Melanocortin Receptor Ligands: New Horizons for Skin Biology and Clinical Dermatology

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The melanocortin (MC) system is probably the best characterized neuropeptide network of the skin. Most cutaneous cell types express MC receptors (MC-Rs) and synthesize MCs, such as α-melanocyte-stimulating hormone (α-MSH), that act in autocrine and paracrine fashion. In human skin cells, activation of adenylate cyclase by MCs occurs at 10^{-6}–10^{-9} M doses of the ligand, but effects are induced in some cell types at subnanomolar concentrations. In addition to the pigmentary action of MCs on epidermal melanocytes, the hair follicle is a source and target for MCs. MCs regulate lipogenesis in sebocytes expressing both MC-1R and MC-5R. In adipocytes, lipid metabolism is modulated by agouti signalling protein, a natural MC-1R/MC-4R antagonist. The anti-inflammatory activity of α-MSH includes immunomodulatory effects on several resident skin cells and antifibrogenic effects mediated via MC-1R expressed by dermal fibroblasts. In human mast cells, α-MSH appears to be proinflammatory due to histamine release. α-MSH exhibits cytoprotective activity against UVB-induced apoptosis and DNA damage, a finding that helps explain the increased risk of cutaneous melanoma in individuals with loss of function *MC-1R* mutations. These findings should improve our understanding of skin physiology and pathophysiology and may offer novel strategies with MCs as future therapeutics for skin diseases.

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Introduction

Melanocortins (MCs), originally characterized as regulators of pigmentation and of cortisol production, are structurally related peptides involved in an ever-increasing list of additional processes such as food intake, energy homeostasis, sexual behavior, exocrine gland function, inflammatory responses, and others (Gantz and Fong, 2003). The natural MCs, ACTH and the melanocyte-stimulating hormones (α-, β-, and γ-MSH), derive from proopiomelanocortin (POMC), which is processed by distinct members of the prohormone convertase family (Seidah *et al.*, 1999) (Figure 1).

It is now established that human skin is a potent source for MCs (Thody *et al.*, 1983; Slominski *et al.*, 1993; Slominski *et al.*, 2000b). Most cutaneous cell types express POMC, prohormone convertases, and release MCs *in vitro* (Chakraborty *et al.*, 1996; Peters *et al.*, 2000; Schiller *et al.*, 2001; Böhm *et al.*, 2005a; Kauser *et al.*, 2005). Expression of MCs and of cortisol by the skin is orchestrated by pro-inflammatory stimuli and corticotropin-releasing hormone analogous to regulation of the central MC system (Slominski *et al.*, 2000a, 2001, 2005c, d; Ito *et al.*, 2005). Since virtually all cutaneous cell types examined so far not only synthesize MCs but also express MC receptors (MC-Rs), the cutaneous MC system is a complex endocrine, paracrine, and autocrine network.

In this review, we will summarize the latest findings on the cutaneous MC system. Emphasis in this collection of data is on the human system and on novel functional data providing the reader with deeper insight into cutaneous physiology and indicating future perspectives for therapeutic intervention with MC peptides. Owing to space limitations of this review, it was impossible to list all cited references in the print version of this review. The interested reader can access the additional references in the Supplementary material.

Biochemistry and pharmacology of melanocortin receptors

The five MC-Rs that have been cloned and characterized belong to the G protein-coupled receptors superfamily (Starowicz and Przewlocka, 2003). They are integral membrane proteins with an extracellular N-terminus, seven

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Abbreviations: AGRP, agouti-related peptide; AP, agouti protein; ASP, Agouti signalling protein; GRK, G protein-coupled receptor kinase; LPS, lipopolysaccharide; MC, melanocortin; Mc-r, (murine) melanocortin receptor; MC-R, (human) melanocortin receptor; MSH, melanocyte-stimulating hormone; POMC, proopiomelanocortin; RHC, red hair and fair skin; TGF-β, transforming growth factor-β; TM, transmembrane; TNF-α, tumor necrosis factor-α

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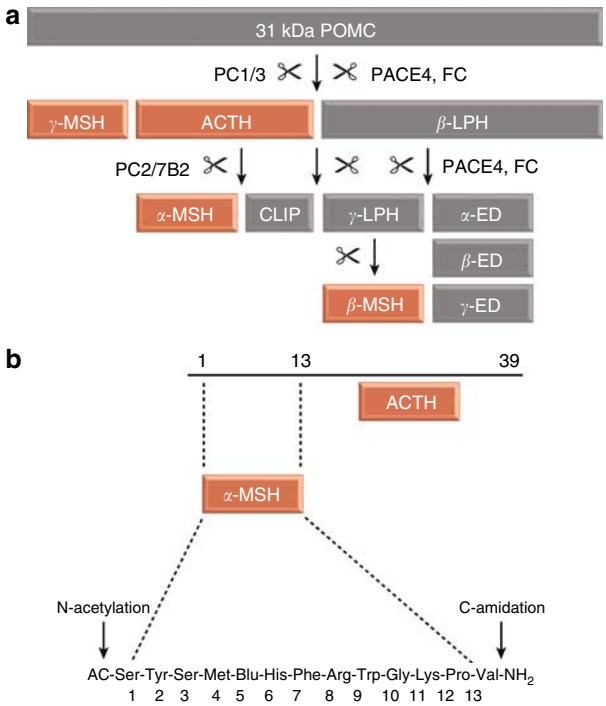

Figure 1. Biosynthesis and biochemical structure of the natural MCs. (**a**) The natural MCs are classically generated from POMC by the proteolytic action of prohormone convertase 1/3 and prohormone convertase 2 together with its cofactor and chaperone 7B2 but furin convertase (FC) and PACE4 can also cleave POMC. This processing yields MCs but also the endogenous opioids (α-, β-, and γ-ED), which do not bind to MC-Rs but to oipioid receptors, and the POMC fragments β- and γ-lipotrophin (LPH) and corticotropin-like intermediate lobe peptide (CLIP). (**b**) Further processing of α-MSH included C-amidation and *N*-acetylation. The pharmacophore of α-MSH resides in the amino acids 4–10. For many immunomodulatory actions of α-MSH, the C-terminal tripeptide of α-MSH, KPV, is also active.

transmembrane fragments, and an intracellular C-terminus. Members of the MC-R subfamily are regulated by both endogenous MC agonists and the natural peptide antagonists Agouti signal protein (ASP) and agouti-related peptide (AGRP). The MC-Rs display overlapping specificities and usually bind several MCs. Only MC-2R shows a strong selectivity for ACTH (Mountjoy *et al.,* 1992). Human MC-1R (Sanchez-Mas *et al.,* 2004) and MC-4R (Gantz *et al.,* 1993a) discriminate poorly between ACTH. α-MSH in murine Mc-1r is more potent than ACTH. α-MSH is the preferred, though not exclusive, MC-5R ligand, while MC-3R is the least selective receptor of the family (Gantz *et al.,* 1993b; Roselli-Rehfuss *et al.,* 1993; Labbe *et al.,* 1994). Attempts have been made to design subtype-specific MC-Rs agonists and antagonists (Holder and Haskell-Luevano, 2004). Recently, non-peptide MC-1R agonists have been reported (Haskell-Luevano *et al.,* 1999; Bondebjerg *et al.,* 2002; Herpin *et al.,* 2003). Concerning the mechanisms of receptor activation, docking models developed for MC-1R and MC-4R suggest that a charged domain, containing conserved acidic residues in transmembrane2 and transmembrane3 (Glu94, Asp117, and Asp121 in MC-1R), interacts with the Arg residue in the His-Phe-Arg-Trp pharmacophore core shared by natural MCs. Site-directed mutagenesis of the MC-1R and MC-4R has also defined the recognition sites of the fully active antagonistic peptides ASP90-132 and AGRP86-132 (Chai *et al.,* 2005). In AGRP (antagonistic to MC-3R and MC-4R) receptor recognition is localized to a 34 residue allowing the design of a fully active mini-AGRP and the development of chimera proteins with potent agonist activity by grafting agonist peptides into the mini-AGRP active loop (Jackson *et al.,* 2005).

MC-Rs undergo post-translational processing and traffic from the endoplasmic reticulum, where they are synthesized, to the plasma membrane. For MC-1R and other MC-Rs, co-translational and post-translational modifications include glycosylation (Sanchez-Mas *et al.,* 2002), acylation (Frandberg *et al.,* 2001; Sanchez-Mas *et al.,* 2005b), and disulfide bond formation (Frandberg *et al.,* 2001; Holst et al., 2002). Recent data suggest that MC-1R (Mandrika *et al.,* 2005) and MC-4R (Biebermann *et al.,* 2003) form dimers or oligomers. MC-1R dimerization has various functional consequences such as dominant-negative effects, or modulation of pharmacological properties (Sánchez-Laorden *et al.,* 2006).

The MC-Rs signal primarily by coupling to the heterotrimeric Gs protein, which activates adenylate cyclase, thus increasing cAMP (Figure 2). However, MC-Rs may trigger additional signalling pathways in some, but not all, cell types. MC-3R activates the inositol triphosphate and Ca^{2+} signalling systems (Konda *et al.,* 1994; Kim *et al.,* 2002; Wachira *et al.,* 2003). Coupling to Ca^{2+} responses was also reported for MC-5R (Hoogduijn *et al.,* 2002), Mc-1r, Mc-3r, Mc-4r, and Mc-5r (Mountjoy *et al.,* 2001), MC-4R (Sabatier *et al.,* 2003), and MC-1R expressed in keratinocytes (Elliott *et al.,* 2004), and melanoma cells (Eves *et al.,* 2003). MC-1R and Mc-1r (Sanchez-Mas *et al.,* 2004) display agonist independent activity. This is consistent with an inverse agonist behavior of ASP and accounts for the direct actions of ASP in the absence of MCs (Graham *et al.,* 1997; Sakai *et al.,* 1997; Siegrist *et al.,* 1997; Suzuki *et al.,* 1997; Ollmann *et al.,* 1998).

MC-R signalling is rapidly attenuated within minutes of agonist exposure. This homologous desensitization has been reported for Mc-4r (Shinyama *et al.,* 2003), Mc-2r (Baig *et al.,* 2001), and Mc-1r and MC-1R in melanoma cells (Sanchez-Mas *et al.,* 2005a). Desensitization of MC-1R is likely mediated by phosphorylation by GRK2 or GRK6, two members of the G protein-coupled receptor kinase family expressed in melanocytes

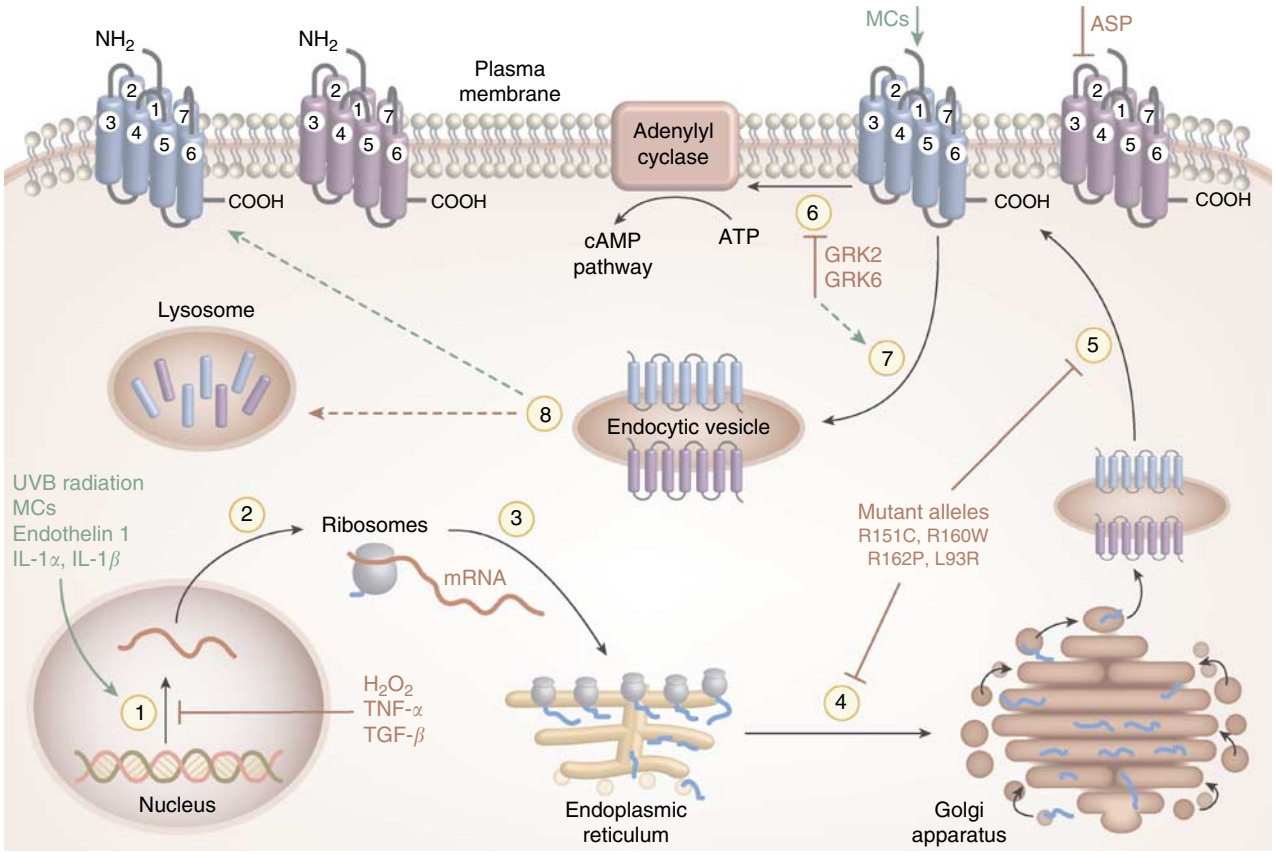

Figure 2. The MC-1R cycle and its regulation. Activatory and inhibitory events are shown in green and red, respectively, with a dashed line whenever they have not yet been fully demonstrated. (1) Transcription of the *MC-1R* gene is stimulated by UV radiation, the melanocortins, IL-1α, and IL-1β, and inhibited by TNF-α, TGF-β, and hydrogen peroxide. (2) The transcript is exported to the cytosol, where translation begins. The structure and properties of the transcript may be influenced by the presence of agonists or antagonists. (3) The nascent protein associates with the endoplasmic reticulum, where translation resumes and post-translational modifications take place. (4) The protein moves to the Golgi apparatus, where post-translational processing is completed. (5) Mature and correctly folded receptor molecules are exported from the Golgi to the plasma membrane. Intracellular traffic of the protein is blocked or impaired by certain mutations, leading to retention of the misfolded units. The exact site of retention remains to be determined, but the most likely possibility is the endoplasmic reticulum. (6) Within the plasma membrane, the functional unit is likely a dimeric form activated by the MCs and inhibited by ASP. On agonist binding, the receptor couples positively to adenylyl cyclase, via the heterotrimeric Gs protein. The active receptor is a substrate of GRK2 and GRK6 that phosphorylate the receptor and block functional coupling. (7) The desensitized receptor and its ligand are internalized in endocytic vesicles. (8) The fate of the internalized receptor remains to be determined, although current evidence suggests lysosomal degradation rather than resensitization and recycling to the plasma membrane.

(Figure 2). Phosphorylation by GRKs is often followed by internalization of receptor-agonist complexes and may trigger new signalling events (Luttrell and Lefkowitz, 2002). Phosphorylation-dependent internalization was demonstrated for Mc-4r (Shinyama *et al.,* 2003), and probably occurs also for MC-1R (Jiménez-Cervantes and García-Borrón, unpublished observations). This is consistent with reports on internal MCs in melanoma cells following their addition to the external medium, and on the appearance of acid-resistant binding (Varga *et al.,* 1976; Adams *et al.,* 1993; Siegrist and Eberle, 1993; Wong and Minchin, 1996; Slominski *et al.,* 2000a). The fate of internalized complexes is uncertain, but rapid translocation to lysosomes without evidence of receptor recycling to the plasma membrane has been reported (Wong and Minchin, 1996). Since MC-1R gene expression is upregulated by the MCs (Slominski *et al.,* 2000a), the density of binding sites in pigment cells continuously exposed to agonists may depend on the balance between two opposing effects that is, receptor sequestration on the one hand, and activation of gene expression on the other. This balance may vary between cell lines accounting for the heterogeneity in the final response (Siegrist *et al.,* 1994).

Melanocortins – regulators of epidermal and follicular melanocytes

There is strong evidence that MCs are important regulators of mammalian skin pigmentation (Abdel-Malek *et al.,* 1999; Slominski *et al.,* 2005b). Indirect evidence for such a role in man originated from the observation that skin pigmentation increases after systemic administration of ACTH, α-MSH, and β-MSH (Lerner and McGuire, 1961). Moreover, supraphysiological blood levels of ACTH or α-MSH lead

to hyperpigmentation in humans (cf Slominski *et al.,* 2000a, 2004b). Patients with POMC null mutations have recently been described that display the red hair and fair skin phenotype (RHC phenotype) (Krude *et al.,* 1998; Krude and Gruters 2000). The unexpected finding of brown/black pigmentation in POMC null mice (Yawsen *et al.,* 1999; Smart *et al.,* 2003; Slominski *et al.,* 2005a) may indicate that eumelanogenesis in these animals is due either to sufficient basal activity of the murine Mc-1r (Sanchez-Mas *et al.,* 2004) or to the existence of non-MC pathway(s) that compensate for MC deficiency, for example, bone morphogenetic protein (Sharov *et al.,* 2005) and opioid signalling or other pathways that modulate intracellular PKA and PKC activity (Kauser *et al.,* 2004, 2005). In higher vertebrates, various *MC-1R* mutants have been unravelled, for example, the chestnut coat color in horses or the blue variant of the arctic fox (Andersson, 2003; Vage *et al.,* 2005) and in man several MC-1R alleles are associated with the RHC phenotype (Valverde *et al.,* 1995; Rees and Healy, 1997; Rees, 2000; Sturm, 2002). The RHC alleles encode proteins with varying degrees of functional impairment, from modest decreases in agonist affinity or coupling efficacy to nearly complete loss of functional coupling (Frandberg *et al.,* 1998; Schiöth *et al.,* 1999; Scott *et al.,* 2002a; Leonard *et al.,* 2003; Ringholm *et al.,* 2004). Aberrant traffic and intracellular retention is a common cause of MC-1R dysfunction associated with the RHC phenotype (Beaumont *et al.,* 2005; Sánchez-Laorden *et al.,* 2006).

UV light is the major environmental stimulus for MCs and MC-1R in the epidermis. UVB light upregulates ACTH/α-MSH and MC-1R in normal epidermal melanocytes (Slominski and Pawelek, 1998; Chakraborty *et al.,* 1999a). MC-1R expression is likewise induced by UV light in normal human keratinocytes (Chakraborty *et al.,* 1999b) and human epidermis *in vivo* (Schiller *et al.,* 2004). This UV light-induced activation of POMC and *MC-1R* promoters may be mediated by p38 stress-activated kinase signalling to upstream stimulating factor-1 (Corre *et al.,* 2004). Therefore, the inductive effects of UV light on MCs and MC-1R expression in epidermal cells *in vitro* and *in situ* suggest that tanning is dependent on activation of the α-MSH/MC-1R system, as first proposed by Pawelek and co-workers (Chakraborty *et al.,* 1999a). Regulation on MC-1R expression by the MCs and other paracrine effectors has been recently reviewed (Slominski *et al.,* 2000a). MC-1R mRNA is upregulated by MCs in normal human melanocytes (Funasaka *et al.,* 1998), and downregulated by ASP (Scott *et al.,* 2002b). A regulatory mechanism based on ligand-dependent modulations of the 5′ untranslated region of *Mc-1r* mRNA has been reported (Rouzaud *et al.,* 2003). Among the cytokines and paracrine factors produced in the skin, endothelin 1 (Scott *et al.,* 2002b), IL-1α/β (Funasaka *et al.,* 1998) upregulate *MC1R* mRNA in normal human melanocytes. Two cytokines that suppress melanogenesis, tumor necrosis factor-α (TNF-α) (Martinez-Esparza *et al.,* 1998) and transforming growth factor-β (TGF-β) (Martinez-Esparza *et al.,* 1997), both downregulate MC-1R expression in normal human melanocytes (Funasaka *et al.,* 1998), and mouse melanoma cells (Martinez-Esparza *et al.,* 1999). Low concentrations of hydrogen peroxide reversibly repress *Mc-1r* expression in mouse melanoma cells (Jimenez-Cervantes *et al.,* 2001). Hydrogen peroxide is a by-product of melanogenesis and reactive oxygen species are formed in UV light-irradiated skin (Nishigori *et al.,* 2004). Finally, early observations suggest that certain melanogenic intermediates may act as chemical signals to upregulate the expression and activity of MC-1Rs (cf Slominski *et al.,* 2004b).

In rodents, α-MSH stimulates follicular melanogenesis by preferentially increasing the synthesis of eumelanin over pheomelanin (Geschwind *et al.,* 1972; Tamate and Takeuchi, 1984; Eberle, 1988; Granholm and Van Amerongen, 1991), in large part via the stimulation of tyrosinase activity at the transcriptional, translational or post-translational levels (Burchill and Thody, 1986; Burchill *et al.,* 1988). In human epidermal melanocytes, α-MSH and ACTH stimulate melanogenesis, dendricity, and proliferation via action at the MC-1R (Hunt *et al.,* 1994a, b, c; Abdel-Malek *et al.,* 1995; Suzuki *et al.,* 1996; Wakamatsu *et al.,* 1997; Tsatmali *et al.,* 1999a, 2002), mediated predominately via the cAMP second messenger system (Busca and Ballotti, 2000).

Recently, involvement of the MC system was detected in the human hair follicle *in situ* and in follicular cell subpopulations *in vitro* (Böhm *et al.,* 2005a; Kauser *et al.,* 2005). These findings are in agreement with the previous detection of POMC mRNA in these hair follicle cell subpopulations (Kauser *et al.,* 2003, 2004). Expression of POMC peptides and MC-1R appears to be highly coordinated during the hair cycle, but perhaps α-MSH and ACTH are not directly involved in the *maintenance* of melanogenesis during the growth or anagen phase of the hair cycle (Slominski *et al.,* 1992; Ermak *et al.,* 1997). This heterogeneous pattern of α-MSH and ACTH expression has also been reported for corticotropin-releasing hormone and the corticotropin-releasing hormone receptor-1 (Ito *et al.,* 2004, 2005; Slominski *et al.,* 2004a) – the chief regulator of pituitary POMC gene expression and the production and secretion of POMC peptides (Slominski *et al.,* 2000a, 2004a). The expression of α-MSH and ACTH may be associated with the ability of some hair bulb melanocytes to survive the apoptosis driven catagen process (Tobin *et al.,* 1999; Commo and Bernard, 2000; Tobin and Paus, 2001). Indeed, in humans, ACTH may stimulate and/or prolong anagen, as overproduction of ACTH or therapeutic administration of ACTH causes acquired hypertrichosis associated with increased pigmentation (Paus, 1998).

Further support for a role of MCs in melanocyte function comes from the observation that both α-MSH and ACTH 1–17 increase melanogenesis, dendricity, and proliferation in follicular melanocytes (Kauser *et al.,* 2005). ACTH 1–17 is more effective at inducing melanogenesis in follicular melanocytes than α-MSH, while α-MSH is most potent at inducing melanocyte

dendricity. This observation concurs with recent findings that ACTH 1–17 is more potent than α-MSH at activating the MC-1R and stimulating epidermal melanogenesis (Tsatmali *et al.,* 1999b). Moreover, the melanogenic and dendritogenic effects of α-MSH and ACTH 1–17 in follicular melanocytes appear to correlate positively with increasing hair color, supporting earlier data showing that α-MSH binding sites maybe linked to hair color (Nanninga *et al.,* 1991).

Melanocortins and lipid synthesis in human sebocytes

A role for α-MSH as a ''sebotrophin'' has been postulated decades ago based on its effect on the rat preputial gland (Thody and Shuster, 1973; Thody and Shuster, 1989), but only recently it was shown that human sebocytes express MC-Rs. Accordingly, MC-5R transcripts were detected in micro-dissected sebaceous glands derived from facial human skin and MC-5R immunoreactivity in human facial sebaceous glands *in situ* (Thiboutot *et al.,* 2000; Hatta *et al.,* 2001). Since sebaceous glands of mice also express Mc-5r and targeted disruption of it results in reduced water repulsion and sebum secretion (Chen *et al.,* 1997), it is possible that MC-5R in man likewise mediates sebogenesis. However, we and others have detected MC-1R in the immortalized human sebocyte cell line SZ95 as well as in primary human sebocytes (Böhm *et al.,* 2002; Zhang *et al.,* 2003). In primary human sebocytes, MC-1R is significantly higher expressed than MC-5R while in SZ95 sebocytes MC-5R expression is undetectable (Böhm *et al.,* 2002). Initial functional studies on human sebocytes revealed that MC peptides at 10^{-8} M increase lipid droplet formation and NDP-α-MSH furthermore stimulates squalene synthesis in these cells (Zhang *et al.,* 2003). Based on these findings, MCs released upon psychoemotional stress may influence the course of acne vulgaris (Zouboulis and Böhm 2004). However, since α-MSH also has anti-inflammatory effects in sebocytes (Böhm *et al.,* 2002), the precise role of MCs and its receptors in human sebaceous gland biology awaits further clarification.

Role of melanocortins in keratinocyte biology

Less is known about the specific influences of MCs on keratinocytes compared to melanocytes. POMC, ACTH, and α-MSH are expressed by normal human epidermal keratinocytes and are upregulated by UVB light *in vitro* (Schauer *et al.,* 1994), suggesting a role of MCs in the tanning response via a paracrine mechanism. The MC-1R is expressed by normal human epidermal and follicular keratinocytes, HaCaT keratinocytes, and malignant human keratinocytes (Luger *et al.,* 2000; Curry *et al.,* 2001; Moustafa *et al.,* 2002; Kauser *et al.,* 2005). Moreover, UVB light and IL-1 are potent upregulators of MC-1R expression in normal and malignant human keratinocytes (Chakraborty *et al.,* 1999b; Luger *et al.,* 2000).

Several lines of evidence suggest that MCs are involved in proliferation and differentiation of keratinocytes. POMC expression is elevated during tissue healing and regeneration (Slominski *et al.,* 1993). In normal interfollicular human epidermis, POMC gene expression and α-MSH immunoreactivity is highest in the differentiated cell layers of the human epidermis (Nagahama *et al.,* 1998; Kono *et al.,* 2001). Expression of POMC peptides and its processing machinery also varies depending on the follicular keratinocyte differentiation status (Kauser *et al.,* 2005). Functional experiments with MSH peptides revealed modulatory effects on keratinocyte proliferation in the epidermis during the telogen phase of the hair cycle, while inhibiting proliferation during anagen (Slominski *et al.,* 1991). In HaCaT keratinocytes α-MSH augmented proliferation (Orel *et al.,* 1997). In these cells, MC peptides at 10^{-7}–10^{-15} M were able to modulate intracellular levels of calcium, a key regulator of keratinocyte differentiation (Elliott *et al.,* 2004). A calcium spike by MC peptides at picomolar doses was not observed in CHO cells stably transfected with the MC-1R (Elliott *et al.,* 2004), suggesting another high-affinity site for MC peptides in HaCaT keratinocytes.

In addition to the above effects, MCs have immunomodulatory actions in human keratinocytes. Similarly to human epidermal melanocytes, melanoma cells (Haycock *et al.,* 1999) and dermal microvascular endothelial cells (Kalden *et al.,* 1999), α-MSH inhibits NF-κB activation induced by pro-inflammatory stimuli in human epidermal keratinocytes, or HaCaT cells (Brzoska *et al.,* 1999; Moustafa *et al.,* 2002). α-MSH also suppressed glutathione peroxidase activity after stimulation with TNF-α or H_2O_2 in HaCaT cells suggesting a protective role for MCs against oxidative stress induced by pro-inflammatory cytokines (Haycock *et al.,* 2000). Recently, α-MSH was shown to reduce prostaglandin E_2 production induced by TNF-α in HaCaT keratinocytes (Nicolaou *et al.,* 2004). Since α-MSH induces IL-10 expression by human keratinocytes (Redondo *et al.,* 1998), UVB light-induced immunosuppression may be mediated in part via MCs released by the epidermis (Schiller *et al.,* 2004).

α-MSH – a modulator of inflammatory responses of the dermal endothelium

Specific and saturable binding sites for α-MSH and MC-1R transcripts were detected in cultured human dermal microvascular endothelial cells (Hartmeyer *et al.,* 1997). Expression of the MC-1R was upregulated in these cells by IL-1β and by α-MSH itself. Interestingly, α-MSH alone increased the expression of IL-8 and GROα *in vitro* indicating that α-MSH under certain conditions can also elicit pro-inflammatory effects (Scholzen *et al.,* 1999). A similar observation was made in human dermal fibroblasts where α-MSH increased IL-8 expression and release (Böhm *et al.,* 1999a; Kiss *et al.,* 1999). On the other hand, α-MSH suppressed IL-1β-induced IL-8 secretion in these cells (Böhm *et al.,* 1999a) consistent with its established anti-inflammatory action. *In vivo* however α-MSH significantly reduced lipopolysaccharide-induced vascular hemorrhage (the so-called Shwartzman reaction) and suppressed expression of vascular cellular adhesion molecule-1 and E-selection *in situ* (Scholzen *et al.,* 2003). *In vitro* expression of these adhesion molecules as well as of intercellular adhesion molecule-1was also sup-

pressed by α-MSH at 10^{-8} and 10^{-12} M in human dermal microvascular endothelial cells stimulated with lipopolysaccharide or TNF-α. These findings highlight an anti-inflammatory activity of α-MSH on the dermal endothelium that may be exploited for future therapy of vasculitis. Furthermore, α-MSH released by the endothelium itself during inflammatory responses (Scholzen *et al.,* 2000) may help to maintain the functional integrity of the endothelial–dermal interface, thereby limiting the extent of endothelial damage during vasculitis.

Fibroblasts – novel target cells for α-MSH in the skin

A growing number of reports indicate that human dermal fibroblasts are target cells of α-MSH. Kiss *et al.* (1995) initially reported a stimulatory effect of α-MSH (10^{-6} and 10^{-8}) in adult human Dermal fibroblasts on matrix metalloproteinase-1. Subsequent studies revealed the presence of MC-1R transcripts, MC-1R immunostaining, and high-affinity binding sites for α-MSH in human dermal fibroblasts derived from newborn foreskin (Böhm *et al.,* 2004). MC-1R transcripts were also detectable in human adult dermal fibroblasts, connective tissue sheath fibroblasts of the hair follicle as well as in dermal papilla cells *in vitro* (Böhm and Luger, 2004; Böhm *et al.,* 2005a). Human dermal papilla cells, a specialized myofibroblast cell type regulating hair follicle activity, expressed both MC-1R and MC-4R (Böhm *et al.,* 2005a). Immunohistochemical studies of nondiseased human skin have disclosed MC-1R immunoreactivity in dermal fibroblasts situated in the periadnexal connective tissue sheaths of the hair follicles (Böhm *et al.,* 1999b, 2004) as well as in follicular dermal papilla cells, which in addition expressed MC-4R (Böhm *et al.,* 2005a).

A role for α-MSH as a regulator of the extracellular matrix of the skin is supported by recent studies demonstrating an effect of α-MSH on collagen synthesis induced by TGF-β_1, a key profibrotic cytokine implicated in the pathogenesis of fibrotic disorders including systemic sclerosis. *In vitro* α-MSH suppressed the TGF-β_1-induced expression of COL(I)α_1,COL(I)α_2 and COL(III)α_2 chains and of procollagen type I-C-terminal peptide (PICP) by human dermal fibroblasts (Böhm *et al.,* 2004). The antagonistic effect of α-MSH on TGF-β_1-induced PICP was abrogated by a synthetic peptide corresponding to the cysteine-rich C-terminal domain of ASP. These data and the stimulatory effect of α-MSH at 10^{-6} to 10^{-9} M on intracellular cAMP in human dermal fibroblasts indicate that α-MSH mediate its effects via the expressed MC-1R. The functional relevance of the above findings was supported using a newborn mouse model in which dermal fibrosis was induced by repetitive injections with high doses of TGF-β_1. α-MSH suppressed interstitial fibrosis induced by TGF-β_1 and reduced the number of vimentin and α-smooth muscle actin positive cells.

The activity of α-MSH on collagen metabolism – in combination with its anti-inflammatory actions – could be of special value for the future therapy of skin disorders where fibroblasts are aberrantly activated. For example, enhanced expression of intercellular adhesion molecule-1 has been reported in fibroblasts from patients with systemic sclerosis (Abraham *et al.,* 1991), but α-MSH can suppress its expression in fibroblastic cells (Böhm *et al.,* 2005a; Hill *et al.,* 2006). The combined anti-inflammatory and anti-fibrogenic activity of α-MSH may also be beneficial in the treatment of inflammatory forms of alopecia, for example, in scarring alopecia but also in alopecia areata in which a breakdown of the immune privilege occurs (Paus *et al.,* 2003).

Melanocortin receptor ligands and human adipocytes

In the last years, enormous progress has been made in dissecting the hypothalamic MC system, its multiple interactions with other neurohormones such as AGRP as well as with adipogenic hormones, and its role as a key regulator of energy homeostasis and food intake (Hoggard *et al.*, 2004; Cheung *et al.,* 2005; Cone, 2005; Della-Fera and Baile, 2005). Both ACTH and α-MSH have been shown to induce lipolysis in mice, rats, hamsters, guinea-pigs, and primates with considerable species variability (Ng, 1990; Bousquet-Melou *et al.,* 1995; Boston, 1999). However, whether MCs directly affect lipid metabolism of human adipocytes is unclear. Several investigators failed to detect a lipolytic effect of MCs in human adipose tissue or in *ex vivo* organ cultures (Bousquet-Melou *et al.,* 1995; Kiwaki and Levine, 2003), while others observed a lipolytic effect of 50 nM ACTH in primary human adipocytes (Xue *et al.,* 1998). In murine adipocytes, the lipolytic activity of ACTH appears to be mediated via *Mc-2r*. *Mc-2r* and to a lesser extent *Mc-5r* RNA expression was detected in murine adipose tissue. In accordance with these data, murine 3T3-L1 fibroblasts express both *Mc-2r* and *Mc-5r* after *in vitro* differentiation into adipocytes (Boston and Cone, 1996). In the human system, conflicting data have been reported regarding the expression of MC-Rs in adipose tissue (Chagnon *et al.,* 1997; Kiwaki and Levine, 2003; Mynatt and Stephens, 2003). Of note, in murine and human skin, MC-2R is detectable (Slominski *et al.,* 1996; Ermak and Slominski, 1997).

Regarding ASP or its murine homolog agouti protein (AP), several studies have demonstrated a regulatory action of this MC-1R and MC-4R antagonist on intracellular calcium in adipocytes (Kim *et al.,* 1997; Shi *et al.,* 2000). AP increased calcium in 3T3-L1 cells as well as in HEK-293 cells transfected with the MC-1R or MC-3R but not in nontransfected cells. AP increased the activity of fatty acid synthase, a key lipogenic gene, in 3T3-L1 cells and in human adipocytes (Claycombe *et al.,* 2000). Long-term treatment of primary human adipocytes with 100 nM AP inhibited basal lipolysis and blocked ACTH-induced lipolysis. However, AP also blocked lipolysis induced by forskolin suggesting that the anti-lipolytic effect of AP not mediated by the cognate MC-R expressed by human adipocytes (Xue *et al.,* 1998). Since ASP is expressed in human adipose tissue (Kwon *et al.,* 1994; Wilson *et al.,* 1995) and most abundantly in mature adipocytes (Xue and Zemel, 2000) ASP may function as an endogenous local modulator of lipid metabolism.

Interestingly, ASP mRNA levels in adipose tissue are elevated in patients with type II diabetes (Smith *et al.,* 2003). Moreover, transgenic mice overexpressing AP in adipose tissue suffer from elevated body weight due to increased fat mass, with accentuation of the obesity when fed a high-fat diet (Mynatt and Stephens, 2001, 2003).

Mast cells – integral players in the cutaneous melanocortin system

More than 20 years ago, it was already reported that very high doses of ACTH and ACTH1-24 (10^{-3}–10^{-6} M) induce a histamine release in rat pleural and peritoneal mast cells (Burt and Stanworth, 1983; Irman-Florjanc and Erjavec, 1984). α-MSH at subnanomolar concentrations likewise was found to act as a secretagogue in human skin mast cells (Grützkau *et al.,* 2000). Whether this effect of α-MSH is MC-R-mediated is unclear since the latter authors did not detect a classical cAMP response of the cells at doses sufficient to activate the MC-1R and MC-5R, both of which are expressed in human skin mast cells and the human leukemic mast cell line HMC-1 (Artuc *et al.,* 1999; Grützkau *et al.,* 2000). In murine bone marrow mast cells, α-MSH at 10^{-8} and 10^{-10} M but not at 10^{-6} M, however, increased intracellular cAMP (Adachi *et al.,* 1999). In contrast to the human system, α-MSH was reported to inhibit histamine release in murine bone marrow mast cells (Adachi *et al.,* 1999) and to exert anti-inflammatory actions such as downmodulation of the mRNA expression of IL-1β, TNF-α and lymphotactin (Adachi *et al.,* 1999), suppression of serum-activated lipopolysaccharide-induced NF-κB activation, and intercellular adhesion molecule-1 expression (Sarkar *et al.,* 2003). In human skin mast cells, α-MSH did not affect the basal mRNA levels of several proinflammatory cytokines (Grützkau *et al.,* 2000). It is possible that the heterogeneous effects of α-MSH on histamine release and cytokine expression in murine and human mast cells are due to species- or tissue-specific differences or reflect the individual experimental conditions.

Whether mast cells can generate MC peptides remains to be shown. Mast cell granules may interact with positively charged groups in the F(ab')2 and/or Fc segments of utilized antibodies via a cation-exchange mechanism to yield false positive immunoreactivity (Ruck *et al.,* 1990). Thus, the initially observed ACTH immunostaining in mast cells of urticaria pigmentosa and other neoplastic mast cell diseases (Horny *et al.,* 1987; Akiyama *et al.,* 1991) awaits further elucidation with current biochemical and molecular tools.

α-MSH – a mediator of cytoprotection

Several lines of evidence suggest that the cutaneous MC system may be part of the innate host defence barrier that ultimately protects the skin and the underlying body against environmental stress, including infectious agents, proinflammatory, and genotoxic stimuli. Indeed, MCs induce melanogenesis to prevent the generation of skin cancer. Furthermore, α-MSH and related peptides were found to exhibit antimicrobial actions (Cutuli *et al.,* 2000). More recently, we reported that α-MSH is capable to protect melanocytes as well as nonmelanogenic cell types of the skin from the most ubiquitous of environmental stressor – UVB (Böhm *et al.,* 2005b). The antiapoptotic effect of α-MSH was linked to reduction in cyclopyrimidine dimers, the major UV-induced DNA photoproduct. The antiapoptotic effects of α-MSH and the reduction in UVB-induced cyclopyrimidine dimers were not observed in melanocytes expressing loss-of function mutation of *MC-1R* (Kadekaro *et al.,* 2005). Moreover, *XPA* fibroblasts with defective nucleotide excision repair were not protected from UVB-induced apoptosis indicating that α-MSH suppresses UVB-mediated apoptosis via enhanced DNA repair (Böhm *et al.,* 2005b). Mechanistically, the protective activity of α-MSH against UVB damage involves the inositol triphosphate kinase-Akt pathway, phosphorylation of the microphthalmia-related transcription factor, and the antiapoptotic protein Bcl_2 (Kadekaro *et al.,* 2005).

These data may explain the increased incidence of cutaneous melanomas in patients with loss of function *MC-1R*, that is, in those individuals with the RHC phenotype. In addition, they support a concept in which α-MSH acts as a regulator of functional integrity of the epidermis and dermis via protecting cells from cell death induced by UVB or cytotoxic cytokines (Hill *et al.,* 2005). In the epidermis, α-MSH may therefore maintain the crucial pigment-producing function of melanocytes, and via reducing UVB-induced DNA damage it may enhance genomic stability of both melanocytes and keratinocytes.

Conclusion and future perspectives

Research in the last few years has provided compelling evidence for a much broader role of MC and their receptors in cutaneous biology than ever thought before (Figure 3). An increasing number of MCR-related genetic diseases and conditions, including the RHC phenotype associated with certain MC1R mutant alleles, are related to the retention within intracellular compartments of misfolded receptor molecules. Studies have demonstrated that cell permeant specific ligands called pharmacological chaperones can bind to the defective protein and partially restore a transport-competent conformation. It is likely that pharmacological compounds belonging to this new class of agents will be developed and will find their place in the treatment of certain skin disorders.

The recent functional studies with MC conducted so far have confirmed in man many *in vitro* and *in vivo* observations in the rodent system, for example, the sebotrophic effect of MCs on the preputial gland of the rat, the central role of MC-1R in coat color regulation of mice, or the effect of AP on lipid metabolism. On the other hand, other novel biological activities of MC peptides emerged by systematically screening the different cutaneous cell types for MC-R expression and testing MCs for biological effects on them. In contrast to the large body of data on the biological actions of MC peptides in human pigment cells, research on MC and MC-Rs in nonmelanocytic cell types of the skin is still in its infancy. However, the promising extra-pigmentary actions of MCs, which are presented in this review, presage a broad

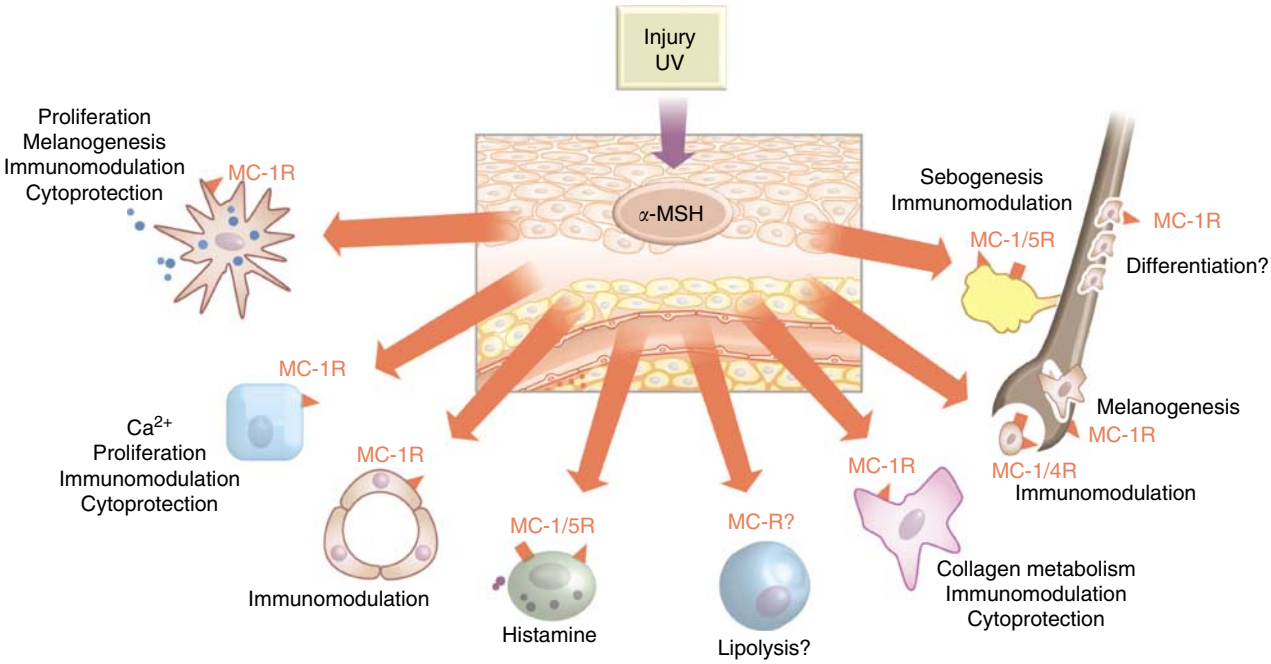

Figure 3. Pleiotropic actions of α-MSH in human skin cells. Depicted are the various skin cell types (*from left to right:* epidermal melanocytes and keratinocytes, endothelial cells, mast cells, adipocytes, fibroblasts, and cells of the pilosebaceous unit, that is, follicular melanocytes and keratinocytes, dermal papilla cells, and sebocytes), which were shown to express MC-Rs and to react with α-MSH. Note that generation of α-MSH is not limited to the epidermis but can be induced in many other cutaneous cell types upon exposure to prototypical stressors. In addition, MC peptides can be delivered to the skin via the classical endocrine pathway.

future potential for MC peptides beyond pigmentation. In addition to the potential use of MC peptides, or even their antagonists, in the treatment of individuals with disturbed sebum secretion, the anti-inflammatory activity of α-MSH combined with its antifibrogenic effects may be particularly interesting in the treatment of patients with inflammatory and fibrotic diseases of the skin including scleroderma, eczema, vasculitis, and alopecia areata to mention only a few. Moreover, the cytoprotective and DNA damage-reducing effects of α-MSH may be of special interest in diseases with increased UV susceptibility, for example, in patients with lupus erythematosus and albinism, respectively. The authors of this review are confident that the cutaneous MC system will continue to teach us further lessons in future, which will attract more researchers to this fascinating field and which will finally help us to treat our patients more efficiently.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

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