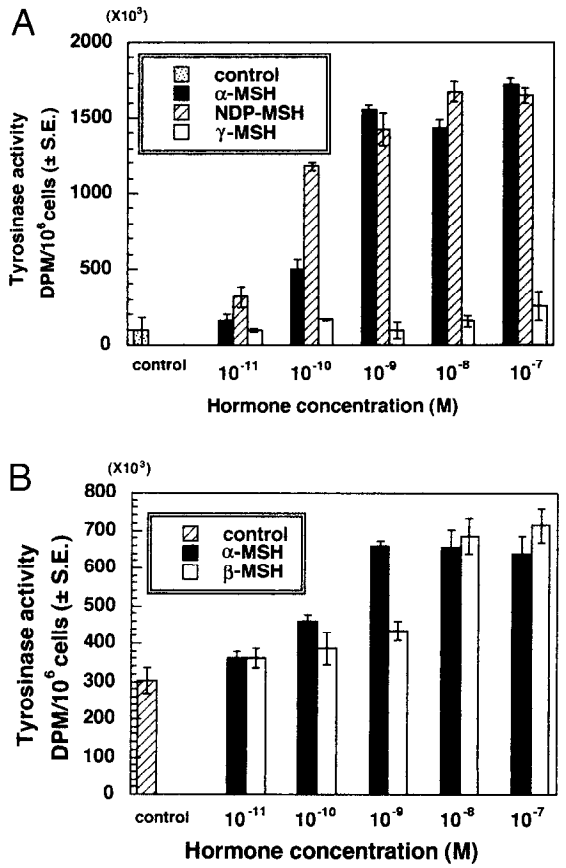

FIG. 6. Stimulation of tyrosinase activity by increasing doses of α-, β-, γ-MSH, and NDP-α-MSH. The data were obtained by measuring tyrosinase activity in the experiments presented in Fig. 5. Tyrosinase activity was measured *in situ* 6 days after treatment with concentrations ranging between 10^{-11}–10^{-6} M of the above hormones, as described in *Materials and Methods*. Each value represents the mean of six determinations (duplicate samples assayed from each of triplicate flasks/group). A, Dose-dependent stimulation of tyrosinase by α-, γ-MSH, and NDP-α-MSH. B, Dose-dependent effects of α- and β-MSH.

increase in melanocyte proliferation and tyrosinase activity (Figs. 5A and 6A). To resolve this discrepancy, we compared the duration of cAMP induction in response to 10^{-7} M α-MSH or γ-MSH in the absence of IBMX. Interestingly, we found that although the elevation of cAMP levels in response to α-MSH continued to increase for at least 24 h of treatment, the stimulation by γ-MSH was evident up to 8 h but was completely abolished by 24 h of treatment (Fig. 7). The cAMP response initiated by binding of α-MSH to the MC1R on human melanocytes is quite different from that induced by α-MSH binding to the murine MC1R on Cloudman melanoma cells. In the latter cells, cAMP levels reached a peak after 30 min of treatment with α-MSH and declined rapidly after 70 min of treatment (32). Our cAMP data, together with the difference in the effects of α-MSH and γ-MSH on proliferation and tyrosinase activity of human melanocytes, suggest that sustained high levels of cAMP might be important for the activation of downstream events that ultimately lead to the mitogenic and melanogenic effects of melanotropins.

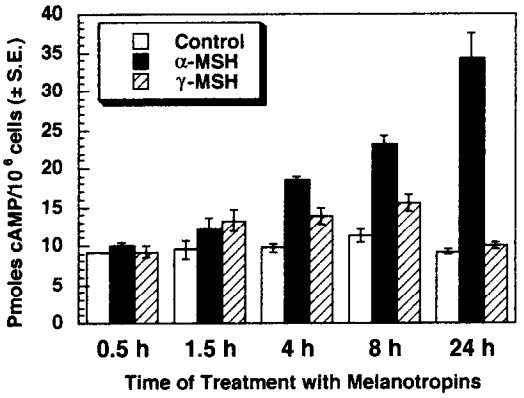

FIG. 7. Time-dependent stimulation of cAMP formation by α-MSH and γ-MSH. Melanocytes were treated with 10^{-7} M α- or γ-MSH for 0.5, 1.5, 4, 8, or 24 h in the absence of IBMX. cAMP levels were quantitated as described in *Materials and Methods*. Similar results were obtained from two experiments using two different melanocyte strains.

Discussion

Until recently, the direct responsiveness of human melanocytes to melanotropins was difficult to demonstrate experimentally despite the reports decades ago that α-MSH, β-MSH, and ACTH cause skin darkening when injected into humans (33, 34). It is now established that human melanocytes express the MC1R (12, 13, 35). Additionally, we have demonstrated that human melanocytes derived from different skin types and age groups (fetal [our unpublished data], neonatal, and adult melanocytes) respond to α-MSH or ACTH with increased proliferation and melanogenesis (21). We have now extended our studies by investigating further the expression of MC1R and comparing the relative potencies of ACTH, α-MSH, β-MSH, and γ-MSH to bind to this receptor, to activate adenylyl cyclase, and to stimulate human melanocyte proliferation and melanogenesis.

The melanocortin receptors represent a subfamily of G protein-coupled receptors with seven transmembrane domains (36). By Northern blot analysis, we detected constitutive expression of MC1R (Fig. 2). Cone *et al.* (36) have reported the presence of a 3-kb and 4-kb mRNA species specific for MC1R in human melanocytes. However, in all our experiments using several human melanocyte strains, we could only detect a single 3-kb mRNA species. We predict that the difference between these results is due to the difference in the melanocyte culture conditions used by us in the present study and by Cone *et al.* We have also observed that brief treatment of melanocytes with α-MSH or ACTH resulted in increased expression (about 2-fold) of the 3-kb MC1R mRNA, an observation which suggests that receptor expression is up-regulated by ligand binding (Fig. 2). This seems to be true for the ACTH receptors as well because ACTH induced an increase in the MC2R mRNA in mouse and human adrenocortical cell lines (37). The latter effect could be mimicked by treatment with dibutyryl cAMP or forskolin, an indicator for a role for cAMP in regulating the expression of the melanocortin receptors. In bovine fasciculata adrenal cells, the expression of the ACTH receptors in response to ligand treatment correlated directly with stimulation of cAMP formation (38). Similarly, an increase in the

transcription of the β_2 adrenergic receptor gene in response to short treatment with epinephrine or dibutyryl cAMP has been demonstrated (39).

We have found that regardless of the skin type from which melanocytes were derived, the potent synthetic analog NDP-α-MSH had the highest binding affinity for the MC1R, followed by α-MSH and ACTH, then β-MSH. Gamma-MSH had the lowest affinity for the MC1R. These results are consistent with the findings of Chhajlani and Wikberg (13) that binding of [^{125}I]NDP-α-MSH to the MC1R expressed by transfected COS cells could be displaced most by cold NDP-α-MSH, followed by α-MSH or ACTH, then β-MSH, and least by γ-MSH. The human MC1R differs markedly from its murine counterpart in its high affinity for ACTH. Although the murine MC1R has a lower binding affinity for ACTH than α-MSH, the human MC1R binds both hormones with equal affinity, as shown by our results (Fig. 3) and those reported by others (12, 13) on cells transfected with the human MC1R gene. Also, the murine and human MC1R seem to differ in their affinity for NDP-α-MSH. This analog is significantly more efficacious than α-MSH in activating the murine MC1R but only slightly more potent than α-MSH in binding and activating the human MC1R (40). This correlates well with previous findings that NDP-α-MSH is at least 100-fold more potent than α-MSH in stimulating tyrosinase activity of the murine Cloudman melanoma cells (41), whereas the difference in the potency of this analog and that of α-MSH in stimulating human melanocytes is not as remarkable (Figs. 3, 4B, 5A, and 6A).

Consistent with the results of receptor binding assays, we found that NDP-α-MSH had a greater effect on cAMP formation than α-MSH and that α-MSH and ACTH had equivalent effects, whereas β-MSH had less effect than either α-MSH or ACTH but a greater effect than γ-MSH. The order of potency of these melanotropins on human melanocytes is similar to that reported by Gantz *et al.* using cells transfected with the human MC1R coding sequence (14). By comparing the binding properties and activation of the MC1R to those of the MC3 and MC4R, we conclude that the MC1R is more similar to the MC4R. The latter receptor is primarily expressed in the brain and is activated equally by ACTH, α-MSH, and β-MSH but significantly less by γ-MSH (15, 42). The MC1R differs markedly from the MC3R, which is activated equally by ACTH, α-, β-, and γ-MSH (14, 16).

The effects of NDP-α-MSH, ACTH, α-MSH, β-MSH on melanocyte proliferation and tyrosinase activity followed the same order of potency as their effects on receptor binding and cAMP formation (*i.e.*, NDP-α-MSH > α-MSH = ACTH > β-MSH > γ-MSH). These results clearly show a direct correlation between the binding to and activation of the MC1R and the ability of the above melanotropins to stimulate human melanocyte proliferation and melanogenesis. From this, we conclude that the biological effects of ACTH, α-MSH, and β-MSH on human melanocytes are specifically mediated by the activation of the MC1R. We have found that γ-MSH, which had the least affinity for the MC1R, could stimulate cAMP formation. This, however, did not result in an increase in melanocyte proliferation or tyrosinase activity. These rather perplexing results led us to compare the duration of stimulation of cAMP formation by α-MSH or γ-MSH. Interestingly, we found that the effect of γ-MSH was short lived in comparison to that of α-MSH. These findings introduce a new concept related to the regulation of melanogenesis by the cAMP induced pathway, namely that not only elevation of cAMP levels but also the prolonged maintenance of these high levels is required for stimulation of tyrosinase activity. Future studies should further clarify the physiological role of melanotropins in the regulation of human pigmentation and their possible participation in certain pigmentary disorders and in the response of human melanocytes to UV light.

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