Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 1789–1793, February 1995
Cell Biology

Mitogenic and melanogenic stimulation of normal human melanocytes by melanotropic peptides

(corticotropin/melanogenesis/cell proliferation)

ZALFA ABDEL-MALEK*†, VIKI B. SWOPE*, ITARU SUZUKI*‡, CAN AKCALI*, M. DANA HARRIGER§¶, STEVEN T. BOYCE§¶, KAZUNORI URABE‖, AND VINCENT J. HEARING‖

*Department of Dermatology, University of Cincinnati College of Medicine, P.O. Box 670592, Cincinnati, OH 45267-0592; ‡POLA Laboratories, Yokohama, Japan; §Shriners Burns Institute, Cincinnati, OH 45220; ¶Department of Surgery, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0558; and ‖Laboratory of Cell Biology, National Institutes of Health, Bethesda, MD 20892

Communicated by Elizabeth S. Russell, The Jackson Laboratory, Bar Harbor, ME, November 4, 1994

ABSTRACT **The significance of melanotropic hormones as physiologic regulators of cutaneous pigmentation in humans is still controversial. Until recently, no direct effect for melanotropins could be demonstrated on human melanocytes. Here we present conclusive evidence that α-melanotropin (α-melanocyte-stimulating hormone, α-MSH) and the related hormone corticotropin (adrenocorticotropic hormone, ACTH) stimulate the proliferation and melanogenesis of human melanocytes maintained in culture in a growth medium lacking any AMP inducer. The minimal effective dose of either hormone is 0.1 nM. In time-course experiments, the increase in cell number and tyrosinase activity became evident after one treatment of the melanocytes with 100 nM α-MSH for 48 hr. The mitogenic effect gradually increased to 50–270% above control, depending on the individual melanocyte strain, with continuous treatment with 100 nM α-MSH for 8 days, whereas the melanogenic effect became maximal (70–450% increase above control) after 4 days of treatment. Western blot analysis of tyrosinase and the tyrosinase-related proteins TRP-1 and TRP-2 revealed that α-MSH increased the expression of those three melanogenic proteins. This was not accompanied by any change in their mRNA levels after brief (1.5–24 hr) or prolonged (6 days) treatment with 100 nM α-MSH, suggesting that the increased expression of these melanogenic proteins was due to posttranscriptional events. These results demonstrate both mitogenic and melanogenic effects of α-MSH and ACTH on human melanocytes. That both hormones are effective at subnanomolar concentrations, combined with the presence of melanotropin receptors on human melanocytes, strongly suggests that these melanotropins play a physiologic role in regulating human cutaneous pigmentation.**

α-Melanotropin (α melanocyte-stimulating hormone, α-MSH) is the physiologic hormone that regulates integumental pigmentation of many vertebrate species. For example, α-MSH induces rapid skin darkening in amphibians and reptiles and stimulates follicular eumelanogenesis in the mouse (1, 2). In addition to the pigmentary effects, other functions for α-MSH and related melanotropins have been described, such as the antagonistic interaction with interleukin 1 (3, 4) and trophic effects on neurons (5, 6).

Injection of high doses of melanotropins into human volunteers resulted in skin darkening (7, 8). Also, the cutaneous hyperpigmentation associated with Addison disease and Cushing disease is thought to be a consequence of hypersecretion of corticotropin (adrenocorticotropic hormone, ACTH) (9). Injection of a superpotent synthetic α-MSH analog into humans increased skin pigmentation without sun exposure (10). These studies suggest a role for melanotropic hormones in stimulating cutaneous pigmentation in humans.

Several groups of investigators have examined the direct effects of melanotropins on cultured human melanocytes. Some found no response to α-MSH, whereas others noted that α-MSH caused a significant increase in intracellular cAMP, without appreciable stimulation of tyrosinase activity and melanin formation (11–13). cAMP inducers, such as cholera toxin, are routinely used in melanocyte growth media. In the presence of these factors human melanocytes fail to respond to melanotropins.

The cloning of the melanocortin receptors, and subsequent receptor binding studies, verified that human melanocytes express one type of melanotropin receptor, MC1 (14–17). It was reported that α-MSH, like other cAMP inducers, enhances the proliferation of nevus cells in a dose-dependent manner (18). Recently it was shown that α-MSH and ACTH are melanogenic but that α-MSH does not affect the proliferation of human melanocytes maintained in a medium devoid of any melanocyte-specific mitogen (19, 20). Others reported that α-MSH is mitogenic but not melanogenic for these cells (17). Further, it has been shown that melanotropins are present in the skin (21).

We now report that α-MSH and ACTH, at physiologic concentrations, are mitogenic as well as melanogenic for cultured normal human melanocytes derived from different skin types. Our results, taken in context of the presence of melanotropins in the skin, strongly suggest a paracrine role of these hormones in regulating human cutaneous pigmentation.

MATERIALS AND METHODS

Melanocyte Culture Conditions. Normal human melanocytes were established in culture from neonatal foreskins or adult skin representing different skin types (22). The complete growth medium consisted of MCDB 153 supplemented with 5% fetal bovine serum, human transferrin (1 μg/ml), insulin (5 μg/ml), α-tocopherol (1 μg/ml), and the melanocyte mitogens recombinant human basic fibroblast growth factor (bFGF, 0.6 ng/ml), phorbol 12-myristate 13-acetate (PMA, 8 nM), and bovine pituitary extract (BPE, 13 ng/ml) as described (23). All the medium components were from sigma except for BPE, which was from Clonetics (San Diego). In all experiments we found it essential to deprive melanocytes of BPE to allow them to respond to α-MSH or ACTH.

Determination of the Mitogenic and Melanogenic Effects of Melanotropins. To compare the dose-dependent effects of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: α-MSH, α-melanocyte-stimulating hormone (α-melanotropin); ACTH, adrenocorticotropic hormone (corticotropin); BPE, bovine pituitary extract; bFGF, basic fibroblast growth factor; PMA, phorbol 12-myristate 13-acetate; IBMX, 3-isobutyl-1-methylxanthine.

†To whom reprint requests should be addressed.

α-MSH and ACTH, melanocytes were deprived of BPE for 48–72 hr prior to and for the entire duration of experiments. Melanocytes were seeded into six-well plates (surface area per well, 9.6 cm^2) at a density of 0.7×10^4 cells per cm^2. Forty-eight hours later, the medium was replaced with fresh medium lacking BPE, and the appropriate concentration of melanotropin (0.01–100 nM) was added to each well (triplicate wells per group). Media with appropriate additives were replaced every other day for a total of 6 days. Twenty-four hours after the last treatment, L [*ring*-3,5-^{3}H]tyrosine (specific activity, 52 μCi/mmol; Amersham; 1 μCi = 37 kBq) was added to the cultures at a concentration of 2.25 μCi/3 ml of medium per well for an additional 24 hr. The medium from each well was then assayed for tyrosine hydroxylase activity by a modification (22) of the charcoal absorption method of Pomerantz (24), and the melanocytes were harvested and counted with a Coulter Counter. The negative control group consisted of melanocytes maintained in the absence of BPE, and the positive control group consisted of melanocytes maintained in complete growth medium.

For analyzing the kinetics of the effects of α-MSH, neonatal melanocytes were plated and treated with 100 nM α-MSH as described above for 2, 4, 6, or 8 days. Twenty-four hours after each treatment with α-MSH, conditioned medium from the appropriate experimental and control groups was assayed for tyrosine hydroxylase activity and the cell number was determined.

Electron Microscopic Analysis. Neonatal melanocytes, treated with or without 100 nM α-MSH for a total of 6 days in monolayer, were fixed in Karnovsky's fixative for 1 hr at room temperature. For histochemical localization of tyrosinase, fixed melanocytes were incubated with 0.1% 3, 4-dihydroxy-L-phenylalanine (L-dopa) in 0.2 M sodium cacodylate buffer (pH 7.3) twice, for a total of 4 hr at 37°C. Cells were then post fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate (pH 7.3), dehydrated, and embedded in an Epon/Araldite mixture for transmission electron microscopy. Thin sections were stained with uranyl acetate and lead citrate prior to examination with a JEOL 100 CXII electron microscope.

Western and Northern Blot Analysis of Tyrosinase, TRP-1 and TRP-2. The levels of three melanogenic proteins, tyrosinase and the tyrosinase-related proteins TRP-1 and TRP-2, were determined by Western blot analysis following treatment with α-MSH, BPE, or cholera toxin and 3-isobutyl-1-methylxanthine (IBMX) for 6 days, by standard methods (22, 25).

The levels of tyrosinase, TRP-1, and TRP-2 mRNAs were determined by Northern blot analysis following brief treatment (1.5, 3, 6, 9, and 24 hr) or prolonged treatment (6 days) of melanocyte cultures with 100 nM α-MSH (26).

RESULTS

Human melanocyte strains established from donors with different skin types respond to α-MSH or ACTH with dose-dependent increases in proliferation and tyrosine hydroxylase activity (Fig. 1). The minimal effective dose for either melanotropin is 0.1 nM, and their effects are evident in neonatal, as well as adult (data not shown), melanocytes derived from Caucasian, African American, or Asian Indian skin. Responsiveness of melanocytes to α-MSH or ACTH can be detected only when BPE is removed from the growth medium. Removal of BPE results in a significant reduction in melanocyte proliferation and melanogenesis. We found variation in the extent of mitogenic and melanogenic stimulation with the above two melanotropins among individual melanocyte strains. Maximal increases in proliferation and tyrosine hydroxylase activity ranged between 50% and 250% and between 70% and 500% above control, respectively. In most experiments tyrosine hydroxylase activity reached a maximum following treatment with 1 nM α-MSH.

The above results were obtained following three treatments of melanocytes with melanotropins for a total of 6 days. Studies on the kinetics of induction of the mitogenic and melanogenic effects of α-MSH showed significant stimulation of proliferation and tyrosine hydroxylase activity following one treatment with 100 nM α-MSH for 48 hr (Fig. 2). The mitogenic effect increased incrementally with continuous treatment with 100 nM α-MSH for up to 8 days, whereas the

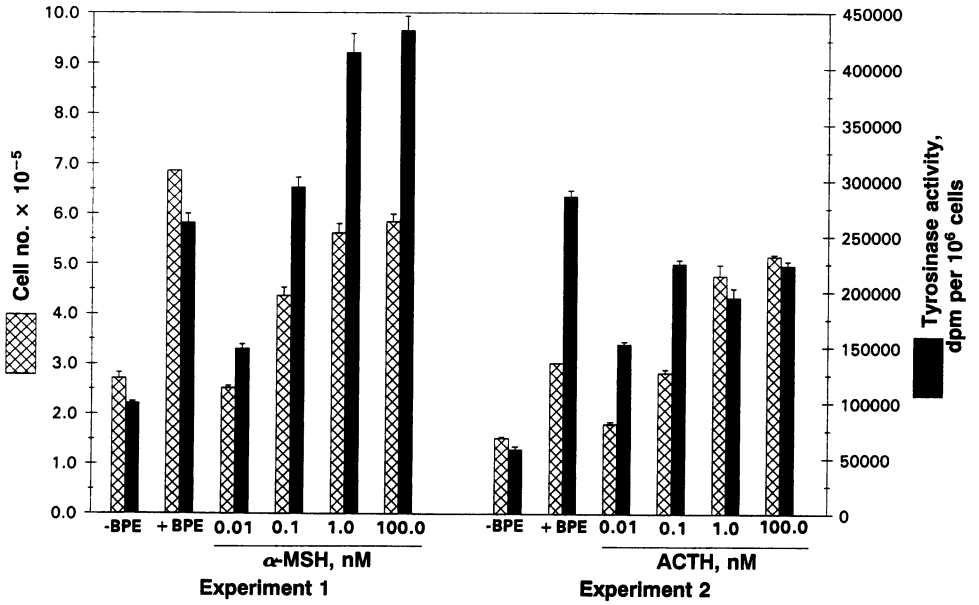

FIG. 1. Dose–response of human melanocytes to α-MSH and ACTH. Melanocytes representative of two cell strains established from two individual neonatal foreskins were deprived of BPE for 48–72 hr prior to the experiments, plated into six well plates and treated with BPE, α-MSH, or ACTH for a total of 6 days. Cell numbers and tyrosine hydroxylase (tyrosinase) activity were then determined. For cell number (cross-hatched bars), each value represents the mean ± SE of triplicate determinations. For tyrosinase activity (solid bars), each value represents the mean ± SE of six determinations. These dose–response experiments were repeated at least four times using neonatal melanocytes and three times using adult Caucasian and African American melanocytes, with similar findings.

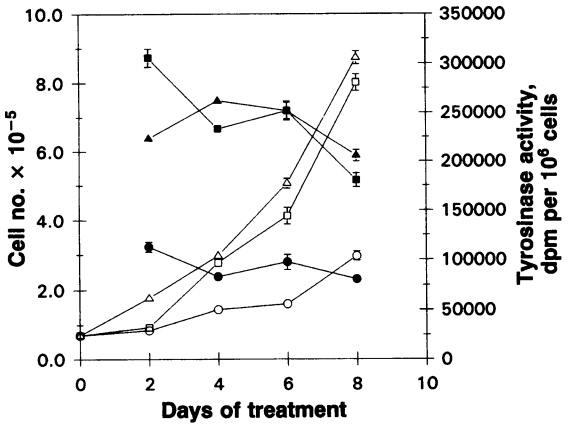

FIG. 2. Kinetics of the responses of neonatal melanocytes to α-MSH. Melanocytes were treated with 100 nM α-MSH or BPE for 2, 4, 6, or 8 days. Open symbols represent cell number and closed symbols represent tyrosinase activity; circles, without BPE; squares, with BPE; triangles, without BPE and with α-MSH. Standard errors < 10% are not shown. This experiment was repeated three times with similar findings, using melanocyte strains from different skin types.

stimulatory effect on tyrosine hydroxylase activity became maximal after 4 days and did not increase further with prolonged treatment with the hormone.

Further investigation of the mitogenic effect of α-MSH, using flow cytometric analysis, revealed that >50% of control melanocytes deprived of BPE were in G_1 phase of the cell cycle and that the addition of α-MSH or BPE for 22 hr increased the percentage of melanocytes in S phase by 2- to 3-fold above control (data not shown).

Treatment with α-MSH or ACTH resulted in marked morphologic alterations of human melanocytes, most evident as increased dendricity (Fig. 3 *A* and *B*). These changes were most pronounced in highly melanotic melanocyte strains derived from individuals with skin types 4, 5, or 6. Electron microscopic studies revealed the presence of more melanized melanosomes and more intense dopa-positive reactivity in the trans-Golgi area, coated vesicles, and melanosomes in α-MSH-treated melanocytes compared with untreated, BPE-deprived melanocytes (Fig. 3 *C* and *D*).

Western blot analysis showed that α-MSH also increased the amounts of tyrosinase and tyrosinase-related proteins TRP-1 and TRP-2 (Fig. 4). These effects are similar to those induced by cholera toxin and IBMX, which concomitantly result in the stimulation of the tyrosine hydroxylase activity, and the expression of tyrosinase, TRP-1, and TRP-2 (27). Northern blot analysis of these same cells showed that α-MSH did not significantly alter the levels of mRNA transcripts for the above three melanogenic proteins following 6 days of treatment (Fig. 5), nor was there any effect on transcription at earlier time points (1.5, 3, 6, 9, or 24 hr) of treatment with 100 nM α-MSH (Fig. 6).

DISCUSSION

The physiologic role of melanotropins in regulating human cutaneous pigmentation has been the subject of considerable debate. While *in vivo* studies clearly demonstrated a hyperpigmentary response in individuals injected with melanotropic hormones (7–10), evidence for a direct effect for melanotropins on normal human melanocytes remained lacking. The recent cloning of the melanocortin receptors, and their expression on normal human melanocytes, has rekindled interest in the biological effects of melanotropins on human melanocytes (14–17).

Previously, several groups reported that human melanocytes did not respond to melanotropins *in vitro* (11–13). We postulate that the failure of melanocytes to respond to melanotropic hormones in those studies was primarily due to the culture conditions under which the cells were maintained. Previously we were also unable to detect any effect of α-MSH, its potent analog [Nle^4,D-Phe^7]α-MSH, or ACTH on human melanocytes cultured in the presence of cholera toxin and IBMX (unpublished data). Others have found that even when the latter two agents were removed from the culture medium, melanocytes remained refractory to melanotropin treatment (11). It is conceivable that treatment with cholera toxin causes permanent intracellular changes, possibly in heterotrimeric guanine nucleotide-binding regulatory proteins (G proteins),

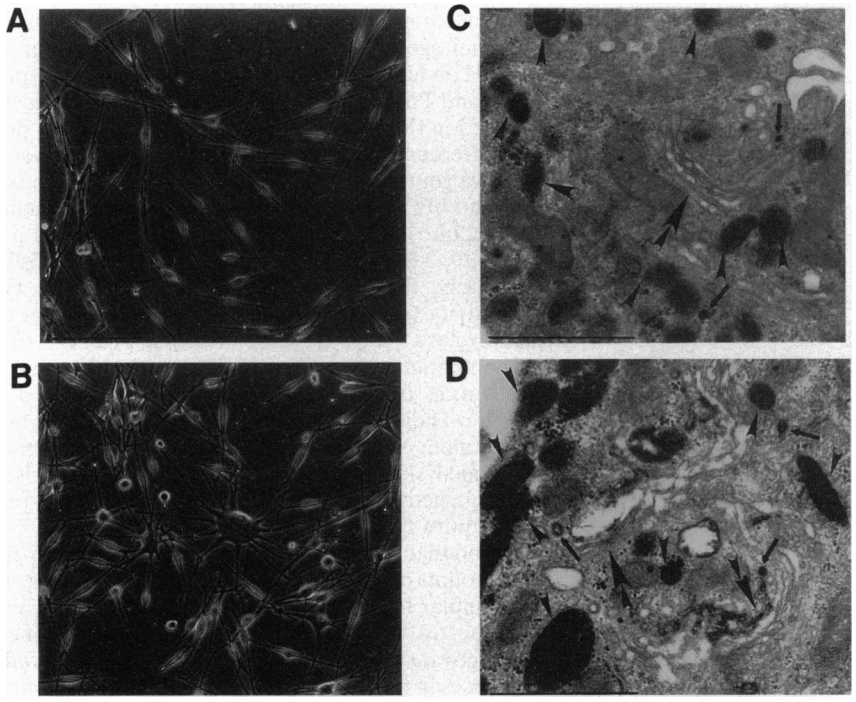

FIG. 3. (*A* and *B*) Light microscopic views of melanocytes deprived of BPE (*A*) or treated with 100 nM α-MSH for 6 days (*B*). (Bar = 24 μm.) (*C* and *D*) Electron microscopic views of dopa-stained melanocytes deprived of BPE (*C*) or treated with 100 nM α-MSH for 6 days (*D*). (Bar = 1 μm.) Arrowheads indicate melanosomes, large arrows indicate Golgi apparatus, and small arrows indicate coated vesicles.

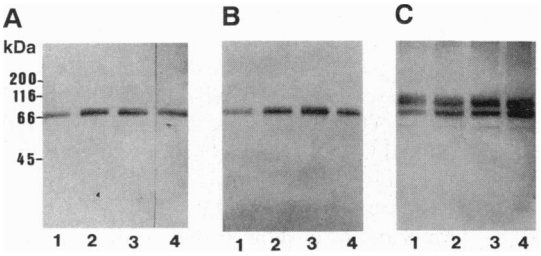

FIG. 4. Western blot analysis of the expression of tyrosinase (*A*), TRP-1 (*B*), and TRP-2 (*C*) in melanocytes treated for 6 days. Lanes 1, without BPE; lanes 2, with BPE; lanes 3, with α-MSH (100 nM); lanes 4, with cholera toxin (20 ng/ml) and IBMX (0.1 mM). The antibodies used were antiserum against hamster tyrosinase; a mouse monoclonal antibody (TA99) raised against human TRP-1; and αPEP-8, an antiserum raised against the carboxyl terminus of synthetic murine TRP-2. This experiment was repeated seven times with similar results.

that render human melanocytes unresponsive to melanotropins, a possibility that requires future investigation.

We have reinvestigated the effects of melanotropins on normal human melanocytes that were established and maintained in culture in a medium containing PMA, bFGF, and BPE (rather than cholera toxin and IBMX) as mitogens. We have found that removal of BPE allows for the responsiveness of melanocytes to melanotropins. By radioimmunoassay we have found that BPE contains, among other constituents, micromolar concentrations of α-MSH, and possibly ACTH, both of which are known to bind G protein-coupled receptors, activate adenylate cyclase, and increase cAMP synthesis (14, 15, 28). Agents that increase intracellular cAMP enhance the proliferation and melanogenesis of cultured human melanocytes (27, 29, 30). This current study has demonstrated that removal of BPE drastically decreases, whereas treatment with α-MSH or ACTH significantly increases, melanocyte proliferation and tyrosinase activity (Figs. 1 and 2). Both melanotropic hormones had similar dose-dependent effects on human melanocytes and a minimal effective dose of 0.1 nM (Fig. 1).

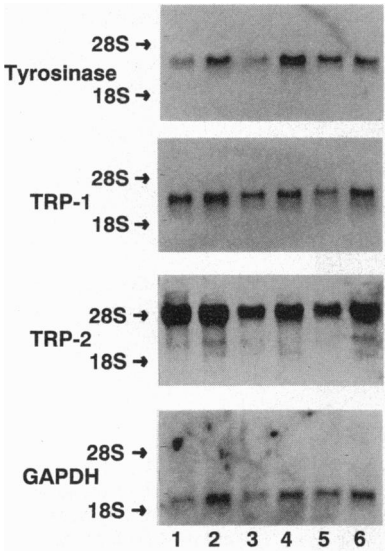

FIG. 5. Northern blot analysis of mRNA transcripts for tyrosinase, TRP-1, TRP-2, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) after treatment of melanocytes for 6 days. Lanes 1 and 4, without BPE; lanes 2 and 5, with BPE; lanes 3 and 6, with 100 nM α-MSH. Lanes 1–3 represent melanocytes from a Caucasian strain and lanes 4–6 represent melanocytes from an African American strain. The same analyses were carried out on two different Caucasian and African American strains with similar results.

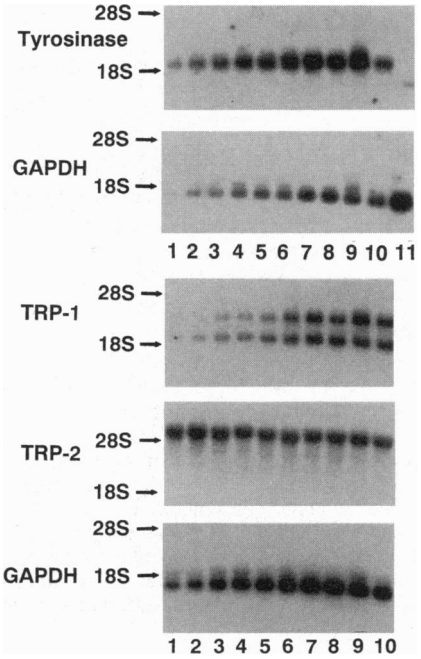

FIG. 6. Northern blot analysis of mRNA transcripts for tyrosinase and the corresponding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and for TRP-1, TRP-2, and their corresponding GAPDH after brief treatment of melanocytes with 100 nM α-MSH. Lanes 1, 3, 5, 7, and 9, without BPE; lanes 2, 4, 6, 8, and 10, with α-MSH for 1.5, 3, 6, 9, and 24 hr, respectively. Lanes 11 in the uppermost two panels represent lysates from human keratinocytes, which served as a negative control.

Surprisingly, Hunt *et al.* (20) reported different dose-dependent responses to α-MSH and ACTH, with biphasic effects for ACTH. Our results seem reasonable, since independent studies on the characterization of the human melanotropin receptor, MC1, have demonstrated that ACTH and α-MSH bind this receptor with similar affinities and have similar dose-dependent effects on adenylate cyclase (15).

Hunt *et al.* (19, 20) have shown that α-MSH and ACTH have melanotropic effects on human melanocytes in culture. However, De Luca *et al.* (17) reported that α-MSH was mitogenic but not melanogenic for these cells. We have consistently observed mitogenic as well as melanogenic effects of α-MSH and ACTH on human melanocytes maintained in the presence of bFGF and PMA and in the absence of BPE (Fig. 1). The discrepancy in the results of different laboratories is probably due to differences in the melanocyte growth media. In the medium we routinely use, the synergistic interaction between α-MSH and bFGF and PMA is expected, since human melanocytes require two classes of mitogens for optimal proliferation (27, 29, 30). One class activates the phosphatidylinositol pathway (e.g., PMA), or a receptor with intrinsic tyrosine kinase activity (e.g., bFGF), and the other activates the adenylate cyclase pathway (e.g., α-MSH, ACTH) (31, 32). α-MSH potentiates the effect of bFGF on the phosphorylation and activation of the extracellular signal-regulated kinase ERK2 (33). The mitogenic effect of α-MSH is associated with the recruitment of melanocytes into S phase, as determined by cell cycle analysis. The molecular mechanisms underlying this effect—e.g., activation of specific kinases and early response genes—require further investigation.

We found that α-MSH stimulates melanogenesis by increasing the amounts of tyrosinase, TRP-1, and TRP-2 (Fig. 4). This effect is similar to that elicited by the concomitant treatment of melanocytes with cholera toxin and IBMX which, like α-MSH, activate the cAMP signal transduction pathway (27). The increases in the amounts of tyrosinase, TRP-1, and TRP-2

are due to posttranscriptional modifications, since no significant changes in the levels of the mRNA transcripts for the three melanogenic proteins were observed following α-MSH treatment (Figs. 5 and 6). Our results are in agreement with those of Naeyaert *et al.* (34), who found no correlation between tyrosinase mRNA levels and melanin content in control and IBMX-stimulated human melanocytes.

The significance of these results is that they describe in detail the direct melanogenic as well as mitogenic responses of human melanocytes to melanotropins and the similarities between the biological effects of α-MSH and ACTH on these cells. Our findings that human melanocytes respond directly to subnanomolar concentrations of these two melanotropins, the presence of MSH receptors on human melanocytes (14–17), and the demonstration that melanotropins are present in the skin (21) suggest a physiologic paracrine role for these hormones in regulating human pigmentation. Irradiation of murine melanoma cells with ultraviolet light activates the melanotropin receptor (35, 36), and mutations in the melanotropin receptor lead to pigmentary changes in several mammalian species (37). That melanotropins might be involved in the sun-induced melanogenic effects on human skin and that mutations in the melanotropin receptor might be responsible for pigmentary abnormalities in humans are exciting possibilities that are yet to be explored.

We wish to thank Drs. H. Yamamoto and T. Takeuchi for kindly providing the TYRS-J probe, Dr. S. Shibahara (Tohaku University) for his generous gift of the TRP-1 pMT4 and the TRP-2 pHDT8 probes, and Dr. William Oetting (University of Minnesota) for donating the pUC19 tyrosinase probe. We are grateful to Dr. Seymour Pomerantz (University of Maryland) for providing the anti-hamster tyrosinase antiserum and to Dr. S. Vijayasaradhi (Rockefeller University) for providing the human TRP-1 cDNA and the TA99 antibody. We acknowledge the assistance of Dr. G. Ghanem, Free University of Brussels, Belgium, in determining the levels of immunoreactive α-MSH in BPE. We thank Ms. Joan Griggs for her excellent secretarial assistance. This work was supported in part by the National Neurofibromatosis Foundation, The Ohio Cancer Research Associates, and Grant 5P30 ES06096-02 from the National Institute of Environmental Health Sciences, National Institutes of Health.

1. Sawyer, T. K., Hruby, V. J., Hadley, M. E. & Engel, M. H. (1983) *Am. Zool.* **23,** 529–540.
2. Geschwind, I. I., Huseby, R. A. & Nishioka, R. (1972) *Recent Prog. Hormone Res.* **28,** 91–129.
3. Lipton, J. M. & Glyn, J. R. (1980) *Peptides* **1,** 15–18.
4. Cannon, J. G., Tatro, J. B., Reichlin, S. & Dinarello, C. A. (1986) *J. Immunol.* **137,** 2232–2236.
5. Verhaagen, J., Edwards, P. M., Jennekens, F. G. I., Schotman, P. & Gispen, W. H. (1986) *Exp. Neurol.* **92,** 451–454.
6. Van Der Neut, R., Hol, E. M., Gispen, W. H. & Bär, P. R. (1992) *Peptides* **13,** 1109–1115.
7. Lerner, A. B. & McGuire, J. S. (1961) *Nature (London)* **189,** 176–179.
8. Lerner, A. B. & McGuire, J. S. (1964) *N. Engl. J. Med.* **270,** 539–546.
9. McGuire, J. S. & Lerner, A. B. (1963) *Ann. N.Y. Acad. Sci.* **100,** 622–630.
10. Levine, N., Sheftel, S. N., Eytan, T., Dorr, R. T., Hadley, M. E., Weinrach, J. C., Ertl, G. A., Toth, K. & Hruby, V. J. (1991) *J. Am. Med. Assoc.* **266,** 2730–2736.
11. Halaban, R., Pomerantz, S. H., Marshall, S., Lambert, D. T. & Lerner, A. B. (1983) *J. Cell Biol.* **97,** 480–488.
12. Ranson, M., Posen, S. & Mason, R. S. (1988) *J. Invest. Dermatol.* **91,** 593–598.
13. Friedmann, P. S., Wren, F., Buffey, J. & MacNeil, S. (1990) *Br. J. Dermatol.* **123,** 145–151.
14. Mountjoy, K. G., Robbins, L. S., Mortrud, M. T. & Cone, R. D. (1992) *Science* **257,** 1248–1251.
15. Chhajlani, V. & Wikberg, J. E. S. (1992) *FEBS Lett.* **309,** 417–420.
16. Donatien, P. D., Hunt, G., Pieron, C., Lunec, J., Taïeb, A. & Thody, A. J. (1992) *Arch. Dermatol. Res.* **284,** 424–426.
17. De Luca, M., Siegrist, W., Bondanza, S., Mathor, M., Cancedda, R. & Eberle, A. N. (1993) *J. Cell Sci.* **105,** 1079–1084.
18. Mancianti, M.-L., Gy. E. & Herlyn, M. (1993) *J. Invest. Dermatol.* **100,** 281S–287S.
19. Hunt, G., Todd, C., Cresswell, J. E. & Thody, A. J. (1994) *J. Cell Sci.* **107,** 205–211.
20. Hunt, G., Todd, C., Kyne, S. & Thody, A. J. (1994) *J. Endocrinol.* **140,** R1–R3.
21. Thody, A. J., Ridley, K., Penny, R. J., Chalmers, R., Fisher, C. & Shuster, S. (1983) *Peptides* **4,** 813–816.
22. Abdel-Malek, Z., Swope, V., Collins, C., Boissy, R., Zhao, H. & Nordlund, J. (1993) *J. Cell Sci.* **106,** 1323–1331.
23. Medrano, E. E. & Nordlund, J. J. (1990) *J. Invest. Dermatol.* **95,** 441–445.
24. Pomerantz, S. (1964) *Biochem. Biophys. Res. Commun.* **16,** 188–194.
25. Hearing, V. J. & Tsukamoto, K. (1991) *FASEB J.* **5,** 2902–2909.
26. Aroca, P., Urabe, K., Kobayashi, T., Tsukamoto, K. & Hearing, V. J. (1993) *J. Biol. Chem.* **268,** 25650–25655.
27. Abdel-Malek, Z., Swope, V. B., Pallas, J., Krug, K. & Nordlund, J. J. (1992) *J. Cell. Physiol.* **150,** 416–425.
28. Wong, G., Pawelek, J., Sansone, M. & Morowitz, J. (1974) *Nature (London)* **248,** 351–354.
29. Eisinger, M. & Marko, O. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2018–2022.
30. Halaban, R., Ghosh, S., Duray, P., Kirkwood, J. M. & Lerner, A. B. (1986) *J. Invest. Dermatol.* **87,** 95–101.
31. Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U. & Nishizuka, Y. (1982) *J. Biol. Chem.* **257,** 7847–7851.
32. Ullrich, A. & Schlessinger, J. (1990) *Cell* **61,** 203–212.
33. Swope, V. B., Medrano, E. E., Smalara, D. & Abdel-Malek, Z. (1994) *Exp. Cell Res.*, in press.
34. Naeyaert, J. M., Eller, M., Gordon, P. R., Park, H.-Y. & Gilchrest, B. A. (1991) *Br. J. Dermatol.* **125,** 297–303.
35. Chakraborty, A. K., Orlow, S. J., Bolognia, J. L. & Pawelek, J. M. (1991) *J. Cell. Physiol.* **147,** 1–6.
36. Bolognia, J., Murray, M. & Pawelek, J. (1989) *J. Invest. Dermatol.* **92,** 651–656.
37. Robbins, L. S., Nadeau, J. H., Johnson, K. R., Kelly, M. A., Roselli-Rehfuss, L., Baack, E., Mountjoy, K. G. & Cone, R. D. (1993) *Cell* **72,** 827–834.