Collagen Metabolism Is a Novel Target of the Neuropeptide α-Melanococyte-stimulating Hormone*

Received for publication, November 17, 2003
Published, JBC Papers in Press, November 28, 2003, DOI 10.1074/jbc.M312549200

Markus Böhm‡‡, Michael Raghunath‡‡, Cord Sunderkötter†, Meinhard Schiller‡, Sonja Ständer†, Thomas Brzoska‡, Thomas Cauvet**, Helgi B. Schioth‡‡§§, Thomas Schwarz‡, and Thomas A. Luger‡

From the †Department of Dermatology and the Ludwig Boltzmann Institute for Cell Biology and Immunobiology of the Skin and the Departments of ‡Experimental Dermatology and §§Medicine, Haematology, and Oncology, University of Münster, 48149 Münster, Germany and the ‡‡Department of Neuroscience, Uppsala University, 751 24 Uppsala, Sweden.

Suppression of collagen synthesis is a major therapeutic goal in the treatment of fibrotic disorders. We show here that α-melanocyte-stimulating hormone (α-MSH), a neuropeptide well known for its pigment-inducing capacity, modulates collagen synthesis and deposition. α-MSH in vitro suppresses the synthesis of collagen types I, III, and V and down-regulates the secretion of procollagen type I C-terminal peptide (PICP) in human dermal fibroblasts treated with the fibrogenic cytokine transforming growth factor-β1 (TGF-β1). α-MSH did not interfere with TGF-β1 signaling, because TGF-β1-induced expression of collagen mRNA was not affected, implying a posttranscriptional mechanism. Human dermal fibroblasts in vitro express a high affinity binding site for MSH, which was identified by reverse transcription PCR and immunofluorescence analysis as the melanocortin-1 receptor (MC-1R). Immunohistochemical studies on normal adult human skin confirmed MC-1R expression in distinct dermal fibroblastic cells. The MC-1R on fibroblasts appears to be functionally relevant because α-MSH increased the amount of intracellular cAMP, and coincubation with a synthetic peptide corresponding to the human Agouti signaling protein abrogated the inhibition of TGF-β1-induced PICP secretion by α-MSH. To assess the in vivo relevance of these findings, a mouse model was used in which dermal fibrosis was induced by repetitive intraepidermal injections with TGF-β1. The inductive activity of TGF-β1 on collagen deposition and the number of dermal cells immunoreactive for vimentin and α-smooth muscle actin was significantly suppressed by injection of α-MSH. Melanocortins such as α-MSH may therefore represent a novel class of modulators with potential usefulness for the treatment of fibrotic disorders.

Fibrotic and sclerotic diseases comprise a large and heterogeneous group of inflammatory, idiopathic, toxic, hereditary, and pharmacologically induced disorders such as hypertrophic scars, keloids, localized scleroderma, systemic sclerosis, scleroderma graft versus host disease of the skin, cirrhosis of the liver, idiopathic and bleomycin-induced lung fibrosis, or cyclopentorpine-induced nephropathy. The therapeutic options are limited, and treatment of these disabling disorders is still a challenge.

A key feature of fibrotic disorders is excessive production of extracellular matrix, mainly type I collagen, followed by a gradual loss of organ function which, in some cases, can be fatal. In recent years it became apparent that transforming growth factor-β1 (TGF-β1), a multifunctional cytokine, is crucially involved in the pathogenesis of fibrotic disorders (1–5). It induces fibrosis by various ways (reviewed in Ref. 6). It enhances the expression of several collagens including types I, III, and V. TGF-β1 decreases the production of matrix-degrading proteases and enhances the synthesis of inhibitors of such proteases. TGF-β1 also increases extracellular cross-linking of collagen by enhancing the expression and the activity of lysyl oxidase (7). These multiple activities explain the potent fibrotic effect of TGF-β1. Therefore, strategies aimed at antagonizing the strong pro-fibrotic effect of TGF-β1 are regarded as providing a promising approach to preventing excessive collagen accumulation in fibrotic disorders (8–10).

α-Melanocorty-stimulating hormone (α-MSH) is a tridecapeptide generated from pro-opiomelanocortin (POMC) by proteolytic cleavage (reviewed in Ref. 11). It was originally isolated from the pituitary gland and characterized as a pigment-inducing factor regulating the coat color of many vertebrate species, but it turned out to regulate many other biological activities with regard to the skin (reviewed in Refs. 11 and 12). The biological activities of α-MSH are mediated by a family of structurally related receptors that are known as the melanocortin receptors (MC-Rs). They belong to the superfamily of G protein-coupled receptors with seven trans-membrane domains, and they activate adenylyl cyclase after ligand binding. Five MC-R subtypes have been cloned that differ in their relative affinities to α-MSH and the other melanocortins (13, 14).

Here we show that, in addition to its multiple biological effects, α-MSH suppresses TGF-β1-induced collagen synthesis by human dermal fibroblasts (HDF) in vitro. This effect is
mediated via the MC-1R. α-MSH also exerts its anti-fibrogenic activity in vivo, because injection of α-MSH into mice reduces TGF-β1–induced fibrosis. Our data establish a role for melanocortins in fibroblast biology and point toward a therapeutic potential of α-MSH and its analogues in the treatment of fibrotic and sclerotic diseases.

### MATERIALS AND METHODS

#### Cells and Culture Conditions—HDF from neonatal foreskin and adult skin as well as normal human melanocytes were purchased from Cell Systems, St. Katharinen, Germany. The human fibrosarcoma cell line HT-1080 was obtained from the American Type Culture Collection (ATCC). Fibroblasts were routinely cultured in RPMI 1640 (PAA, Colbe, Germany), 1% glutamine, 1% penicillin/streptomycin (both from PAA), 10% fetal calf serum (FCS) (Biochrom, Berlin, Germany), and 1% H11032.
were treated with pepsin to destroy non-collagenous proteins (24). Proteins in either fraction were precipitated using methanol/chloroform and processed for SDS-PAGE (5% acrylamide; acryl/bisacryl, 37.5:1). All loaded aliquots were calibrated for cell mass so that all slots contained pepsin-treated collagen derived from the same amount of cells. Slab gels were fixed, and radiolabeled collagens were detected by autoradiography (25). Representative gels were subjected to densitometry using the Biostep Phoretix Grabber (Bistep, Jahnisdorf, Germany).

**Determination of Procollagen I C-terminal Peptide—**The amounts of procollagen I C-terminal peptide used as a marker for procollagen I secretion were determined using a commercially available ELISA (TaKaRa, Shiga, Japan). HDF were seeded into 12-well tissue culture plates at a density of 250,000 cells per well. Confluent HDFs were then deprived of FCS for 2 days and subsequently stimulated with α-MSH (10⁻¹⁰⁻¹⁰⁻¹¹ M), TGF-β₁ (10 ng/ml), or both agents in the presence of 50 μg/ml ascorbate. In some experiments, cells were coincubated with a synthetic peptide corresponding to the amino acids 87–132 of the human Agouti signaling peptide (Phoenix Pharmaceuticals, Belmont, CA) at a 10-fold molar excess. Culture supernatants were harvested after 48 h, centrifuged, and frozen at −70 °C until use. Statistical evaluation from triplicate wells was performed using the Student’s t test.

**Mouse Model for Cutaneous Fibrosis—**For in vivo evaluation of the anti-fibrogenic effect of α-MSH, a mouse model described previously by Shinozaki et al. (26) was used with slight modifications. Accordingly, cutaneous fibrosis was induced by intracutaneous injections of 800 ng of TGF-β₁ into the neck of newborn Balb/c mice on three consecutive days. Treatment groups (four groups of three mice each) consisted of mice injected with TGF-β₁, α-MSH (25 μg), TGF-β₁ plus α-MSH, and the solvent (0.1% BSA in PBS) in which TGF-β₁ had been solubilized. On day 4, mice were sacrificed, and 4-mm punch biopsies were taken from the sites of injection for immunohistochemical analysis.

**Immunohistochemistry—**After fixation in 4% paraformaldehyde and embedding in paraffin, biopsies from mouse skin were processed with the following stains: (i) hematoxylin and eosin; (ii) van Gieson stain, in which collagen appears red; and (iii) resorcin-fuchsin stain, according to Weigert, in which elastic tissue appears black. For collagen staining, the sections were treated with 1 mg/ml pepsin (Sigma) in 0.5M acetic acid, washed, and incubated with a rabbit antibody against collagen type I (1:100; DPC Biermann, Bad Nauheim, Germany) for 1 h. For the staining of vimentin, sections were microwave-treated to unmask epitopes, followed by incubation with a polyclonal antibody from Abcam (Cambridge, UK) for 30 min at 37 °C. For the staining of α-smooth muscle actin, a monoclonal antibody from Dumn Labortechnik (Asbach, Germany) was incubated for 1 h at 2 μg/ml without prior unmasking. Immunohistochemistry for MC-1R in sections of normal adult human skin (n > 5) was performed exactly as outlined previously (17, 21). Sections were developed by the indirect immunoperoxidase technique using 3-amino-9-ethylcarbazole (Sigma) as a chromogen. Negative controls included incubation with control IgG at the same protein concentration as the primary antibody, omission of the first antibody, or pre-incubation with the immunogenic peptide in 10-fold weight excess in the case of MC-1R immunostaining. Vimentin immunostaining and α-smooth muscle actin immunostaining in sections of mouse skin were quantitatively assessed by counting the number of immunoreactive interfollicular dermal cells in three high power fields (∗400). Means ± S.D. from 3–4 independent experiments were analyzed by analysis of variance.

**RESULTS**

**α-MSH Modulates Collagen Expression by HDF in Vitro—**We addressed the question of whether α-MSH can modulate the key function of HDF, namely the expression and secretion of collagen. To this end, cultured, normal HDF from neonatal foreskin were treated with α-MSH, TGF-β₁, or both substances. The amounts of collagen present in cell lysates and culture supernatants were separately determined after metabolic labeling, pepsin digestion, and SDS-PAGE.

TGF-β₁, a well known inducer of collagen synthesis (6), increased the amount of secreted collagens in the culture medium (Fig. 1A). α-MSH alone appeared to reduce the extracellular amount of collagens I, III, and V by 50–70% as determined by densitometry (Fig. 1A, and data not shown). This reduction was not due to intracellular retention, although there was some increase in α₁(I) chains that we attribute to a higher synthesis rate. The intracellular bands showed no delayed migration and, thus, excluded significant posttranslational overmodification due to abnormal intra-endoplasmic retention (Fig. 1A). Most strikingly, α-MSH dramatically reversed the stimulatory effects of TGF-β₁ on the extracellular collagen presence with the strongest effects on collagens I and III and somewhat milder effects on collagen V (Fig. 1A). In the conspicuous absence of intracellular retention, these findings implicate either intracellular or extracellular proteolytic degradation or a combination of both.

To further substantiate the activity of α-MSH on collagen synthesis and/or secretion, we determined the amount of procollagen I C-terminal peptide (PICP) in the culture media of HDF stimulated with α-MSH, TGF-β₁, or both agents. The addition of TGF-β₁ led to a dramatic increase of PICP in the culture medium by >500%. In accordance with the modulatory effect of α-MSH on the TGF-β₁-induced collagen biosynthesis and subsequent secretion, we found significantly reduced secreted amounts of PICP by HDF (677.9 ± 60.1 pg/ml versus 1313.9 ± 136 pg/ml; p < 0.005) (Fig. 1B). α-MSH alone, in contrast, did not affect the basal amounts of secreted PICP. These findings suggested either an intracellular or an extracellular cause for the reduction of secreted procollagen I.

**Modulation of Collagen Synthesis by α-MSH Is Not Mediated by Reduced mRNA Expression—**We next wondered if the mod-
competitive radioligand binding using an iodinated synthetic collagen suggested the presence of specific binding sites in HDF treated with TGF-β1 (Table II). Similar results were obtained when HDFs were stimulated with 10 nM α-MSH (M), 10 ng/ml TGF-β1 (T), or both agents (T + M) for 12 h or left untreated (N/A). Relative mRNA levels were measured by real-time PCR and normalized for GAPDH. Data are means ± S.E. from three independent experiments.

| Gene product | Relative mRNA expressiona |
|--------------|---------------------------|
|              | N/A M T T + M             |
| Col α1(I)    | 22.2 ± 1.1 22.9 ± 2.7 55.6 ± 9b 55.5 ± 2.3 |
| Col α2(I)    | 81.6 ± 9.6 95.2 ± 14.4 149.2 ± 20.3b 135.2 ± 23.9 |
| Col α3(III)  | 28.8 ± 2.8 28.6 ± 3.8 76.2 ± 14.8b 52.3 ± 3.8 |

a Relative mRNA levels were measured by real-time PCR and normalized for GAPDH. Data are means ± S.E. from three independent experiments.

ulotary activity of α-MSH on collagen synthesis is regulated at the transcriptional level. HDF from neonatal foreskin were stimulated with α-MSH, TGF-β1, or both agents for 12 h. The relative mRNA levels for the α1(I) and α2(I) chains of collagen I (alleles COL1A1 and COL1A2, respectively) and for the α3(III) chains for collagen III (allele COL3A1) were subsequently determined by quantitative real-time PCR. TGF-β1 significantly increased the mRNA levels of collagen type I α1 and α2 as well as that of collagen type III α3, as compared with non-treated cells (Table II). The observed rate of increase in the amount of these collagenas by TGF-β1 was in accordance with earlier reports (27). Despite some variation, neither α-MSH alone nor coinubcation of α-MSH and TGF-β1 caused a significant reduction in the relative levels of the collagen mRNAs (Table II). Similar results were obtained when HDFs were treated with TGF-β1 and α-MSH for 24 h (data not shown). These findings show that α-MSH does not interfere with TGF-β1 signaling and that α-MSH may affect collagen expression at the posttranscriptional level.

Detection of High Affinity Binding Sites for MSH on HDF—The identified effects of α-MSH on the amount of extracellular collagen suggested the presence of specific binding sites in HDF. Therefore, we examined HDF from neonatal foreskin for competitive radioligand binding using an iodinated synthetic α-MSH analogue, NDP-MSH. Displacement was performed with an unlabeled ligand at varying concentrations, and COS-1 cells were used as a negative control. HDF exhibited a specific and saturable binding kinetic with 125I-NDP-MSH. The affinity of the radioligand was similar to COS-1 cells transfected with the human MC-1R (Fig. 2). The Kᵢ values were 0.058 ± 0.012 nM for the HDF and 0.086 ± 0.033 nM for COS-1 cells transfected with the human MC-1R, the latter value being similar to previous studies (19). HDF, therefore, exhibited similar affinity but slightly lower expression levels of high affinity MSH binding sites than did COS-1 cells transfected with MC-1R. These data strongly suggested that α-MSH binds to specific surface receptors on the surface of HDF, which appear to mediate its biological action.

Expression of MC-1R in HDF in Vitro and in Situ—To investigate in detail the expression of MC-Rs in HDF, we performed RT-PCR analysis using primers against all known MC-Rs (Table I). MC-1R was the only MC-R expressed in HDF derived from neonatal foreskin (Fig. 3A). Similarly, HDF derived from adult human skin expressed MC-1R at the RNA level (data not shown). The MC-1R amplification product of HDF comigrated exactly with that of normal human melanocytes used as a positive control (Fig. 3A). The identity of the amplification product in HDF (416 bp) was determined by DNA sequencing and found to be identical with the mRNA sequence of MC-1R as deposited in the National Center for Biotechnology Information (Table I, and data not shown). In contrast to MC-1R, no other MC-R was expressed in HDF as shown by RT-PCR (Fig. 3A).

The amplification products of the positive controls were all of the expected size (Fig. 3A and Table I) (15–17).

To examine the expression of the MC-1R at the protein level in HDF in vitro, we next performed immunofluorescence studies. For melanoma cells in culture, it has been reported that binding sites for MSH are confined to certain areas on the cell surface (17, 28), whereas in other cutaneous cell types MC-1R immunoreactivity appeared more randomly distributed (17). Immunofluorescence studies with HDF fixed either in paraformaldehyde (data not shown) or methanol gave similar images. MC-1R antigenicity was visible as a characteristic punctate staining randomly distributed with accentuation in the cell periphery (Fig. 3B). Control experiments with pre-immune serum or neutralization with the immunogenic peptide used for generation of the anti-MC-1R antibody did not produce any staining (Fig. 3B).

To check if expression of MC-1R is maintained in HDF in situ, skin sections of normal adult human skin were processed for immunohistochemistry. MC-1R immunoreactivity was absent in interfollicular dermal fibroblasts at the light microscopic level. However, in distinct fibroblastic cells of the connective tissue sheath of hair follicles, MC-1R immunoreactivity was consistently detectable (Fig. 3C). MC-1R immunostaining in these cells had a punctate pattern and was localized mainly in the cytoplasm (Fig. 3C). Immunostaining with an antibody against vimentin confirmed the nature of these cells as fibroblasts (data not shown). Pre-incubation with the antigenic peptide or pre-immune serum, in contrast, did not produce any staining (Fig. 3C). As reported previously (21), MC-1R immunoreactivity was also detected in distinct epithelia of the skin appendages, for example of the outer root sheath hair follicle keratinocytes (Fig. 3C). Collectively, the data from these studies demonstrate that MC-1R expression is not restricted to HDF in culture but is also detectable in situ in distinct dermal fibroblast populations of normal human skin.

Functional Coupling of MC-1R Expressed in HDF—To investigate the functioning of the identified MC-1R on HDF, we
performed cAMP measurements of cells stimulated with varying doses of α-MSH. It has been shown previously that all members of the MC-R family are G protein-coupled receptors whose interaction with the ligand results in stimulation of adenylyl cyclase. α-MSH increased the amount of intracellular cAMP in a dose-dependent manner as compared with non-treated cells (Fig. 4). This effect was maximal at 10⁻⁸ M (p < 0.05) and similar to stimulation of HDF with 0.1 μM forskolin. Concentrations of α-MSH higher than 10⁻⁸ M did not lead to significant changes in the amount of intracellular cAMP as compared with non-stimulated HDF (Fig. 4).

To clarify if MC-1R mediates the inhibitory action of α-MSH on TGF-β₁-induced collagen synthesis in HDF, we performed blocking experiments with a synthetic peptide corresponding to the amino acids 87–132 of human Agouti signaling peptide (ASIP), a natural and highly potent antagonist of MC-1R but also of MC-4R (29). Cysteine-rich C-terminal ASIP fragments were shown previously to be as potent as full-length ASIP (30). Because HDF express only MC-1R (Fig. 3A), we hypothesized that the synthetic ASIP peptide would block the antagonistic effect of α-MSH on collagen synthesis induced by TGF-β₁. Co-incubation of the synthetic ASIP fragment at 10⁻⁷ M plus α-MSH at 10⁻⁸ M and TGF-β₁ (10 ng/ml) completely abrogated the antagonistic effect of α-MSH on PICP secretion (Fig. 5). In contrast, the synthetic ASIP fragment alone did not affect secretion of PICP in a significant manner (Fig. 5). As outlined above, α-MSH alone or coinucubation of TGF-β₁ plus the synthetic ASIP did not exert any modulatory effect on PICP secretion (data not shown). Taken together, these data strongly support the concept that α-MSH, via acting on MC-1R, modulates fibroblast activity or collagen synthesis, respectively.

**α-MSH has Anti-fibrogenic Activity in Vivo**—We next wished to know if α-MSH can also modulate collagen synthesis and secretion in vivo. Therefore, we employed an animal model in which cutaneous fibrosis is elicited by repetitive intracutaneous injections of high doses of TGF-β₁ (26). We chose newborn mice because they contain significantly less collagen in their skin than adult mice, thus rendering the former suitable for evaluation of fibrogenic and anti-fibrogenic stimuli. Accordingly, newborn mice were injected into the neck for three consecutive days with TGF-β₁ (800 ng), α-MSH (25 μg), α-MSH plus TGF-β₁, or PBS. On day 4, punch biopsies were taken from the injection sites and subjected to biochemical and histological analysis. In contrast with samples from adult murine skin, the
hydroxyproline content in the newborn mice samples was below the detection limit to allow collagen analysis by this approach (data not shown). Therefore, we used semiquantitative histochemical and immunohistochemical analysis to assess the effect of α-MSH on TGF-β1-induced skin fibrosis. When compared with PBS, injections with TGF-β1 induced dermal thickening and fibrosis as well as increased numbers of collagen fibers as shown by hematoxylin and eosin and Van Gieson stains and also by immunohistochemistry using an anti-collagen type I antibody (Fig. 6, A, D, and G versus B, E, and H). Elastic fibers in the skin of untreated mice, in contrast, were sparse and were detected primarily in the dermal vasculature with no increase upon injection with TGF-β1 (data not shown). Injections with α-MSH alone did not produce any changes as compared with mice treated with PBS/BSA (data not shown). On the other hand, coinjection of mice with TGF-β1 plus α-MSH resulted in a significant reduction in the amount of extracellularly deposited collagen as compared with mice injected with TGF-β1 alone (Fig. 6, C, F, and I versus B, E, and H). To further corroborate the anti-fibrogenic activity of α-MSH in vivo, we examined the in situ number of dermal cells immunoreactive for vimentin, an established fibroblast marker, as well as for α-smooth muscle actin (α-SMA), a fibroblast activation and myofibroblast trans-differentiation marker. It has been shown previously that α-SMA is strongly induced in fibroblasts by TGF-β1 in vitro (31). Enhanced in situ expression of α-SMA has also been associated with increased tissue injury and the progression of interstitial fibrosis (32–34). As compared with mice injected with PBS/BSA, TGF-β1 significantly increased the number of dermal cells immunoreactive for both vimentin and SMA. This effect was strongly antagonized by coinjection of TGF-β1 plus α-MSH (Table III; p < 0.001 for vimentin; p < 0.02 for α-SMA). In accordance with the above data, α-MSH alone did not have any modulatory activity on the number of vimentin or α-SMA positive cells in murine skin (data not shown). These findings demonstrate that the modulatory activity of α-MSH on TGF-β1-induced collagen synthesis and deposition is not confined to HDF in vitro but is also operational in vivo.

**DISCUSSION**

We have shown here that the neuropeptide α-MSH antagonizes the action of TGF-β1 on collagen synthesis in HDF in vitro as well as in a mouse model of cutaneous fibrosis in vivo. This represents an analogy to the antagonistic action of this neuropeptide on interleukin-1-mediated responses that appears to be affected by suppression of NF-κB activation (35). It has been shown previously that the TGF-β1-mediated effect on type I procollagen α1 transcription in rat kidney fibroblasts depends on the expression of connective tissue growth factor (CTGF), a downstream target of TGF-β1, that is negatively regulated by cAMP (36). Because α-MSH increases intracellular cAMP, we originally hypothesized that treatment with α-MSH would lead to reduced connective tissue growth factor expression and, consequently, to reduced mRNA expression of collagen types I and III. However, the lack of any effect of α-MSH on the mRNA levels of types I and III collagen precludes an interference of α-MSH with the signal transduction of TGF-β1. The latter conclusion is in accordance with findings showing that α-MSH in HDF neither inhibits TGF-β1-induced phosphorylation or blocks the nuclear translocation of Smad2/3 (data not shown). It is possible that the observed differences between the aforementioned findings and our data are due to cell-specific differences (i.e., HDF versus rat kidney fibroblasts) or differences in the individual experimental setting (e.g., the use of artificial cAMP versus natural cAMP inducers).

The fact that α-MSH does not interfere with TGF-β1-mediated transcription of collagen type I suggests a posttranscriptional mechanism for the suppressive effect on collagen synthesis, e.g., translational repression or enhanced extracellular proteolytic degradation. Regarding the latter, α-MSH may suppress the activity of the C- and N-terminal procollagen proteinases that remove the propeptides from secreted procollagen. This would lead to a reduced formation of collagen fibers. Alternatively, α-MSH might stimulate degradation of secreted procollagen by activating members of the matrix metalloproteinase (MMP) family, for example MMP-1 (37) and/or other members such as MMP-2 and MMP-9. Because C- and N-proteinases are only marginally active in fibroblast cell cultures (38), the observed reduction of collagens in the culture medium may point to extracellular degradation of single procollagen trimers. In addition to the above potential mechanisms, it is possible that α-MSH affects the intracellular free pool of selected amino acids required for collagen synthesis. Recently, it was demonstrated that selected environmental changes such as hypoxia inhibit proline uptake while leaving methionine uptake relatively unaffected (39). With regard to α-MSH, however, nothing is known about the potential influence on the uptake and transport of amino acids. Further studies are thus necessary to elucidate the molecular mechanism by which α-MSH modulates collagen synthesis.

The presence of MC-1R in HDF as shown by radioligand binding, RT-PCR, and immunofluorescence in this paper explains a number of previously reported activities of α-MSH on human fibroblasts. It was reported that α-MSH can block the interleukin-1-induced production of prostaglandin E in a lung fibroblast cell line (40). We have shown that α-MSH in vitro increases the secretion of interleukin-8 and modulates the activation of the transcription factors NF-κB and AP-1 in HDF (41).

The inhibitory action of α-MSH on TGF-β1-induced collagen synthesis and/or secretion by HDF in vitro and its anti-fibrogenic activity in vivo adds another dimension to the broad spectrum of biological activities of this neuropeptide. The skin itself contains the full capacity to produce POMC peptides (42), and HDF in vitro have recently been shown to generate immunoreactive amounts of adrenocorticotropic and α-MSH (43). However, transgenic mice with a signaling-deficient MC-1R are not sclerodermic (44). In light of our findings, it is puzzling that peripheral blood levels of POMC peptides are elevated in pa-
tients with systemic sclerosis (45), and increased in situ expression of POMC has been detected in inflammatory cells of keloids (46). It remains to be determined whether these findings are part of the cutaneous stress response (11) or are related to the involvement of α-MSH in collagen metabolism. Because the amounts of α-MSH used in our studies were higher than the plasma concentration of this neuropeptide in man, the exact role of α-MSH in fibroblasts under physiologic and pathophysiologic conditions requires further investigation.

Our findings on the modulating activity of α-MSH on collagen synthesis finally highlight a novel biological activity that may be exploited in the treatment of fibrotic disorders. α-MSH is a small molecule with a molecular mass of 1.66 kDa. Preliminary data have been shown that nickel-induced contact dermatitis in humans can be suppressed by a topical α-MSH (100 μM) (11). It is known that systemic or intradermal injection of α-MSH or its analogue, NDP-MSH, into humans is well tolerated and has little toxicity (47, 48). The latter α-MSH derivative was found to be 10–1000-fold active than α-MSH, depending on the applied bioassay (49). In the past, a variety of α-MSH analogues with increased potency and prolonged activity have been synthesized (49). These bioactive peptides include minimal fragment analogues of α-MSH containing the core sequences 6–9 and 7–9. The truncated α-MSH peptides are active at micromolar concentrations and are MC-R subtype-specific. The low molecular weight of such peptide fragments may render them suitable for transdermal delivery in vivo.

Acknowledgments—We thank Ilka Wolff, Cordula Focke, Zhuo Li, and Ursula Schulte for expert technical assistance.

REFERENCES

1. Kawakami, T., Iln, H., Xu, W., Smith, R., LeRoy, C., and Trojanowska, M. (1998) J. Invest. Dermatol. 110, 47–51
2. Tuan, T. L., and Nichter, L. S. (1998) Mol. Med. Today 4, 19–24
3. Khalil, N., and Greenberg, A. H. (1991) CIBA Found. Symp. 157, 194–207
4. Shihah, F. S. (1996) Semin. Nephrol. 16, 536–547
5. Cazaja, M. J., Weiner, F. R., Flanders, K. C., Giambone, M. A., Wind, R., Espey, L., and Zern, M. A. (1989) J. Cell Biol. 108, 2477–2482
6. Massague, J. (1990) Annu. Rev. Cell Biol. 6, 597–641
7. Ferre-Filho, E. J., Choi, Y. J., Han, X., Takala, T. E., and Trackman, P. C. (1996) J. Biol. Chem. 271, 30797–30803
8. McCormick, L. L., Zhang, Y., Tootell, E., and Gilliam, A. C. (1999) J. Immunol. 163, 5693–5699
9. Yamamoto, T., Takagawa, S., Katayama, I., and Nishioka, K. (1999) Clin. Immunol. 92, 6–13
10. Hill, C., Flyvbjerg, A., Rasch, R., Bak, M., and Logan, A. (2001) J. Endocrinol. 170, 647–651
11. Slominski, A., Wortsman, J., Lager, T. A., Paus, R., and Solomon, S. (2000) Physical. Rev. 80, 979–1020
12. Bohn, M., and Lager, T. A. (2000) Horm. Res. 54, 287–283
13. Mountjoy, K. G., Robbins, L. S., Mortrud, M. T., and Cone, R. D. (1993) Science
Collagen Metabolism Is a Novel Target of the Neuropeptide α-Melanocyte-stimulating Hormone
Markus Böhm, Michael Raghunath, Cord Sunderkötter, Meinhard Schiller, Sonja Ständer, Thomas Brzoska, Thomas Cauvet, Helgi B. Schöth, Thomas Schwarz and Thomas A. Luger

J. Biol. Chem. 2004, 279:6959-6966. doi: 10.1074/jbc.M312549200 originally published online November 28, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M312549200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 49 references, 12 of which can be accessed free at http://www.jbc.org/content/279/8/6959.full.html#ref-list-1