Corticotropin Releasing Hormone and Proopiomelanocortin Involvement in the Cutaneous Response to Stress

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Sloinski, Andrzej, Jacobo Wortsman, Thomas Luger, Ralf Paus, and Samuel Solomon. Corticotropin Releasing Hormone and Proopiomelanocortin Involvement in the Cutaneous Response to Stress. Physiol Rev 80: 979–1020, 2000.—The skin is a known target organ for the proopiomelanocortin (POMC)-derived neuropeptides α-melanocyte stimulating hormone (α-MSH), β-endorphin, and ACTH and also a source of these peptides. Skin expression levels of the POMC gene and POMC/corticotropin releasing hormone (CRH) peptides are not static but are determined by such factors as the physiological changes associated with hair cycle (highest in anagen phase), ultraviolet radiation (UVR) exposure, immune cytokine release, or the presence of cutaneous pathology. Among the cytokines, the proinflammatory interleukin-1 produces important upregulation of cutaneous levels of POMC mRNA, POMC peptides, and MSH receptors; UVR also stimulates expression of all the components of the CRH/POMC system including expression of the corresponding receptors. Molecular characterization of the cutaneous POMC
gene shows mRNA forms similar to those found in the pituitary, which are expressed together with shorter variants. The receptors for POMC peptides expressed in the skin are functional and include MC1, MC5 and \(\mu\)-opiate, although most predominant are those of the MC1 class recognizing MSH and ACTH. Receptors for CRH are also present in the skin. Because expression of, for example, the MC1 receptor is stimulated in a similar dose-dependent manner by UVR, cytokines, MSH peptides or melanin precursors, actions of the ligand peptides represent a stochastic (predictable) nonspecific response to environmental/endogenous stresses. The powerful effects of POMC peptides and probably CRH on the skin pigmentation, immune, and adnexal systems are consistent with stress-neutralizing activity addressed at maintaining skin integrity to restrict disruptions of internal homeostasis. Hence, cutaneous expression of the CRH/POMC system is highly organized, encoding mediators and receptors similar to the hypothalamic-pituitary-adrenal (HPA) axis. This CRH/POMC skin system appears to generate a function analogous to the HPA axis, that in the skin is expressed as a highly localized response which neutralizes noxious stimuli and attendant immune reactions.

I. PHYSIOLOGY OF THE SYSTEMIC RESPONSE TO STRESS: ROLE OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

The vertebrate brain is endowed with the functional control of the endocrine system, which proceeds through highly organized structures. This complex neuroendocrine function involves a myriad of pathways and humoral mediators that are characteristically activated in response to external (environmental) or internal changes sensed as stressful. In chronic sustained stresses, the humorally mediated involvement of the hypothalamic-pituitary-adrenal (HPA) axis is most prominent. Activation of this pathway by the stress-sensing central circuits or the central action of proinflammatory cytokines proceeds through the hypothalamic production and release of corticotropin releasing hormone (CRH), which stimulates pituitary CRH receptors (5, 27, 110, 118, 232, 313, 421, 430) (Fig. 1). CRH enhances the production and secretion of the anterior pituitary-derived propiomelanocortin (POMC) peptides melanocyte stimulating hormone (MSH), ACTH, and endorphin (17, 110, 208, 267, 268) (Fig. 1). Upon release into the systemic circulation, ACTH reaches the adrenal gland and activates the MC2 receptors (MC2-R), inducing thereby the production and secretion of corticosterone (rodents) or cortisol (humans). These are powerful anti-inflammatory factors that counteract the effect of stress and buffer tissue damage. Moreover, the same steroids act to terminate the stress response by interacting directly with central nervous system (CNS) or anterior pituitary receptors to attenuate CRH and POMC peptide production (110, 444). The underlying purpose of this adaptive response is the stabilization and restoration of general homeostasis (27, 42, 110, 208, 232, 267, 268, 415).

II. THE SKIN AS A SHOCK ORGAN FOR ENVIRONMENTAL STRESSES

The skin, the largest body organ, is strategically located as a barrier between the external and internal environments, being permanently exposed to noxious stimulators such as bursts of radiation (solar, thermal), mechanical energy, or chemical and biological insults. Because of its functional domains and structural diversity, the skin must have a constitutive mechanism for...
dealing with those stressors while cellular/tissue damage is still localized and of low magnitude, i.e., before triggering the systemic response. Ideally, such a defense mechanism should possess stress-sensing capability of very high sensitivity, because of the relatively low intensity of the continuous pleiotropic physicochemical and biological insults affecting the skin. Thus the skin stress response mechanism may be envisioned as efficiently recording fluctuating environmental information; once a critical threshold is reached, this would trigger an organized biological response. Hence, this cutaneous stress response mechanism must be efficient, self-regulated in intensity and field of activity, and endowed with the capability of differentiating environmental noise from biologically relevant signals (365, 376, 378). Those properties imply continuous recognition and integration of appropriate signals, fast response to activation, and high degree of specificity, all to rapidly reestablish tissue homeostasis (Fig. 2). Desirable responses could involve, for example, the stimulation of local biosynthetic pathways for the manufacturing of buffering molecules to counteract the damaging effect of physical, biological, or chemical insults (Fig. 3) (365, 376, 378, 382).

III. CUTANEOUS RESPONSE TO STRESS: LOCAL NEUROENDOCRINE SIGNALS

Whereas spatially and temporally the responses to stress of skin and CNS may be totally dissociated, their similarity in functional relevance raises the possibility of shared mediators. In this regard, it is known that mammalian skin, a well-characterized target for the POMC-derived ACTH and MSH peptides, does contain POMC (220, 376, 438, 445). It has been further shown that the skin has the intrinsic capability to actually produce POMC as well as CRH peptides and to also express the corresponding receptors. Cutaneous modulation of CRH and POMC production could be mediated by proinflammatory cytokines, as is the case at the systemic level. Thus the skin expresses an equivalent of the HPA axis that may acts as a cutaneous defense system, operating as coordinator and executor of local responses to stress (Figs. 2 and 3) (362, 365, 376, 378).

Against the background above, we review evidence documenting production and regulation of POMC and CRH peptides, with expression of the corresponding receptors in mammalian skin. Cutaneous mechanism regulating CRH and POMC gene expression are compared with their central homologs, and the function of local CRH and POMC derived peptides is discussed within the context of the skin response to stress. Information is provided on the experimental characterization of receptors for CRH and POMC peptides in skin cells, regulation of their expression, and definition of signal transduction pathways. Potential interactions within the epidermal and follicular keratinocyte compartments occurring between the cutaneous equivalent of the hypothalamic-pituitary axis and the skin immune and pigmentary systems are analyzed against experimental data and clinical information already available. This review ends by setting the stage for future research, basic and clinical, on cutaneous mediators in response to physical, chemical, or biological stressors.

IV. CORTICOTROPIN RELEASING HORMONE

A. CRH Expression: Intracranial

CRH, the most proximal element of the HPA axis, has been already sequenced and its gene cloned (348, 413,
Additional intermediates may include the 16-kDa NH2-41-aa-long (4.7 kDa) CRH peptide (166, 267, 268, 294, 295). The CRH gene is composed of two exons separated by an intron (344, 348, 413). The first exon encodes most of the 5′-untranslated region in the mRNA, while the second exon contains the prohormone sequence and the 3′-untranslated region. CRH transcripts in the rodent and human brain are ~1.4 and 1.5 kb long, respectively (348, 413). The 3′-flanking region contains four polyadenylation addition signals (AATAAA). The 5′-flanking region contains DNA sequences responsible for tissue specific expression, second messenger binding, and glucocorticoid regulation sites (166, 267, 268). Translation of exon 2 generates the 196-amino acid (aa)-long pro-prepro-CRH, in which the first 26 aa represent the signal peptide, cleaved in the rough endoplasmic reticulum to generate pro-CRH-(27—196) (49, 166, 204, 295). Unmodified pro-CRH-(27—196) has 18 kDa molecular mass, that increases to 23 kDa after posttranslational modifications (294, 295). Endoproteolytic processing of pro-CRH within the trans-Golgi-network and secretory granules generates the final 41-aa-long (4.7 kDa) CRH peptide (166, 267, 268, 294, 295). Additional intermediates may include the 16-kDa NH2-terminal pro-CRH, pro-CRH-(125—149; 8 kDa), and pro-CRH-(125—151; 3 kDa). The enzymes involved in this process are the convertases PC1 and PC2 (49).

Expression of the CRH gene is controlled by cAMP-dependent protein kinase A (PKA), calcium/calmodulin-dependent protein kinase C (PKC) pathways (4, 98, 166, 267, 268, 430). In addition, transcription factors associated with cytokine signaling can also activate the CRH promoter (395). A list of stimulators of CRH production at the central level includes the neurotransmitters serotonin, acetylcholine, histamine, norepinephrine, and epinephrine (166, 192, 199, 261, 267, 268); the neuropeptides arginine vasopressin (AVP), ANG II, neuropeptide Y, cholecystokinin, activin, and enkephalin; the cytokines interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α, which can also stimulate CRH production (27, 50, 166, 267, 268, 305, 395, 418, 420, 421); and leptin (165). Among the negative regulators of CRH production, the most important are glucocorticoids; estrogens and GABA also share this activity (166, 267, 268, 305, 395, 418). In addition, secretion of CRH is inhibited by dynorphin, substance P, somatostatin, and galanin (166).

CRH is produced predominantly in the paraventricular nucleus (PVN) of the hypothalamus and delivered into portal capillaries converging in the anterior lobe of the pituitary (5, 166, 267, 268, 418). In addition, autonomic neurons of the PVN projecting to the brain stem and spinal cord supply CRH to the sympathoadrenal system and, through neurons projecting to the pituitary, CRH is involved in osmotic regulation not connected with stress (5, 267). In anterior pituitary corticotrophs, CRH regulates POMC gene activity and production/release of ACTH, β-endorphin, and MSH peptides (5, 166, 267, 268). In addition to the hypothalamus, CRH is produced in other areas of the brain (5, 166). CRH production has even been shown in the anterior pituitary, where it may act as a paracrine stimulator of POMC production, as a growth factor for corticotrophs, and as a modulator of expression of the CRH-R1 gene (132, 239, 309, 413, 433). CRH is also involved in functional modulation of the immune system (27, 180, 268a, 331, 392, 394, 418, 430), the reproductive system (100, 267, 297, 301), and the cardiovascular system (19, 112, 267, 316, 320), and it acts as a major catabolic peptide in the hypothalamus, inhibiting food intake, increasing energy expenditure, and producing sustained weight loss (165).

B. CRH Expression: Peripheral

The CRH gene is widely expressed in extracranial tissues but at levels much lower than in hypothalamus. Expression of the gene has been detected in endometrium, placenta, uterus, ovary, testes, spleen, immune system, pancreas, liver, stomach, small and large bowel, adrenal gland, thyroid gland, and skin (13, 27, 46, 87, 94, 100, 180, 184, 195, 253, 267, 268, 268a, 298–303, 305, 318, 321, 335, 344, 355, 359–361, 394, 398, 418, 430, 432, 456). Peripheral processing of pro-CRH into CRH appears to be similar at the peripheral and central levels, as shown in placenta, endometrium, uterus, and immune system (49, 297, 301, 398, 456). In addition to its regulation by similar factors as in the brain (100, 166, 267, 268, 297, 299, 301, 305, 332, 416, 430, 456), CRH production in peripheral tissue is also enhanced by prostaglandins, epidermal growth factor, and platelet growth factor and decreased by nitric oxide (NO) and progesterone (297, 299, 301, 305, 319, 456). Accumulating evidence indicates that CRH produced in uterus and placenta may play an important role in the normal progression of pregnancy (235, 297, 301, 456). CRH is a potent immunomodulator; depending on cellular target, CRH can inhibit or stimulate local immune function (9, 142, 195, 268a, 305, 334, 418, 421, 430). CRH can modify vascular functions (19, 116, 267, 316, 320) and also act as a local growth factor (239, 245, 357, 379, 417, 433). Expression of the CRH-related urocortin gene with production of urocortin peptide has also been documented in peripheral tissues such as placenta, uterus, immune system, stomach, small and large bowel, pancreas, adrenal gland, testis, heart, and skin (18, 262, 263, 298, 380).
C. CRH Expression: Skin

1. Rodent

CRH was first detected in the skin by immunocytochemistry as CRH immunoreactivity (CRH-IR) in the C57BL6 mouse, in the pilosebaceous unit of the hair follicle and epidermis (360). CRH-IR was further localized to keratinocytes of the basal epidermis, the outer root sheath (ORS), and the matrix region of developing hair follicles (321). In hair follicles, the highest intensity of stain was seen during the anagen IV/VI stages and the lowest levels in catagen and telogen skin (321). CRH-IR was also found in the nerve bundles and perifollicular neural network B throughout the entire hair cycle (321). A schematic drawing of hair cycle coupled CRH and CRH-R expression in murine skin is presented in Figure 4.

Actual identification of CRH was accomplished with two separate techniques based on reverse-phase HPLC combined with CRH RIA (321, 357). Tissue levels of CRH measured by RIA showed hair cycle-dependent fluctuation being highest in anagen II/IV skin (67 fmol/g wet wt) and lowest in catagen and telogen skin (36 fmol/g wet wt) (321). Corresponding serum concentrations of CRH-IR were 8.6 fmol/ml during anagen III and 5.6 fmol/ml in telogen (321). Surprisingly, however, the corresponding CRH mRNA was consistently below the limit of RT-PCR detectability in any of the different phases of hair cycle (telogen, anagen, and catagen) (360). Because CRH-IR is found in the nerve bundles and perifollicular neural network B throughout the entire hair cycle, and CRH concentrations are higher in tissue than serum (321), we have proposed that in the mice CRH is imported into the skin, entering through descending (afferent) nerves. Such transport would provide a mechanism to precisely regulate domains of local CRH-dependent POMC production (321, 357, 360).

The lack of expression of the CRH gene in skin was further confirmed by the results of RT-PCR assays repeated with larger number of cycles and followed by Southern blot hybridization to CRH cDNA; again, these tests failed to show CRH mRNA in mouse skin, despite its presence in brain control (104). Thus the abundance of CRH-IR in the skin and its nerve bundles in the absence of cutaneous CRH gene expression imply that at least in the

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**FIG. 4.** Expression of CRH and CRH-R1 antigens throughout hair growth cycle in mice. CRF, CRH; APM, arector pili muscle; DP, dermal papilla; E, epidermis; FBN, follicular neural network B; HS, hair shaft; IRS and ORS, inner and outer root sheaths, respectively; Mel, melanocytes; SG, sebaceous gland. [From Roloff et al. (321), with permission from the FASEB Journal.]
mice, CRH may reach the skin through descending nerves that target well-defined compartments (360). An alternative explanation, that skin cells could express a hitherto undetected CRH variant or a related CRH gene, is undergoing current testing. In this regard, immunoreactivity corresponding to the related peptide urocortin was detected at varying levels in mouse skin; the highest concentrations were observed in telogen and the lowest in late anagen VI skin (380).

2. Human

The CRH gene, although not expressed in mice skin, is nevertheless clearly demonstrable in human skin by RT-PCR amplification of the predicted 413-bp transcript representative of the CRH exon 2 (355, 359, 361). In the latter experiments, the sources of human skin RNA tested were biopsy specimens of scalp, compound melanocytic nevus and basal cell carcinoma, and cultured cell lines of normal and malignant melanocytes and squamous cell carcinoma (355, 359, 361). Melanoma and squamous cell carcinoma cells showed a CRH mRNA transcript 1.5 kb long by Northern blot hybridization (361). CRH peptides were also detected in facial skin, cultured human melanocytes, HaCaT keratinocytes, squamous cell carcinoma, and melanoma cells after RP-HPLC separation followed by monitoring of the eluted fractions with specific anti-CRH RIA (355, 361). Most recently, expression of CRH gene was found in a large panel of cultured melanocytes, nevocytes, and melanoma cells, with the highest intensity in malignant melanocytes (121). Indirect immunofluorescence studies localized the CRH antigen to keratinocytes of the epidermis and hair follicle, dermal blood vessels, skeletal muscle, and nerve bundles of human scalp (357). Expression of the related urocortin gene and production of the corresponding urocortin peptide was also shown in human skin (380).

In normal melanocytes, production of CRH peptide could be stimulated with ultraviolet radiation B (UV-B; wavelength 290–320 nm) (355); and in melanoma and squamous cell carcinoma cells, CRH production responded to forskolin, an agent that raises intracellular cAMP levels (361). Conversely, dexamethasone inhibited cAMP production through CRH-R2 (355, 419), whereas the human CRH-R2 variant has been only detected in brain (194). Human and rat CRH-R1 genes contain 14 and 13 exons, respectively (330, 419), whereas the human CRH-R2α gene contains 12 exons (207). In addition to CRH receptors, CRH binding proteins of 322 aa have been characterized; these are partially associated with plasma membranes or circulate in the blood (74, 185, 218, 293, 307). CRH binding proteins have high affinity for CRH and are postulated to inactivate extracellular CRH, thus preventing its interaction with receptors. This would be the mechanism for neutralization of the large amounts of circulating CRH present during pregnancy (74, 185, 218, 235, 293, 300, 307).

The CRH signal is transduced into cAMP and calcium-activated metabolic pathways via interaction with the CRH-R (5, 74, 100, 267, 268, 388). Activation of adenylate cyclase induces production of cAMP, and subsequently of PKA-dependent pathways. CRH-R activation of phospholipase C induces production of inositol trisphosphate (IP₃), which leads to activation of PKC-dependent pathways. There are data suggesting that CRH signal transduction is directly coupled to calcium channels (100, 198, 388). The CRH signal is transduced more efficiently into stimulation of cAMP production through CRH-R1 than CRH-R2 (74, 442). For example, CRH and urocortin are more potent than sauvagine and urotensin I in activation of adenylate cyclase activity through CRH-R1, although urocortin, sauvagine, and urotensin I are more potent than CRH in the stimulation of cAMP production through CRH-R2 (74, 442). The affinity of CRH for CRH-R1 is similar to that of urocortin and sauvagine, whereas CRH-R2 affinity for CRH is significantly lower than for urocortin, sauvagine, and urotensin I (5, 74, 388).

CRH-R have so far been identified in adrenal glands, testes, ovaries, prostate, kidney, liver, gut, spleen, circulating immune cells, synovium, heart, skeletal muscle,
uterine myometrium, vascular endothelium, arterial smooth muscle, endometrium, placenta, and skin (5, 13, 19, 74, 86, 100, 139, 150, 151, 163, 181, 190, 200, 267, 268, 296, 297, 301, 302, 305, 318, 320, 321, 334, 338, 357, 388, 391, 400, 410, 456). Molecular and pharmacological characterization of CRH receptors have shown that CRH-R2 is predominantly expressed in peripheral tissues, e.g., heart, skeletal muscle, smooth muscle of arterial wall, vascular endothelium, uterus, placenta, and immune cells (74, 139, 151, 163, 190, 200, 297, 301, 318, 334, 388, 391). Notwithstanding, CRH-R1 has been also detected in intrauterine tissue, placenta, and immune cells (94, 139, 181, 297, 301, 318, 396, 409). The signal transduction pathway activated through peripheral CRH receptors is coupled to the stimulation of cAMP production and subsequent activation of PKA (74). However, involvement of calcium-activated pathways by phospholipase C or membrane-bound calcium channels has also been reported (19, 109, 186–188, 198, 242, 357, 388). In addition, the NO/cGMP-dependent pathway appears to mediate CRH-R activated vasodilation in human fetal-placental circulation (86), and a sustained vasodilator effect of CRH and sauvagine on rat mesenteric artery through stimulation of NO production has been reported (19).

CRH-R located in heart and vasculature mediate the inotropic, vasodilatory, and antiedema effects of CRH or CRH-related peptides (19, 74, 112, 268, 316, 320, 440, 441), whereas receptors present on immune cells mediate complex immune/inflammatory responses (27, 305, 418, 430). Although central CRH (neuroendocrine) effects are immunoinhibitory (27, 268, 305, 418), in peripheral tissues CRH effect on immune balance is complex (27, 142, 180, 268a, 305, 331, 333–335, 418, 430, 440, 441). In fact, CRH may have an opposite immunoactivating effect in periphery, by stimulating proinflammatory reactions (180, 305, 418, 430), although an anti-inflammatory effect for exogenously applied CRH has also been described (142). Paez Pereda et al. (268a) showed that CRH can either stimulate or inhibit IL-1 production depending on monocyte activation status; therefore, it is likely that activation-dependent signal transduction through CRH receptors may explain sometimes contradictory phenotypic effects in immune cells. Locally produced CRH may regulate steroid production in gonads and adrenal glands via a paracrine mechanism of action (56, 102, 103, 150, 387). In placenta and uterus, CRH receptors that include CRH-R1 and CRH-R2 are involved in maintenance of pregnancy as well as initiation and maintenance of labor (235, 297, 301, 456). In this setting, CRH receptors are activated through paracrine or autocrine mechanisms by locally produced CRH or urocorcin (297, 301). This action requires precise time- and tissue-restricted differential expression of CRH receptors in intrauterine tissues (86, 87, 94, 297–303, 318, 319, 396, 456). Regulation of CRH activity may be also achieved by the production of CRH binding protein (CRH-BP) that may inactivate or inhibit local CRH signaling in placenta, amnion, chorion, and maternal decidua (293, 297–301). Near term there is a rapid surge in circulating CRH in human pregnancy that coincides with a decrease of plasma CRH-BP levels (72, 235, 293).

B. CRH Receptors: Skin

1. Rodent

Searching for mRNA coding for CRH receptors using RT-PCR amplification of RNA isolated from C57BL6 mouse skin has clearly shown the predicted 407-kb product representative of the CRH-R1 mRNA (360). The transcript was present throughout the entire hair cycle, even during the telogen phase (360). In the hairless mice CRH-R1 mRNA was also detected; RT-PCR assay with Southern blot hybridization to murine CRH-R1 cDNA showed its presence in the epidermis (357). Studies with full thickness skin in the C57BL6 mice showed a 2.7-kb-long CRH-R1 mRNA transcript by Northern hybridization; it was readily detectable in anagen skin and below detectability in telogen skin (360). In the same model, CRH-R1 protein was localized by indirect immunofluorescence to keratinocytes of the ORS, hair matrix, dermal papilla of anagen VI hair follicles, keratinocytes of inner root sheath (IRS), and ORS of early catagen; in contrast, skin immunoreactive CRH-R1 was very low in telogen skin (Fig. 4) (321). These results suggest that both transcription and translation of the CRH-R1 gene are hair cycle dependent. Most recently, RT-PCR assays using primers described in Reference 318 identified a 615-bp fragment common to the α- and β-variants of the mouse CRH-R2 cDNA (318; Fig. 5). Sequencing of the cloned segment showed an almost perfect match with the published mouse CRH-R2 genebank sequence; the single difference was a change of G to A found at position 675 in the known sequence (base 7 in the experimental sequence). This base permutation did not affect the amino acid sequence in the encoded peptide fragment that was exactly the same as the known sequence.

CRH did exhibit specific binding to skin, as detected by autoradiography with a 125I-[Tyr]-ovine CRH (oCRH) tracer (321). CRH binding was localized to keratinocytes of hair follicles and the epidermis and dermal papillar of almost perfect match with the published mouse CRH-R2 genebank sequence; the single difference was a change of G to A found at position 675 in the known sequence (base 7 in the experimental sequence). This base permutation did not affect the amino acid sequence in the encoded peptide fragment that was exactly the same as the known sequence.
and anagen VI skin (321, 357). It is possible that autoradiography may have detected both CRH-R1 and CRH-R2 because of the low specificity of the technique. Because the distribution of CRH-R1 is hair cycle dependent, CRH-R1 may predominate in epidermal and follicular keratinocytes, whereas CRH-R2 gene expression would predominate in extrafollicular compartments such as panniculus carnosus.

A) CRH EFFECT ON HAIR GROWTH AND EPIDERMIS. CRH participation in the regulation of hair growth was evaluated in a well-characterized skin organ culture system (272, 273), in which DNA synthesis in epidermal and dermal compartments reflects predominantly proliferation of keratinocytes, either epidermal or follicular (357). In this model, addition of CRH to telogen and anagen IV skin was found to stimulate DNA synthesis in epidermal keratinocytes, without measurable effect on the dermal compartment (357). However, in anagen II, CRH had the opposite activity on epidermal DNA synthesis, exerting an inhibitory effect, whereas dermal DNA synthesis was enhanced (357). These experiments suggest strong hair cycle restriction in the expression of CRH actions on epidermal and follicular keratinocyte proliferation. Thus experiments with exogenous CRH show variable effects depending on the cellular population targeted and on the hair cycle-dependent expression of CRH-related receptors. Contributing factors in determining the effect of exogenous CRH could include the endogenous production of CRH and CRH-related molecules and the CRH-activated production of ACTH and MSH.

Preliminary in vivo experiments in mice showed that CRH released slowly from subcutaneous implants placed in the direct vicinity of telogen hair follicles effectively resulted in follicular arrest with extended telogen (resting) stage (R. Paus, K. Fechner, and L. Mecklenburg, unpublished data). It is not yet clear whether hair follicle cycle arrest is a direct action of CRH or whether it simply reflects a systemic effect of serum corticosterone. High serum levels of CRH could substantially raise corticosterone levels through stimulation of the HPA axis (444). High systemic levels of glucocorticoid would clearly suffice as the explanation for the profound anagen-inhibitory effect of CRH implants (cf. Refs. 272–274).

B) CRH EFFECTS ON THE VASCULAR AND IMMUNE SYSTEMS. Intradermally injected CRH induces local mast cell degranulation in rats and mice (409). This effect is dose dependent, mediated by the amidated form of CRH, and inhibited by the nonpeptide CRH-R1 antagonist antalarmin. Because CRH-R1 mRNA has been detected in mast cells, those effects may be mediated through CRH-R1 (409). CRH-induced degranulation of mast cells is accompanied by increased vascular permeability (reduced by treatment with H1-receptor antagonists), leading to the suggestion that this cutaneous effect of CRH may be primarily addressed at enhancing local vascular permeability (409). However, others have found that direct topical application of CRH to cutaneous or mucosal tissue had instead vasoconstrictive and anti-inflammatory effects (134, 237, 440). Particularly interesting are the studies of CRH on animal models of tissue injury (134, 237, 333–335, 440, 441). Thus when CRH is injected subcutaneously or intravenously to rats with thermal injury, fluid accumulation is decreased by $>50\%$ in injured skin of treated animals, independent of the functional activity of the HPA axis. This action has been explained by CRH-induced decrease in negative interstitial fluid pressure on traumatized tissues, thereby reducing edema. Moreover, local injection of CRH reduced doxorubicin-induced inflammation in the eyelid of rabbits and decreased the severity of skin injury (237). In the latter study, CRH did not alter vascular permeability but reduced the expected acute influx of monocytes and macrophages and protected the skin overlying the injection site, substantially reducing the extent of injury. Other studies have shown that in the rat urocortin has significantly higher potency than CRH, inhibiting heat-induced cutaneous edema (422). Because $\alpha$-helical CRH-(9–49) reversed the inhibition of edema produced by either urocortin or CRH, at doses that did not affect ACTH secretion, it has been suggested that those effects are mediated through CRH-R2 (422). Additional CRH effects on inflammation
include antinociceptive actions and acceleration of wound healing (333–335, 440, 441).

There is indirect evidence for CRH activity on the local regulation of blood flow through effects on the cutaneous vasculature. These would be mediated by interaction with CRH receptors present on smooth muscle of the arterial wall. For example, vasorelaxing effects have been shown in rat mesenteric arteries that imply mediation via CRH-R2 on vascular smooth muscle (320). Other studies have demonstrated the presence of CRH binding sites of high and low affinity on endothelial cells: $K_d$ of $2 \pm 0.2 \times 10^{-10}$ and $1.77 \pm 0.14 \times 10^{-6}$ M and $B_{\text{max}}$ of $0.79 \pm 0.095$ and $0.97 \pm 0.12$ fmol/mg protein, respectively, for high- and low-affinity binding sites (116). The expression of those receptors was accompanied by an inhibitory effect of CRH on IL-1α-induced prostacyclin and prostaglandin synthesis through inhibition of phospholipase A2 and cyclooxygenase (114–116). Most recently, a dual vasodilatory effect of CRH and sauvagine on mesenteric artery was described: a short direct effect on smooth muscle, followed by sustained endothelium-dependent vasodilation (19). The last effect was associated with stimulation of NO production. Therefore, by analogy with the mesenteric bed (19, 114, 116, 320) and vasculature in the placenta and brain (86, 316, 338), it is possible that CRH would also act through a similar pathway on cutaneous vascular cells, endothelial or smooth muscle, with possible effects on local inflammation, hemostasis, and/or coagulation.

C) CRH EFFECTS ON MALIGNANT MELANOCYTES. The hamster melanoma cell line provided further insight into the mechanism of CRH action in the skin (109, 357). This cell system expresses the CRH-R1 gene and CRH binding sites. The melanoma CRH-R1 mRNA transcript is $\sim 2.5$ kb long, being $0.2$ kb shorter than that detected in normal skin (357). CRH had demonstrable biological effects that consisted of a rapid and dose-dependent increase in intracellular calcium concentration. This effect was reduced by preincubation with the CRH antagonist $\alpha$-helical CRH-(9–41) and actually inhibited by depletion of extracellular calcium with $3 \text{mM EGTA}$. Therefore, CRH signal transduction appears to be coupled, at least partly, to activation of calcium channels (109). The CRH-related peptides sauvagine and urocortin also induced increases in intracellular calcium concentration but at concentrations $\sim 1,000$-fold higher.

2. Human

In human skin biopsy specimens, CRH-R1 mRNA was identified by RT-PCR followed by Southern blot hybridization and found in normal scalp, compound nevus, basal cell carcinoma, and perilesional facial skin (357, 359, 361). The CRH-R1 gene was also expressed in normal and malignant melanocytes (121, 357, 359, 361) and keratinocytes (359, 361); the expression was upregulated by UVB irradiation or 12-O-tetradecanoylphorbol 13-acetate (TPA) treatment (359). CRH-R1 gene expression was high in melanoma and squamous cell carcinoma cells (121, 361).

A) BIOCHEMICAL CHARACTERIZATION OF CRH RECEPTORS. Human melanoma cells and HaCaT keratinocytes exhibit specific binding sites for CRH; application of CRH or of the related sauvagine or urocortin peptides induces rapid and significant increases in intracellular calcium (109, 357). The effect appears to be specific for these peptides, since the neuropeptides $\beta$-endorphin, $\alpha$-MSH, and ACTH, which also interact with G protein-linked receptors, had either no or minimal effects (357). The calcium stimulatory activity in human melanoma cells is higher for CRH than urocortin or sauvagine peptides, being detected already at concentrations as low as $10^{-12}$ and $10^{-10}$ M in a dose-dependent manner (109). Similar to hamster melanoma, the CRH induced intracellular calcium accumulation in these human cell lines was inhibited by the CRH antagonist $\alpha$-helical CRH-(9–41) and by extracellular calcium depletion with EGTA (109). Because human melanoma cells express CRH-R1 mRNA, it was postulated that the above effects are mediated through CRH-R1 (109). Studies on the A 431 human squamous cell carcinoma cells showed that the structurally related peptides CRH, sauvagine, urotensin I, and mystixin-7 and mystixin-11 stimulated cytosolic calcium accumulation and IP$_3$ production (186–188). These pharmacological studies suggest therefore that the increases in cytosolic calcium are due to both calcium influx from the extracellular compartment, through calcium channels coupled to CRH receptors and pertussis toxin-sensitive G proteins, and to mobilization of intracellular calcium through intracellular calcium-independent increase in IP$_3$ (186–188). CRH and CRH-related peptides can also stimulate cAMP production and tyrosine phosphorylation in A 431 carcinoma cells, albeit with a different pattern for each group of peptides tested (186–188), suggestive of differential CRH receptor subtype activation (187). Most recently, we have provided evidence for the existence of CRH receptors in human keratinocytes and defined CAMP-mediated CRH-stimulated pathway in these cells, with demonstrable inhibitory activity on cell proliferation (379).

CRH receptors were also identified in human dermal fibroblasts, where two binding sites with different affinities were found (115). The high-affinity binding site had $K_d$ of $20 \pm 0.22$ pM and $B_{\text{max}}$ of $1.95 \pm 0.22$ fmol/mg protein, and the low-affinity class of receptor had $K_d$ of $160 \pm 17$ nM and $B_{\text{max}}$ of $2.38 \pm 0.27$ fmol/mg protein (115). CRH receptor expression was accompanied by the phenotypic effect of CRH blockade of the IL-1α stimulated prostacyclin (PGI$_2$) and PGE$_2$ production (114, 115). Because CRH inhibits IL-1α-stimulated PGI$_2$ and PGE$_2$ synthesis in bovine aortic endothelial cells through probable...
inhibition of phospholipase A2 and cyclooxygenase (116), it is highly possible that a similar regulatory mechanism may be operative in dermal endothelial cells of human skin.

B) CRH EFFECTS ON CELL PROLIFERATION. CRH has heterogeneous effects on cell proliferation. Thus, in HaCaT keratinocytes, cell growth is inhibited after addition of \(10^{-11} - 10^{-8} \text{ M} \) CRH to the culture medium (379). In contrast, in melanoma cultures, the effect is biphasic in that short-term incubation (6 h) with CRH (\(10^{-10} - 10^{-8} \text{ M}\)) inhibits DNA synthesis in either serum-containing or serum-free media, whereas long-term incubation (3–4 days) at high CRH concentration (\(10^{-5} \text{ M}\)) stimulates the growth of melanoma cells (357, A. Slominski and B. Zbytek, unpublished data). Both stimulation and inhibition of cell proliferation by CRH have been described in AT-20 pituitary cells (239, 433), whereas cell growth is inhibited in CRH-treated mammary cancer cells (417). CRH at concentrations of \(10^{-10} - 10^{-6} \text{ M}\) had no effect on melanin synthesis in cultured melanoma cells (Slominski and Zbytek, unpublished data).

VI. PROOPIOMELANOCORTIN

B. POMC Expression: Intracranial

The pituitary gland has been recognized as an important source of melanotropic factors (6, 14), defined as \(\alpha\)- and \(\beta\)-MSH and \(\beta\)-endorphin (cf. Refs. 110, 141, 143, 201, 444). The structures of the POMC gene and of the POMC protein have been well established (cf. Refs. 75, 110, 141, 143, 444). The POMC gene is expressed predominantly in the anterior pituitary where it comprises \(\sim 30\%\) of all mRNA (282). POMC mRNA concentration is one to two orders of magnitude higher in pituitary than in brain (17).

In general, mammals transcribe only one POMC gene per haploid genome (99), although the mouse genome contains two nonallelic POMC \(\alpha\)- and \(\beta\)-genes (425). The mouse POMC \(\alpha\)-gene is transcribed in pituitary and brain; it is located on chromosome 12 and shares high homology with human, bovine, and rat POMC genes (424). The POMC \(\beta\)-gene has \(<90\%\) aa sequence homology (predicted from cDNA sequence) with \(\alpha\)-POMC (425). The POMC \(\beta\)-gene is not expressed in pituitary, has the characteristics of a pseudogene, and is located on chromosome 19 (425). Lower vertebrates including lamprey, fish, and frogs also express two forms of POMC gene that have different degree of homology depending on the species (12, 402). The human POMC gene is located on chromosome 2 (25).

The mammalian POMC gene contains three exons and two introns; exon 1 corresponds to the 5'-untranslated mRNA; exon 2 contains a part of the 5'-untranslated mRNA, sequences of the signal peptide, and start sequences of the NH2-terminal fragment (NT); and exon 3 codes for the COOH terminus of NT, joining peptide (JP), ACTH, \(\beta\)-lipotropin (LPH), and for 3'-untranslated mRNA (17, 25, 59, 75, 99, 143, 170, 222). In the pituitary and brain, the primary transcripts are spliced out to generate a POMC mRNA of \(\sim 1.1 \text{ kb}\) (17, 25, 99, 170, 222). Shorter and longer POMC transcripts also exist but are found only in extrapituitary tissues that include skin (17, 76, 84, 91, 99, 167, 353, 371). The POMC mRNA size heterogeneity has been explained by alternative splicing, variation in the length of the poly(A)+ tail, or use of alternate transcription initiation sites (17, 76, 84, 91, 99).

POMC mRNA is translated into a single 30-kDa protein product, the precursor of ACTH, endorphins, melatropins (MSH), and lipotropins (LPH) (25, 59, 170, 383). These neuropeptides are differentially generated through successive cell-specific processing steps and posttranslational modifications that include endo- and exopeptidase cleavage, amidation, and acetylation (7, 8, 17, 20, 25, 59, 170, 383). As indicated in Figure 6, posttranslational processing of POMC yields a large number of biologically active peptides. POMC is processed in the corticotrophs of the anterior pituitary to form ACTH, \(\beta\)-LPH, and NT, with smaller amount of \(\gamma\)-LPH and \(\beta\)-endorphin (59). Further processing occurs in the intermediate lobe: NT to \(\gamma\)-MSH, ACTH to \(\alpha\)-MSH and corticotrophin-like intermediate lobe peptide (CLIP), and \(\beta\)-LPH to \(\beta\)-MSH, \(\beta\)-endorphin, \(\beta\)-endorphin-(1–26) and \(\beta\)-endorphin-(1–27). All of the peptides formed in this manner undergo amidation, mono- and diacetylation, phosphorylation, glycosylation, and methylation before processing of POMC is completed (7, 8, 17, 20, 25, 59, 170, 383). In the pituitary, the biosynthetic processing of the POMC precursor starts with specific cleavage by the convertases PC1 and PC2 at the post-pairs of basic residues (Lys-Arg) and (Arg-Arg) (59, 90, 345, 455). The convertase PC1 cleaves POMC to produce a 16-kDa NT, the JP, and the COOH-terminal peptides ACTH and \(\beta\)-LPH with some \(\beta\)-endorphin (25, 59). PC1 is expressed solely in corticotrophs determining their commitment to the production and secretion of ACTH. In melatropins, the coexpression of convertases PC1 and PC2, as well as the expression of carboxypeptidase E, and the amidating and N-acetylating enzymes determine that this cell type produces essentially \(\alpha\)-MSH and \(\beta\)-endorphin (25, 59, 90, 106, 170, 270, 345, 383). PC2 convertase cleaves the first 14 aa of the ACTH sequence to generate ACTH-(1–14)OH peptide and \(\beta\)-LPH to \(\beta\)-MSH and \(\beta\)-endorphin (25, 59). The ACTH-(1–14)OH after COOH-terminal amidation generates desacetyl-\(\alpha\)-MSH, a step required for biologic activity (25, 59, 383). Interestingly, whereas subsequent \(\alpha\)-N-acetylation at the NH2 terminus, that results in \(\alpha\)-MSH, enhances the melanocyte stimulating bioactivity of the tridecapeptide (25, 59, 383), and acetylation of \(\beta\)-end inhibits its opioid activity (25, 59). The O-acetylation of \(\alpha\)-MSH forms \(N,O\)-diacetyl-\(\alpha\)-MSH. It
has been suggested that PC1 or PC2 may be also involved in the generation of γ-MSH peptides; however, the mechanism of the reaction and the potential involvement of other enzymes remain to be clarified (90, 345).

Production and secretion of POMC peptides is organ specific, and in the pituitary it is lobe specific and under multihormonal control with main stimulatory input by CRH and AVP, and inhibitory regulation by adrenal glucocorticoids (17, 25, 59, 99, 110, 141, 170, 192, 222, 439). Epinephrine also has ACTH stimulatory activity, and the cAMP-PKA system appears to be the main intracellular signaling pathway for this pituitary action (10) that stimulates ACTH secretion, similar to the effect of pituitary adenylate cyclase-activating peptide (PACAP) and vasoactive intestinal polypeptide (VIP) (11). PACAP and VIP enhance separately the CRH stimulation of the POMC gene, but without additive effect. Other factors that stimulate production of POMC peptides by the anterior pituitary are PACAP, VIP, serotonin, oxytocin, ANG II, bradykinin, and leptin, whereas GABA decreases it (11, 17, 25, 59, 81, 124, 165, 170, 222, 243, 327, 383, 414). In the intermediate pituitary, neither glucocorticoids nor CRH has any effect on POMC gene expression, although CRH can selectively induce release of α-MSH (17, 25, 59, 170, 222, 383). α-MSH release from the intermediate pituitary is stimulated by β-adrenergic agonists, whereas negative regulators of this process are dopaminergic and α-adrenergic agonists, PGEl, and branched amino acids (17, 25, 59, 170, 222, 329, 383).

The proinflammatory cytokines IL-1, IL-2, IL-6, TNF-α, and INF-γ stimulate pituitary POMC gene expression and production of POMC peptides (17, 25, 27, 32, 33, 118, 170, 305, 313, 383, 421). However, the mechanism of this regulation is complex. For example, IL-1 stimulates POMC promoter activity in AtT-20 cells in a bimodal manner (weak short-term effects followed by strong long-term effects) (182). A similar effect was found for TNF-α; IL-6 had a robust stimulatory long-term effect, whereas INF-γ had acute stimulatory effects followed by a marked inhibitory effect. The effect of the cytokines on POMC gene expression is mediated by the tyrosine phosphorylation cascade (182). IL-1, IL-6, and TNF-γ can also significantly potentiate the stimulatory effect of CRH on POMC expression (17, 25, 33, 118, 170, 305, 313, 383, 421). Leukemia inhibitory factor (LIF) can stimulate POMC gene expression and secretion by corticotrophs (80), acting synergistically with CRH to potentiate POMC transcription and production of ACTH (15). Most recently, it has been proposed that LIF and IL-1β activate the suppressors of cytokine signaling (SOCS) pathway, which would inhibit POMC gene expression and ACTH secretion, thus
acting as a negative-feedback mediator in cytokine activity on the neuro-immuno-endocrine axis (16).

B. POMC Expression: Peripheral

Transcription and translation of the POMC gene have been detected in most peripheral tissues including placenta, uterus, gonads, thyroid, pancreas, adrenals, gastrointestinal tract, lungs, spleen, immune system, and skin (17, 27, 32, 33, 117, 170, 220, 226, 238, 258–260, 297, 301, 305, 365, 376, 383, 445). These extracranial sites produce shorter and longer POMC transcripts, in addition to the 1.1-kb message (17, 84, 91, 99, 167, 353, 371). That heterogeneity may represent alternative splicing, variation in the length of the poly(A)* tail, or use of alternative transcription initiation sites (76). It has been questioned whether mRNA smaller than 1.1 kb are translatable, since they lack the signal peptide that enables the end products to exit the cell (85, 167). In this regard, a POMC mRNA of only 0.8 kb found in placenta, testes, and ovary has been associated with the production of secretory POMC peptides (cf. Refs. 3, 167, 301, 383). Furthermore, in vitro assays have shown that short POMC transcripts can indeed be translated (85); possible mechanisms allowing translation of peptides and proteins lacking the signal sequence have been recently described (197). It appears that most tissues have the ability to produce POMC but at levels much lower than in pituitary (17, 27, 32, 33, 59, 170, 297, 305, 376, 383, 456). In placenta and testes, production of POMC peptides is stimulated by CRH (3, 297, 301, 302). In the immune system, POMC expression and peptide release are stimulated by CRH, AVP, lipopolysaccharide (LPS), and IL-1 and inhibited by glucocorticoids (27, 33, 183, 305, 421).

Because of the significance of the immune system in the skin (43), it could participate as a local site of production of the POMC peptides β-endorphin, ACTH, and α-MSH. In fact, full-length POMC transcripts have been detected in splenic mononuclear cells (MNC), which process POMC to ACTH through the same pathway as in corticotrophs (225). Also, lymphocytes and macrophages form predominantly β-endorphin and ACTH-(1—9), with smaller amounts of ACTH-(1—24), ACTH-(1—25), ACTH-(1—26), and β-endorphin-(144—146). T lymphocytes of human and murine origin as well as lymphoma-derived T-cell lines both express POMC mRNA and release the POMC peptides α-MSH and ACTH (55, 144, 224, 225, 259, 393). In human lymphocytes, POMC mRNA transcripts of 0.8, 1.2, and 1.5 kb have been detected (393). Similarly, murine γ/δ epidermal T lymphocytes express a truncated form of POMC and release α-MSH (108). The presence of ACTH immunoactivity has been clearly demonstrated in rodents T-helper cells, cytotoxic T cells, and B cells (224). There is also evidence suggesting differential processing of POMC in lymphoid tissues, perhaps due to the presence of distinct proteases that are selectively expressed in LPS-treated B cells where a truncated form of ACTH is detected (144). Neutrophils, however, have only been shown to express a 9.5-kb POMC mRNA transcript and do not release α-MSH (393). Recently, CRH was found to be synthesized and released by lymphocytes where it up-regulates POMC mRNA production (144, 305, 385, 393, 407). Cells in the monocytic lineage that contain POMC and produce melanocortins include human peripheral blood monocytes, epidermal Langerhans cells, cell lines derived from myeloid malignancies (U937), and rodent peritoneal macrophages (55, 206, 216, 225, 238, 240, 247). Basal production of melanocortins by monocytes is low but increases significantly upon stimulation with LPS, mitogens (concanavalin A), or tumor promoters (phorbol 12-myristate 13-acetate) (224, 247). In addition to immunocompetent cells, other cells known to release immunomodulating cytokines such as cardiac muscle cells, fibroblasts, and keratinocytes have been found to synthesize and release POMC peptides and thereby contribute to the development of an immune response (220, 241, 337, 408).

In humans, increased production of POMC peptides has been detected during the course of arthritis, in viral and parasitic infections, and in inflammatory skin diseases such as atopic eczema (32, 61, 62, 135, 136, 144, 258, 259, 384, 443). α-MSH injected systemically has immunosuppressor activity blocking IL-6- and TNF-α-induced fever, inhibiting the development of adjuvant-induced arthritis, and preventing endotoxin-induced hepatitis by antagonizing various cytokines and chemokines (60, 61, 83, 152, 210), suggesting its participation in the immune responses against viral infections. Indeed, POMC elements may bind POMC transcription factors present in the human immunodeficiency virus-1 genome, in cytomegalovirus, and in some oncogenes (c-fes, MAT-1). Therefore, signals known to mediate the activation of POMC transcription factors such as corticotropin releasing factor, tumor promoters, or ultraviolet light may activate viruses or oncogenes and thus facilitate infection and possibly tumor development (208). α-MSH also antagonizes the neuroendocrine effects of CRH and IL-1 (347, 423).

C. POMC Expression: Skin

MSH was the first POMC peptide detected directly in the skin (411), which expresses the POMC gene and produces also ACTH and β-endorphin (cf. Refs. 220, 365, 376, 378, 445). POMC gene transcription and translation in the mammalian skin was originally observed in the C57BL6 mice (370, 371). Subsequently, POMC gene expression has been found in human skin, normal and pathological, and in cutaneous cell culture systems (57, 67, 71, 96, 104, 107,
TABLE 1. Expression of POMC gene and production of POMC peptides in skin cells

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>POMC mRNA</th>
<th>POMC Peptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal keratinocytes</td>
<td>Present</td>
<td>α-, β-, γ-MSH, ACTH, β-endorphin, β-LPH</td>
</tr>
<tr>
<td>Follicular keratinocytes</td>
<td>Present</td>
<td>α-, β-MSH, ACTH, β-endorphin</td>
</tr>
<tr>
<td>Epidermal melanocytes</td>
<td>Present</td>
<td>α-, β-MSH, ACTH, β-endorphin</td>
</tr>
<tr>
<td>Follicular melanocytes</td>
<td>ND</td>
<td>Absent†</td>
</tr>
<tr>
<td>Dermal nevocytes</td>
<td>Present</td>
<td>α-, β-MSH, ACTH, β-endorphin</td>
</tr>
<tr>
<td>Sebocytes</td>
<td>Present</td>
<td>α-, β-MSH, β-endorphin</td>
</tr>
<tr>
<td>Sweat gland cells</td>
<td>ND</td>
<td>Absent†</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>Present</td>
<td>α-MSH, ACTH, β-endorphin</td>
</tr>
<tr>
<td>Langerhans cells</td>
<td>Present</td>
<td>α-MSH, ACTH</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Present</td>
<td>α-MSH, ACTH</td>
</tr>
<tr>
<td>Macrophages</td>
<td>Present</td>
<td>α-MSH, ACTH</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Present</td>
<td>α-MSH, ACTH</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>Present</td>
<td>ND</td>
</tr>
<tr>
<td>Adipocytes</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>ND</td>
<td>ACTH</td>
</tr>
<tr>
<td>Smooth muscle‡</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Nerves</td>
<td>NA</td>
<td>α-, γ-MSH, ACTH</td>
</tr>
</tbody>
</table>

MSH, melanocyte stimulating hormone; LPH, lipotrophin; POMC, proopiomelanocortin; ND, not done; NA, not applicable. † Below detectability by immunocytochemistry. ‡ Below detectability by in situ hybridization and immunocytochemistry.


1. Rodent

Immunoreactive α-MSH has been detected in rodent skin from Wistar, Hooded, and Norwegian Brown rats, gerbils, and hairless mice (411). Cutaneous α-MSH (mean concentrations, expressed as ng/g wet tissue wt) has ranged from a low of 0.3 (Norwegian Brown rat) to a high of 3.2 (gerbil), values consistently lower than the corresponding hypothalamic and pituitary concentrations (411). Nevertheless, the skin levels are similar to the levels measured in extrahypothalamic-pituitary regions of the brain (411). Skin α-MSH peptide concentrations are unaffected by hypophysectomy, at least in rat, gerbil, and mouse skin (411). Moreover, skin peptide concentration is not modified by daily injection of α-MSH that increase 7- to 10-fold the α-MSH plasma levels of hypophysectomized or intact rats. Therefore, MSH peptides detected in the skin are not of pituitary or plasma origin (411). Instead, these data and direct evidence obtained in the C57BL6 mouse (see below) indicate that α-MSH present in rodent skin is the result of local production. Our own molecular studies in mouse skin have shown that the POMC gene is actually transcribed, translated (371), and further processed to the POMC peptides ACTH, MSH, and β-endorphin (122, 356, 371). Also, the hairless mice express POMC mRNA in skin, as shown by RT-PCR and Northern blot hybridization of epidermal mRNA (G. Ermak and A. Slominski, unpublished data).

A) POMC GENE EXPRESSION AND POMC PEPTIDES PRODUCTION IN C57BL6 MOUSE SKIN. The POMC transcript of C57BL6 mice skin is 0.9 kb long, and the POMC protein, detected with an anti-β-endorphin antibody, has 30–33 kDa molecular mass (371). The truncated form of POMC mRNA has been detected in epidermis and in epidermal Thy-1+ dendritic cells in the C57BL6 mouse skin (107, 108). A combination of Northern and Western blot analysis with immunocytochemistry detected both mRNA transcript and POMC protein during the active growth phase of the hair cycle (anagen) (122, 356, 360, 371). Because POMC gene expression was not detected (371) or was very low (360) in the resting phase of the hair cycle (telogen), the experiments were interpreted as indicative of hair cycle-dependent gene expression and thus raised the possibility of POMC product involvement in skin appendage physiology (104, 273, 356, 368).

In additional studies, the exon 3 of the POMC transcript was found to be constitutively expressed in skin, being low in telogen and significantly upregulated in the growth phase of the hair cycle (anagen) (360). An attempt to determine the size of POMC mRNA on anagen skin using pituitary and brain RNA as standards give inconclusive results. This was due to the large discrepancy in tissue gene expression (per unit of mRNA), with levels at least 10,000-fold higher in pituitary than skin. Nevertheless, a truncated 0.9-kb POMC transcript was still present throughout the entire anagen, and an additional 1.1-kb POMC transcript was detected in anagen IV (360). Another attempt was then made to detect full-length POMC mRNA at a latter stage of the hair cycle (anagen VI skin) (104), when melanogenic activity reaches its peak (368, 369, 373). RT-PCR amplification was performed with primers spanning exons 1–3 and exons 2–3 (signal peptide and coding region) or exon 3 (coding region only), followed by Southern hybridization to murine POMC cDNA. Only a 475-bp transcript from exon 3 was detected; fragments of 810 and 738 bp corresponding to regions spanning exons 1–3 and exons 2–3 were present in pituitary and brain controls but absent in anagen skin (104). Thus, in anagen VI skin, the truncated 0.9-kb transcript may be the sole or predominant POMC species (104), yet it is the appearance of a 1.1-kb POMC transcript in anagen IV (day 5 after anagen induction) that correlates with the detection of a 30- to 33-kDa POMC protein on same day specimens; the POMC protein was not found in telogen or during the preceding days of anagen development (371). Therefore, selective expression of the 1.1-kb transcript (during anagen IV) may be the predominant pathway that leads to synthesis of the POMC protein in mouse skin (104). Nevertheless, truncated POMC mRNA transcripts may be translated (85), and peptides and proteins lacking
the signal sequence may still be translocated (197). Thus the 0.9-kb POMC mRNA cannot be entirely excluded as a source of POMC peptides.

Glucocorticoids are the physiological suppressors of pituitary POMC production (99, 110, 141, 170, 383, 444), and correspondingly, the synthetic glucocorticoid dexamethasone attenuates POMC mRNA production in anagen skin (104). Topical dexamethasone treatment is accompanied by decreased mRNA coding for the cutaneous MC1 (receptor for α-MSH) and for tyrosinase, and tyrosinase activity is correspondingly decreased (104). Topical application of dexamethasone also produces massive catagen development and rapid inhibition of melanogenesis (104, 273, 274, 372). Thus murine hair growth and attendant melanogenesis may be jointly regulated by local POMC peptide production/MC1 receptor stimulation (104), and suppression of this local pathway by glucocorticoids would explain the observed termination of both hair growth and follicular melanogenesis.

In mouse skin extracts, the POMC products detected by RP-HPLC coupled to specific RIA assays include β-endorphin, α-MSH, and ACTH peptides (122, 273, 356). Thus accumulated evidence indicates that mouse skin produces POMC, which is then processed to its secretory neuropeptides; triggering of this expression sequence may be linked to hair cycle (122, 231, 273, 356, 360, 368, 371). Accordingly, β-endorphin concentrations increase during anagen development, reach a peak in anagen VI, and decline during follicle involution (catagen); the lowest levels are reached in the resting phase (telogen) (122, 371). The pattern for ACTH immunoreactivity is similar to β-endorphin, e.g., low levels in telogen with a rise during anagen to a peak in anagen VI skin (356). These episodic variations in local levels of POMC peptides also correlate with expression of the convertases PC1 and PC2, from a high level in anagen VI to being low in telogen (230). The pattern of predictable intermittent cutaneous accumulation of ACTH, MSH, and β-endorphin peptides indicates precise regulatory control that determines actual production rates; whether the peptides degradation rates are also under regulatory control remains to be tested.

The differential expression of POMC products is not only associated with functional changes (hair cycle phase) but is also anatomically restricted (cellular compartment specific). In this regard, immunocytochemistry studies have shown that β-endorphin staining is primarily limited to sebocytes (Fig. 7), ACTH stain to epidermal and ORS keratinocytes, and to skeletal muscle, whereas β-MSH stains follicular and epidermal keratinocytes, sweat gland ducts, and sebocytes (122, 230, 231, 273, 356, 371). These immunostains also show the already mentioned temporal dependence on hair cycle phase, with lowest levels in telogen and progressive increases to a peak in anagen VI (122, 231, 371). α-MSH is detected throughout the entire hair cycle mainly in nerve bundles and perifollicular nerves ending, although it is additionally expressed in keratinocytes of ORS and hair matrix during anagen IV (273). POMC mRNA has been detected by in situ hybridization localized to the the dermal sebaceous glands in skin from the anagen III-VI phase of the hair cycle, whereas epithelial expression of the same POMC mRNA, first detected in scattered keratinocytes of the epidermis and ORS of hair follicles of anagen I, increases during progression to anagen VI (230, 231). The convertases PC1 and PC2, present at very low levels in epidermal keratinocytes of telogen skin, increase in anagen VI, with PC1 showing the greatest change (230). In the pilosebaceous unit, only PC1 is detected in telogen skin, whereas PC2 becomes detectable in anagen VI skin (230). Thus a close temporal correlation exists between localization and intensity of POMC mRNA expression, expression of the POMC processing convertases PC1 and PC2, and detection of POMC derived peptides.

B) POMC GENE EXPRESSION AND POMC PEPTIDE PRODUCTION IN RODENT MELANOMAS AND CELL CULTURE SYSTEMS. In the amelanotic rodent melanoma lines, Bomirski AbCl hamster and Cloudman S91 mouse clone 6, Northern hybridization analysis detected POMC mRNA transcripts of 3.5, 1.5, and ~1–1.1 kb (353). Expression of POMC mRNA in both cell lines correlated with detection of a 30-kDa POMC protein by Western blotting and by cytoplasmic immunostaining with antibodies against β-endorphin and γ2-MSH (353, 367). These results indicate that hamster and mouse melanoma cells can transcribe and translate the POMC gene. POMC mRNA production in the AbCl line was stimulated by exposure to L-DOPA (353). Of potential oncologic relevance, the amelanotic Bomirski transplantable hamster melanoma and melanotic variants are linked by common origin (41, 353, 367). In comparative analysis, POMC mRNA was expressed only in the rapidly growing amelanotic Ab variant, whereas POMC mRNA was not detected in the two slower growing Ma and MI melanotic variants (353). We have therefore suggested that in this system POMC gene expression may represent an autoregulatory mechanism whose expression stimulates progression to the malignant phenotype (367).

The melanocytic cell lines that produce α-MSH include LND2 and different subclones of B16 and Cloudman S19 melanomas, normal nontransformed (Melan A) and ras transformed (LTRras1–3, PAGTori and PAGT5) mouse melanocytes, and human melanoma lines (67, 71, 128, 223, 457). In the B16 melanoma line, immunoreactive α-MSH (α-MSH-IR) has been further identified by RP-HPLC as desacetyl-α-MSH (223). The highest concentrations of α-MSH-IR were found in the least differentiated, most metastatic melanoma cell lines and in ras-transformed melanocytes (223). In mice transplanted with various B16 melanomas, only the highly metastatic variant was α-MSH positive, with the IR located predominantly in the peripheral invading zones (457). This was in contrast...
to levels below the limit of detectability of the assay in the low metastatic F1 variant, which grows as a noninvasive tumor. Characterization of POMC mRNA from the S-91 melanoma cell line PS-1-HGPRT-1 showed three transcripts of ~6.5, 3.5, and 1.1 kb (71). These corresponded, respectively, to nonspliced primary transcription product, alternatively spliced product, and mature POMC. In the same melanoma cell line, UVB stimulated POMC gene expression and production and release of ACTH and α-MSH in a dose-dependent manner (71).

Because all of the above models of melanoma express MSH receptors, the observed production of POMC peptides could represent an autostimulatory mechanism, which accelerates growth and aggressive malignant behavior. The general trend is that POMC gene is more actively transcribed, or produces more POMC-derived α-MSH in the least differentiated, most malignant hamster and mouse melanoma cells and in ras-transformed melanocytes (223, 353, 367, 457). Deregulated intrinsic expression of the POMC gene appears then as a common factor preceding malignant transformation of melanocytes and/or melanoma progression to less differentiated faster growing forms (223, 353, 367, 457). Thus local expression of POMC peptides triggered by undetermined factors could alter melanoma cell phenotype promoting tissue invasion. POMC peptides would exert this action through auto-, intra-, or paracrine mechanisms; alternatively, POMC peptides may affect surrounding tissues such as immune elements or vascular system to further promote tumor growth and metastatic cascade.

In addition to being present in melanoma, POMC peptides are also produced by several nonmelanoma rodent skin cell lines (71, 108, 220, 223). For example, the immortalized (BALB/c) PAM 212 keratinocytes, in which
production of ACTH and MSH is stimulated by UVB or dibutyryl cAMP (71), and immune cells such as epidermal Thy-1+ lymphocytes, Langerhans cells, and circulating cells of monocytic lineage express the POMC gene and produce POMC peptides (108, 220).

2. Human

MSH was initially detected as immunoreactivity in extracts of normal human skin and identified by RP-HPLC in isolated epidermis as the desacetyl-, monoacetyl-, and diacetyl forms (411, 438); this established the background for further studies on the local expression of POMC peptides. With the use of immunocytochemistry on frozen sections of normal human skin, α-MSH was found in epidermal melanocytes and Langerhans cells, and ACTH was found in differentiating keratinocytes (438). Pathological specimens of human skin evaluated by immunocytochemistry also showed POMC-derived antigens in dermal and epidermal compartments (381). The POMC peptides expressed included ACTH, α-MSH, and β-endorphin, although a consistent pattern did not emerge (381). In normal corporal skin (obtained after surgery), POMC peptides were detected in dermis but not epidermis. Dermal POMC peptides were present in anagen hair follicles, where they accumulated in keratinocytes of ORS and hair matrix. In human scalp, POMC peptides were seen in both epidermal and follicular keratinocytes (381). The possible discrepancy with the results of Wakamatsu et al. (438), who did detect POMC peptides in epidermis of normal corporal skin, may be explained by the lower sensitivity for detection in the Formalin-fixed paraffin-embeded tissues (381). Direct RIA analyses in normal human skin showed that immunoreactive MSH levels were indeed very low or below the level of detectability (214). The same group found high MSH levels in melanoma lesions, which, together with our detection of ACTH, α-MSH, and β-endorphin in a wide range of skin diseases suggest that POMC peptide expression represents a disease-related phenomenon (381). Such a possibility received strong support from observations in keloids, a rather homogeneous primary reactive skin disorder, where POMC products were consistently detectable (10 of 11 cases) and localized to keratinocytes and mononuclear cells (381). Because POMC mRNA was detectable by RT-PCR in biopsy specimens from normal scalp, basal cell carcinoma, and compound nevus, cutaneous POMC peptides appear to originate from local production (359). Together these findings suggest that POMC peptides are produced and stored locally in lesional (especially reactive) skin cells as a component of the cutaneous response to injury.

More recently, it was confirmed that human skin cells could actually produce POMC peptides either in vivo (57, 168, 169, 211, 212, 220, 255, 363, 381, 438) or in cell culture systems (67, 96, 107, 189, 337, 341, 342, 354, 359, 408, 438, 446). In this regard, cultured keratinocytes and melanocytes produce POMC peptides and release α-MSH and ACTH into the medium (67, 189, 337, 354, 446). Production of α-MSH and ACTH can be significantly upregulated at the protein and mRNA levels upon treatment with phorbol 12-myristate 13-acetate, IL-1, and UV light (67, 96, 219–221, 337, 341, 342, 446).

A) Expression of POMC gene and production of POMC peptides in vivo. Production of POMC peptides in vivo was strongly suggested by the immunocytochemical studies performed in skin biopsy specimens and cultured cells and was supported by the corresponding detection of POMC mRNA. In situ hybridization experiments with normal human skin showed strong POMC mRNA expression in epidermal melanocytes and keratinocytes, whereas in the dermis, POMC mRNA was detected at much lower levels, in endothelial and perivascular cells (57). Thus these studies confirmed previous work implicating epidermal and dermal cells as source of cutaneous POMC peptides (57, 107, 168, 169, 189, 212, 219, 255, 337, 354, 359, 381, 389, 408, 438, 446). Of note, γ-MSH-IR was shown in nerve fibers in the basal layers of epidermis, in the upper dermis, close to Merkel cells, in Meissner’s corpuscles, around ORS of the hair follicle, in nerve bundles of the deep dermis, and to a lesser degree around sweat glands and blood vessels (169, 212). Because POMC peptides have been detected in the circulating immune system (27, 32, 33, 169, 219–221, 247, 305), together with cutaneous nerves, they may represent additional sources of POMC peptides in the skin.

Most resident skin cells express in situ α-MSH and ACTH, β-MSH, β-endorphin, and γ-MSH peptides (Table 1), arising from different portions of the POMC protein precursor. The peptide nature of the immunocytochemically detected antigens was confirmed with RP-HPLC analyses that did demonstrate the presence of desacetyl-α-MSH, mature α-MSH, and ACTH peptides in extracts of human epidermis (438) and in perilesional and lesional skin from areas of basal cell carcinoma (363). Thus these analyses performed in biopsy specimens, in conjunction with the cell culture studies, demonstrate that POMC precursor protein is produced in situ, being processed locally to the final secretory peptides. Expression of MSH and ACTH was accompanied by epidermal expression of the POMC processing enzymes PC1 and PC2 convertases, with PC2 showing tendency to coexpress with α-MSH (438). It therefore appears that POMC processing may be cell type regulated similar to pituitary corticotrophs and melanotrophs, i.e., skin cells may specialize in the production of either ACTH or α-MSH as initially proposed (376).

The detection of POMC peptides in skin is common to a broad spectrum of apparently unrelated conditions, from the neoplastic melanocytic nevi, melanoma, basal cell carcinoma, and squamous cell carcinoma to the in-
flammatory keloids, psoriasis, and scarring alopecia (381). This finding suggests that deregulated local production of POMC may be a common reactive phenomenon in cutaneous pathology (381), a possibility supported by the results of studies on the progression of pigmented lesions (127–129, 131, 211, 214, 215, 255, 381). In semiquantitative study, POMC-derived antigens were below the limit of detectability by immunocytochemistry in normal skin melanocytes and in benign blue nevi (255). In contrast, common and dysplastic melanocytic nevi showed weak positivity for α-MSH, ACTH, and β-endorphin antigens in 14, 33, and 44% of the specimens, respectively, with a tendency for higher expression levels in dysplastic nevi (255). In melanoma, POMC antigens were easily detectable in >50% of cases, and the stain was also more intense and diffuse in the more aggressive forms: the nodular type (pure vertical growth phase), the vertical growth phase of superficial spreading type, acral lentiginous types, and metastatic melanomas (211, 255). Direct measurement of α-MSH with RIA found that concentrations in skin melanomas were variable from 0.21 to 2.32 pmol/g wt and in lymph node metastasis from 0.31 to 4.25 pmol/g wt (214). The α-MSH-IR detected in melanomas was chromatographically heterogeneous; only a small fraction represented authentic α-MSH peptide, whereas most of the immunoreactivity corresponded to more hydrophobic species of larger molecular weight (128, 131). The larger molecular weight α-MSH-IR species probably have preserved COOH-terminal amino acid sequence and may contain the whole 1–13 α-MSH sequence (128, 131).

Immunocytochemistry studies performed in 30 specimens from basal cell carcinoma patients showed heterogeneous distribution of immunoreactive α-MSH, β-MSH, and ACTH peptides in tumor cells, lesional and perilesional areas, epidermal and follicular keratinocytes, dermal mononuclear cells, and extracellular matrix (363). The frequency of peptide detection was similar in lesional and perilesional areas and in tumor cells. RP-HPLC studies combined with RIA monitoring of eluted fractions documented that ACTH and α-MSH of lesional and perilesional areas corresponded to the authentic peptides; an additional peptide eluted at the same time as desacetyl-α-MSH. POMC mRNA was also detected by RT-PCR in extracts of perilesional and lesion skin (363). Most interestingly, the MC1-R gene encoding the receptor activated by MSH and ACTH was also expressed in the same lesional and perilesional skin, suggesting paracrine/autocrine mechanisms of action for the peptides. Perhaps POMC peptides facilitate basal cell carcinoma development and/or progression (363).

Ghanem et al. (127) found that melanoma patients have increased serum levels of α-MSH-IR, and these could be positively correlated with progression of clinical stage or level of invasion. This suggests that local tissue MSH could eventually spill into the blood. The possibility of POMC spillover from the skin is also suggested by studies in severe atopic dermatitis and psoriasis (125, 135, 136, 252). These patients showed increased serum β-endorphin levels; however, because of the previous immunocytochemical detection of POMC peptides in psoriatic skin (381), it was suggested that circulating β-endorphin could be generated, at least partly, in lesional skin (135).

POMC peptide production was evaluated by indirect immunofluorescence in skin biopsies from 16 patients with vitiligo lesions and 13 matched healthy controls (212). Keratinocytes from control skin and from involved and uninvolved skin of vitiligo patients expressed α-, β-, and γ-MSH (212). MSH-IR was, however, higher in keratinocytes and extracellular compartments of involved vitiligo skin than in control skin. The adnexal structures (sweat glands and pilosebaceous orifices) showed no difference in the intensity of stain (212). Plasma α-MSH levels were similar in vitiligo and control patients, whereas β-endorphin levels were higher in vitiligo, failing to show the normal circadian rhythmic change (252).

B) EXPRESSION OF POMC GENE AND PRODUCTION OF POMC-DERIVED PEPTIDES IN VITRO. Expression of the POMC gene has been noted in cells of epidermal and dermal origin that include normal melanocytes and keratinocytes (57, 66, 96, 107, 189, 219, 337, 354, 359, 438, 446), Langerhans cells (247), fibroblasts (408), human dermal microvascular endothelial cells (HDMEC) (341, 342), circulating immune cells (34, 35, 40, 41, 72, 258–260), and malignant melanocytes and keratinocytes (67, 128, 354, 363, 411). Gene expression was accompanied by detection of POMC-derived ACTH, α-MSH, and β-endorphin immunoreactivities in cultured cells and culture medium. Characterization of α-MSH and ACTH peptides by RP-HPLC analyses of cell extracts in normal and malignant melanocytes and in keratinocytes showed multiple forms of POMC peptides (128, 131, 337, 354, 363, 411, 438), which included ACTH-(1–10), acetylated ACTH-(1–10), ACTH-(1–17), ACTH-(1–39), desacetyl-α-MSH, and α-MSH (354, 363, 411, 438). Western blot analyses have confirmed the production of β-LPH and β-endorphin in normal keratinocytes (446), whereas normal melanocytes and squamous cell carcinoma cell β-endorphin was detected by RP-HPLC analyses combined with specific RIA assay (354). In melanoma and squamous cell carcinoma cells, ACTH, α-MSH, and β-endorphin were determined by RP-HPLC-RIA (411); one of the ACTH-IR peaks eluted at the same time as ACTH-(1–13) (354). These observations suggested that processing of POMC to β-endorphin, ACTH, and α-MSH is a constitutive property of skin cell type, regardless of whether they are benign or malignant. Production of POMC peptides in keratinocytes and melanocytes was found to be under regulatory control, being stimulated by UVB, selected cytokines, and other factors (47, 64, 67, 220, 337, 446) (see below). Also, HDMEC stimulated by UVB and UVA produce and release α-MSH and ACTH (341,
3. Regulation

It is generally accepted that translation of the POMC mRNA requires the full-length transcript composed of three exons, where exon 1 contains the signal peptide (17, 99, 170, 383). In human skin cells, the size of the POMC transcript is of 1.2 or 1.3 kb, similar to that detected in the hypothalamus and pituitary (220, 337, 446). In contrast, in rodent skin, a shorter 0.9-kb form predominates, although a full-length 1.1-kb transcript is also detected (104, 360, 371). It is not yet clear which of these forms is translated in rodent skin, since it is possible that the shorter 0.9-kb POMC could be translatable (85) because of mechanisms that would allow the translation of peptides and proteins lacking the signal sequence (197). Cloning of POMC mRNA fragments from human skin has shown only 86% homology with the pituitary transcript, with no differences in the region coding for ACTH and β-endorphin (57). This only partial POMC mRNA homology raises the question of whether additional nonallelic forms of POMC are expressed in the skin. It must be noted that lower vertebrates express two forms of POMC gene (12, 402), whereas in the mouse genome, two nonallelic α- and β-POMC genes have been identified (425).

Production of the final ACTH, MSH, and endorphin peptides is a complex multistep process that requires POMC processing by prohormone convertases (PC1 and PC2). The PC1 gene is expressed in human keratinocytes, where it is upregulated by UVB (47, 48), whereas Schoelzen et al. (341) showed that dermal microvascular cells express PC1 mRNA, which is again stimulated by UV, and by IL-1 and α-MSH. Immunocytochemistry studies showed that the PC1 and PC2 enzymes are expresssed in the human skin, in epidermal keratinocytes and melanocytes (438), and in the anal mucosa (159). In the C57BL/6 mouse, the enzymes are expressed in follicular and epidermal keratinocytes and sebaceous gland (230). The overall processing of POMC in skin cells yields multiple forms of ACTH, desacetyl-α-MSH, α-MSH, β-LPH, and β-endorphin (122, 131, 223, 337, 354, 356, 363, 411, 438, 446), but whether similar posttranslational modifications of intermediates of POMC, as in pituitary and brain, e.g., amidation, acetylation, glycosylation, and phosphorylation, would also occur in the skin remains to be defined.

A list of the factors that can stimulate or inhibit production of POMC peptides in the skin is presented in Table 2. The lack of cutaneous access to the rich source of neuroendocrine factors represented by the hypothalamus suggests that control of full expression of the POMC gene in the skin is probably different from the pituitary. It is possible that the immune system, also involved at the central level, could play a more dominant regulatory role on POMC peptides production in the skin. In this regard, the proinflammatory cytokine IL-1 has a significant stimulatory/inductive effect on POMC gene expression in resident skin cells and circulating immune cells including macrophages (27, 32, 33, 57, 219–221, 337, 445, 446). Indeed, IL-1 increases production of POMC mRNA and ACTH, α-MSH, and β-LPH peptides in cultured normal and malignant epidermal melanocytes and keratinocytes (67, 219–221, 337, 445, 446). In HDMEC, expression of POMC gene is also stimulated by IL-1 (341, 342). Another cytokine, TNF-α, stimulates production of POMC mRNA in normal dermal fibroblasts (408), whereas transforming growth factor (TGF)-β inhibits it in the same cell system and in keratinocytes, but not in keloidal fibroblasts (408). Neither TNF-α nor endothelin-1 affects POMC peptide production in melanocytes and keratinocytes (67). Further confirming the selectivity of cytokine action, the release of α-MSH by transformed human keratinocyte line is not altered by the cytokines IL-2, IL-6, TGF-β, and interferon (IFN)-γ. Thus selected cytokines and growth factors produced in the skin can act locally, stimulating or inhibiting POMC gene expression and peptide production in a cell type-restricted fashion.

In rodent skin, the expression of POMC gene and production of POMC peptides appear to be regulated by the same biological clock that controls hair cycle (104, 122, 273, 356, 360, 368, 371). Although the effector messengers of that clock remain the subject of numerous hypotheses, a pacemaker, local or distant, would generate the signals that periodically stimulate and inhibit cutaneous POMC gene expression (104, 272, 273, 276, 356, 360, 368, 371, 372, 376). Because the anagen-induced cutaneous POMC gene expression is inhibited by topical application of the synthetic glucocorticoid dexamethasone, which also terminates hair growth, it is possible that the dexamethasone-induced attenuation of POMC gene activ-

### Table 2. Modulators of POMC gene expression in the skin

<table>
<thead>
<tr>
<th>Agent</th>
<th>POMC Production (mRNA and/or Peptides)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokines and growth factors</td>
<td></td>
</tr>
<tr>
<td>IL-1</td>
<td>Stimulated</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Stimulated</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>Stimulated</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Inhibited</td>
</tr>
<tr>
<td>Physicochemical factors</td>
<td></td>
</tr>
<tr>
<td>Ultraviolet radiation</td>
<td>Stimulated</td>
</tr>
<tr>
<td>Dibutyryl cAMP</td>
<td>Stimulated</td>
</tr>
<tr>
<td>TPA and PMA</td>
<td>Stimulated</td>
</tr>
<tr>
<td>L-Dopa</td>
<td>Stimulated</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Inhibited</td>
</tr>
</tbody>
</table>

TPA, 12-O-tetradecanoylphorbol 13-acetate; PMA, phorbol 12-myristate 13-acetate; IL-1, interleukin-1; TNF-α, tumor necrosis factor-α; TGF-β, transforming growth factor-β.
ity may be mediated by a direct effect on the cutaneous clock (104).

A major cutaneous stressor, UVR, can stimulate/induce cutaneous POMC gene expression as shown in human and rodent normal and malignant cultured keratinocytes and melanocytes (48, 64, 67, 71, 96, 220, 221, 337, 446). The response to UVR was found to be dose dependent when assessing ACTH and \( \alpha \)-MSH and secretion in normal and malignant keratinocytes (67, 71, 219–221, 337, 446) and of \( \beta \)-LPH and \( \beta \)-endorphin in keratinocytes (446). These effects were accompanied by correspondingly increased POMC mRNA production. A similar UVR-induced stimulation of POMC gene expression, with increased ACTH and \( \alpha \)-MSH production and release to the culture media, was observed in rodent transformed keratinocytes and melanoma cells (64, 71). Most recently, UVB and UVA treatment of HDMEC resulted in increased expression of the POMC gene with increased production and release of ACTH and \( \alpha \)-MSH (341, 342). Humans and horses exposed to sunlight exhibit increases in the circulating levels of \( \alpha \)-MSH and ACTH (129, 157, 158), and experimental whole body exposure to UVR increases \( \beta \)-LPH and \( \beta \)-endorphin serum levels (23, 205). UV-absorbing topical sun blockers abrogate the \( \beta \)-LPH response (23), implicating mediation by a photoreaction. Although the pituitary gland was postulated as the source of these humoral responses, the experiments cited suggest a stress-sensing role for the skin and also support the skin as an alternate source for circulating neuropeptides.

Because of the significance of its interaction with the skin, the contribution of UVR to the production of nuclear signals deserves further examination. Theoretically, UVR could activate the genome directly or, indirectly via action on membrane-bound signaling processes, production of second messengers, or via pathways activated by oxidative stress (cf. Ref. 378). In support of the latter possibility, N-acetyl-cysteine (NAC), a precursor to glutathione that acts as an intracellular free radical scavenger, abolished the UVR-stimulated POMC peptide production in human keratinocytes and melanocytes (67). It was then postulated that NAC may inhibit POMC peptides production through attenuation of the oxidative stress triggered by UVR (64, 67). The tyrosine phosphorylation inhibitors tyrphostin and genistein had no effect on basal or UVR-stimulated POMC production (67). Exposure to dibutyryl cAMP had stimulatory effects on basal \( \alpha \)-MSH and ACTH production in a number of cell lines, which include transformed (BALB/c) PAM 212 keratinocytes and Cloudman S91 melanoma cells, PS-1-HGPRT-1 clone, and normal and transformed human keratinocytes and melanocytes, suggesting involvement of cAMP-activated pathways in that process (67, 71). Finally, phorbol 12-myristate 13-acetate and TPA enhanced POMC gene expression in melanocytes and keratinocytes (219, 220, 337, 446), suggesting involvement of PKC-activated pathway(s). Thus oxidative stress, cAMP, and PKC all appear to participate in the regulation of POMC gene expression.

VII. SKIN AS A TARGET FOR PROOPIOMELANOCORTIN PEPTIDES

A role for POMC peptides in the regulation of skin pigmentation is suggested by observations in the POMC knock-out mice model; surprisingly, these animals survive to adulthood (450). Lack of POMC gene expression is characterized by the main phenotypic traits of severe obesity and adrenal insufficiency; also prominent is a defect in fur pigmentation (450). This pattern is strikingly similar to the clinical picture of patients with pituitary POMC gene mutations that generate allelic forms with defective production of POMC protein (154, 196). Affected individuals present with red hair pigmentation, severe early-onset obesity, and adrenal insufficiency. There is also a large body of clinical information on POMC peptides excess syndromes that confirm the skin as a target for POMC-derived peptides (cf. Refs. 101, 113, 137, 141, 171, 201, 202, 204, 220, 221, 244, 282, 283, 352, 376, 384, 397, 412, 445). For example, humans with pathologically increased levels of plasma ACTH (Addison disease) or excessive ACTH production by tumors (Nelson syndrome) have hyperpigmentation and skin atrophy (101, 113, 137, 141), while the administration of MSH or ACTH peptides stimulates melanogenesis (201, 202, 204). Prolonged administration of synthetic ACTH in humans induces acne, skin atrophy, hyperpigmentation, and hypertrichosis (101, 113, 137, 141), whereas elevated serum concentrations of \( \alpha \)-MSH are associated with skin pigmentation (289). Additional research performed on human and animal models (see sect. vii, A and C) indicates that also immune, epidermal, adnexal, vascular, and dermal structures represent additional targets for POMC peptides.

A. Melanocortin Receptors

It is known that the phenotypic effects of POMC peptides are mediated via interaction with cell surface receptors linked to the G protein (101, 110, 141, 257, 444). MSH and ACTH activate melanocortin (MC) receptors, whereas \( \beta \)-endorphin acts predominantly on \( \mu \)- and \( \delta \)-opioid receptors. The expression of MC and endorphin receptors in different skin compartments is listed in Table 3; the following section discusses the skin MC receptors.

Investigations performed in frog melanophores showed that \( \alpha \)-MSH interacts with cell surface receptors to induce cAMP production and the final phenotypic effect of skin darkening (2, 141, 201, 350). In S91 melanoma cells, MSH interacts with specific cell surface receptors, activates adenyl cyclase activity, and increases intracellular cAMP concentrations (201, 202, 204). A role for POMC peptides in the regulation of skin pigmentation is suggested by observations in the POMC knock-out mice model; surprisingly, these animals survive to adulthood (450). Lack of POMC gene expression is characterized by the main phenotypic traits of severe obesity and adrenal insufficiency; also prominent is a defect in fur pigmentation (450). This pattern is strikingly similar to the clinical picture of patients with pituitary POMC gene mutations that generate allelic forms with defective production of POMC protein (154, 196). Affected individuals present with red hair pigmentation, severe early-onset obesity, and adrenal insufficiency. There is also a large body of clinical information on POMC peptides excess syndromes that confirm the skin as a target for POMC-derived peptides (cf. Refs. 101, 113, 137, 141, 171, 201, 202, 204, 220, 221, 244, 282, 283, 352, 376, 384, 397, 412, 445). For example, humans with pathologically increased levels of plasma ACTH (Addison disease) or excessive ACTH production by tumors (Nelson syndrome) have hyperpigmentation and skin atrophy (101, 113, 137, 141), while the administration of MSH or ACTH peptides stimulates melanogenesis (201, 202, 204). Prolonged administration of synthetic ACTH in humans induces acne, skin atrophy, hyperpigmentation, and hypertrichosis (101, 113, 137, 141), whereas elevated serum concentrations of \( \alpha \)-MSH are associated with skin pigmentation (289). Additional research performed on human and animal models (see sect. vii, A and C) indicates that also immune, epidermal, adnexal, vascular, and dermal structures represent additional targets for POMC peptides.

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tion-dependent effect. At low concentrations, a results, e.g., in human melanomas on different melanoma models have generated conflicting bilization of intracellular calcium (51). However, studies stimulated IP 3 production, and at high concentrations, it effect on IP 3 (162), whereas others showed a concentra-
tion-dependent on the presence of calcium in the receptor mi-
sagation of PKC by the receptor even in the absence of the ligand (105).

Table 3. Expression of MC and opioid receptors

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>MC Receptor</th>
<th>Opioid Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal keratinocytes</td>
<td>MC1</td>
<td>μ-Opiate receptor</td>
</tr>
<tr>
<td>Follicular keratinocytes</td>
<td>MC1</td>
<td>μ-Opiate receptor</td>
</tr>
<tr>
<td>Epidermal melanocytes</td>
<td>MC1, MC2†</td>
<td>ND</td>
</tr>
<tr>
<td>Follicular melanocytes</td>
<td>MC1</td>
<td>ND</td>
</tr>
<tr>
<td>Dermal nevocytes</td>
<td>MC1</td>
<td>ND</td>
</tr>
<tr>
<td>Sebocytes</td>
<td>MC5</td>
<td>μ-Opiate receptor</td>
</tr>
<tr>
<td>Sweat gland cells</td>
<td>MC1, MC5</td>
<td>μ-Opiate receptor</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>MC1</td>
<td>Absent</td>
</tr>
<tr>
<td>Langerhans cells</td>
<td>MC1</td>
<td>ND</td>
</tr>
<tr>
<td>Monocytes</td>
<td>MC1</td>
<td>ND</td>
</tr>
<tr>
<td>Macrophages</td>
<td>MC1</td>
<td>ND</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>MC1</td>
<td>ND</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>MC1</td>
<td>Absent</td>
</tr>
<tr>
<td>Adipocytes</td>
<td>MC2, MC5</td>
<td>Absent</td>
</tr>
</tbody>
</table>

MC, melanocortin; ND, not done. † Detected by RT-PCR.

The postreceptor pathways activated by α-MSH bind-
ing to the MC5-R in B lymphocytes involve stimulation of the Janus kinase 2 (JAK2) and signal transducers and activators of transcription (STAT1) pathways (52), as well as transmission through the same phosphorylation pathway used by cytokines and growth factors. In cells of monocytic lineage, α-MSH acts on MC1-R through cAMP-activated pathways to suppress the activation of nuclear transcription factor NFκB induced by inflammatory agents (227). Activation of NFκB may be required for expression of cytokines and adhesion molecules (229). Activation of MC1-R in immune cells inhibits NO and neopterin production as well as prostaglandin synthesis (209, 312, 390).

α-MSH may also be involved in stimulation of melanogenesis (51, 271), and some investiga-
tors have suggested that MSH receptor signal trans-
duction may be coupled to phospholipase C-activated production of IP 3 and diacylglycerol with subsequent mobilization of intracellular calcium (51). However, studies on different melanoma models have generated conflicting results, e.g., in human melanomas α-MSH was found indeed to stimulate IP 3 production (399), whereas in ham-
ster melanoma, the MSH signal transduction and subse-
quent phenotypic effect were linked to cAMP without any evidence of IP 3 production (366). A similar discrepancy is found in adrenal cortex. Some authors observed that α-MSH stimulated exclusively cAMP production without effect on IP 3 (162), whereas others showed a concentra-
tion-dependent effect. At low concentrations, α-MSH stimulated IP 3 production, and at high concentrations, it instead stimulated cAMP production (177, 178). The latter investigators further postulated that the steroidogenic action of α-MSH, but not ACTH, involved stimulation of PKC and tyrosine kinases as the result of phospholipase C activation (179). To further search for second messengers of MC2 receptor (MC2-R) different from cAMP, ACTH was modified by the attachment of α-nitrophenylsulfenyl to a tryptophan residue (NSP-ACTH). NPS-ACTH was found to rapidly stimulate intracellular calcium mobilization and production of 15-lipoxygenase metabolites of arachidonic acid in bovine fasciculata-recticularis cells (451). Therefore, calcium and lipoxygenase metabolites may act as additional second messengers of ACTH in bovine adrenal cells, and the ACTH-(11—24) region may be important in this regulation (451). However, it is still unclear whether the reported effects reflect the activation of solely MC2-R or, of other receptors as for example, MC5 that has high affinity for ACTH and is also expressed in the adrenal cortex (248, 431). Pharmacological studies with cloned MC receptors have shown that their signal transduction is coupled to activation of adenyl cyclase (82, 88, 123, 248–251, 256, 257). Although modest activation of IP 3 production by MSH binding to MC3 was reported (88, 257), there is no evidence that cloned MC1 and MC2 receptors would be coupled to other than cAMP second messenger generating systems (88).

The postreceptor pathways activated by α-MSH bind-
ing to the MC5-R in B lymphocytes involve stimulation of the Janus kinase 2 (JAK2) and signal transducers and activators of transcription (STAT1) pathways (52), as well as transmission through the same phosphorylation pathway used by cytokines and growth factors. In cells of monocytic lineage, α-MSH acts on MC1-R through cAMP-activated pathways to suppress the activation of nuclear transcription factor NFκB induced by inflammatory agents (227). Activation of NFκB may be required for expression of cytokines and adhesion molecules (229). Activation of MC1-R in immune cells inhibits NO and neopterin production as well as prostaglandin synthesis (209, 312, 390).

MSH receptors have been detected and characterized in rodent melanoma cells (64, 68–70, 95, 101, 161, 175, 176, 244, 265, 266, 277–279, 281, 283–285, 351, 352, 358, 364, 366, 434, 435), normal and malignant human melanocytes (1, 93, 101, 119, 120, 130, 161, 164, 257, 352, 401), keratinocytes (31, 35, 64–66, 220, 264, 363), and other cutaneous cells and extracutaneous tissues including brain, adrenal gland, gonads, and immune cells (29, 34, 45, 79, 88, 141, 147, 209, 220, 249, 251, 312, 390). In vivo experiments on mice showed wide distribution of MSH binding sites, to almost all organs (406). The work of the pigment biology group at Yale University has been fundamental in the characterization of cell-surface MSH recep-
tors (38, 95, 193, 201, 244, 277–279, 284, 285, 287, 434, 435, 447–449). In fact, the observation that after binding the ligand, the MSH receptor is internalized and translocated to the Golgi region (434) represents the first demonstration of receptor internalization after binding of a peptide hormone. Ultrastructural and biochemical assays showed translocation of the MSH-MSH-R complex from the cell surface to melanosomes and demonstrated the presence of actual intracellular MSH binding sites (68, 101, 244, 279,
MOLECULAR CHARACTERIZATION OF MELANOCORTIN RECEPTORS

Melanocortin receptors were characterized in crosslinking experiments that identified a membrane-bound receptor of ~45 kDa in a number of melanocytic lines (101). Cloning of the MC1-R and MC2-R receptors was also performed with mRNA isolated from melanocytes (82, 250). Currently, there is a family of MC receptors that includes five members MC1-R to MC5-R, that share high sequence homology (cf. Refs. 88, 171, 251). All five genes belong to the superfamly of seven transmembrane G protein-coupled receptors (82, 88, 171, 248–251); these are 298–372 amino acids long and have short intracellular domains. MC receptors are coupled to an adenylyl cyclase, with possible additional coupling in MC3-R to phospholipase C (cf. Refs. 82, 88, 123, 248–251). The MC receptors have different pharmacological profiles of activation by MSH and ACTH peptides (82, 88, 248–251, 290, 317, 339, 340). In rodents, the receptor for MSH, MC1-R, has high affinity for MSH, low for ACTH and \( \beta \)-MSH, and lowest for \( \gamma \)-MSH (88, 248, 250, 251, 317). In humans, MC1-R has equally high affinity for MSH and ACTH and lower affinity for \( \beta \)-MSH and \( \gamma \)-MSH (82, 88, 123, 250, 251). The receptor for ACTH, MC2-R, exhibits absolute specificity for ACTH (88, 250). MC3-MC5-R show equal affinity for MSH and ACTH, whereas MC3-R or \( \gamma \)-MSH receptor has high affinity for \( \gamma \)-MSH (cf. Refs. 88, 123, 248–251). MC1 receptors have been detected in skin, brain, and immune system; MC2-R in adrenal gland, adipose tissue, and skin; MC3-R in brain, placenta, gut, and thymus; MC4-R in brain; and MC5-R in brain, adipose tissue, muscles, esophagus, thymus, prostate, skin, and exocrine glands that include Harderian, lacrimal, and specialized (preputial) or nonspecialized sebaceous glands (45, 79, 82, 88, 104, 123, 141, 161, 209, 220, 248, 250, 251, 257, 317, 362, 400, 404, 431). The distribution of MC1-R, MC2-R, and MC5-R in skin is presented in Table 3. The mRNA for MC1-R and MC2-R have been detected in rodent skin by Northern blot hybridization (104). MC2-R mRNA was detected by RT-PCR in human skin biopsy specimens of compound melanocytic nevus and in cultured normal and malignant melanocytes (362). Of note, in human melanocytes, ACTH appears to be more potent than MSH in stimulation of melanogenesis (161).

MC1-R has a prominent role in regulation of mammalian pigmentation (88, 101, 141, 171, 248, 250, 257, 397). In rodents, MC1-R is encoded by the extension locus (E), which has four alleles resulting from point mutations (88, 257, 317). These allelic forms differ in their responsiveness to MSH and may even constitutively activate adenylyl cyclase without interacting with the ligand. Similar regulatory features have been described in other species (88, 171, 257). In humans, mutations at the MC1 locus generate allelic forms of the receptor which differ in ligand specificity and most importantly in phenotypic effects concerning hair and skin pigmentation (88, 171, 257, 386, 397, 428). It has also been proposed that defective MC1-R function may be associated with an increased incidence of melanoma (429).

MC1-R is also a target for other regulatory proteins. For example, the agouti signaling protein (AS), which is expressed in skin, inhibits the binding of MSH to MC1-R, with resulting inhibition of MSH-stimulated cAMP production and melanogenesis (cf. Refs. 88, 171, 251, 257, 397, 401). AS also acts as antagonists to the other MC receptors, MC2-R to MC5-R (cf. Refs. 88, 171, 251). AS bound to MC1-R can inhibit cAMP-mediated activation and melanogenesis, independently of exogenously added MSH (171, 257, 401). Because signal transduction in MC1-R is coupled to \( G_s \) but not \( G_i \) protein, AS could antagonize auto- or intracrine effects of endogenously produced MSH or ACTH. Alternatively, binding of AS to MC1-R could generate changes in the receptor immediate environment that would activate other, unrelated receptors.

Agouti-related protein (AGRP), which shares sequence homology with AS, is a naturally occurring antagonist of MC3 to MC5 receptors that may be involved in the hypothalamic control of feeding behavior (452). AGRP probably acts through competitive inhibition of MSH binding to MC3 to MC5 receptors and inhibition of MSH-induced cAMP production (452). Additional antagonists of MC receptors are dynorphin peptides (313).

REGULATION OF MC-R EXPRESSION

Several years ago Pawelek et al. (283) proposed that UVB-induced melanogenesis was mediated through up-regulation of the MSH receptor system. This was based on observations of upregulated expression of functional MSH receptors by UVB that amplified the melanogenic effect of exogenous MSH in a dose-dependent manner both in vivo and in cell culture systems (31, 38, 40, 48, 64, 68, 71, 283, 378). UVB action appeared to involve arrest of...
the cell cycle at the G2 phase, when cultured rodent melanocytes express maximal MSH receptor activity and responsiveness to MSH (64, 283). UVB stimulates expression of MC1-R in normal and malignant human and rodent melanocytes and in keratinocytes (38, 64, 68, 71, 119, 120, 378). When the skin is exposed to UVR, thermal, chemical, or biological insults, it responds with enhanced production of cytokines, growth factors, ACTH, MSH, and intermediates of melanogenesis (365, 378). In normal and malignant melanocytes, many cytokines such as IL-1α, IL-1β, endothelin-1, adult T-cell leukemia-derived factor/thioredoxin (ADFLTRX), INF-α, INF-β, INF-γ, dibutyryl cAMP, and the hormones α-MSH, β-MSH and ACTH can upregulate expression of the MC-1 gene and of functional cell-surface MSH receptors (31, 64, 67, 70, 71, 95, 119, 120, 175, 176, 400). IL-1 can also stimulate MC-1 receptor expression in normal and malignant human keratinocytes (31, 64) and HDMEC (341, 342). α-MSH, β-MSH, and ACTH as well as factors raising intracellular cAMP have similar effects in transformed keratinocytes (31) and HDMEC (341, 342). Melanin precursors such as L-tyrosine and phosphorylated isomers of L-DOPA also stimulate expression of MSH receptors and melanogenic responsiveness to β-MSH (233, 364, 377). Retinoic acid, while stimulating MSH receptor expression, inhibits MSH-induced melanogenesis (69, 265). In addition, retinoic acid and vitamin E inhibit MSH-sensitive adenylate cyclase activity in mouse melanoma cells (328). TNF-α inhibits MC1 expression in melanocytes (119).

B. Opioid Receptors

Most recently expression of μ-opioid receptors that bind with high-affinity β-endorphin was detected in cultured human epidermal keratinocytes (30). In human skin, in situ hybridization and immunocytochemistry has shown that the receptors are localized to keratinocytes of the epidermis and ORS of the hair follicles and to peripheral epithelial cells of sebaceous glands and secretory portion of sweat glands (30). The expression of μ-opiate receptors was downregulated by both the antagonist naltraxone and the agonist β-endorphin (30). Presence of the related ζ-opioid receptor has been documented in human and mouse skin (453). Furthermore, β-endorphin acts as a local pain modulator (148).

C. Phenotypic Effects of POMC Peptides

1. Pigmentary system

The stimulatory role of melanocortins on mammalian pigmentation has been discussed extensively (101, 126, 141, 155, 161, 171, 201, 203, 213, 244, 257, 284, 336, 352, 374). There is a general agreement that the POMC peptides with the most significant melanogenic activity are ACTH, α-MSH, and β-MSH; the activity of γ-MSH is low, and that of endorphins and CLIP is undetectable (101, 141, 161, 236, 257, 358, 400). Structurally ACTH and MSH peptides have in common the amino acid sequence -Tyr-Met-His-Phe-Arg-Trp- that contains the tetrapeptide His-Phe-Arg-Trp critical for melanotropic activity (141, 160). The functional relevance of β-MSH in mammals has been debated, since some considered it an artifact of β-LPH proteolytic degradation (343). However, Bertagna et al. (26) have demonstrated convincingly that β-MSH is a normal product of POMC processing in human nonpituitary tissues. β-MSH stimulates melanogenesis in cell culture and skin pigmentation in humans (202, 400) and rodents (101, 201, 244, 279, 306). γ-MSH peptides have low melanogenic activity in murine and hamster malignant melanocytes (358) as well as in frog and lizard melanosomes (101, 141, 257). Although the overall role of γ-MSH peptides in the regulation of mammalian pigmentation may be small, selected γ-MSH peptides could still modulate pigmentation indirectly, by modifying the cellular response to other melanotropins. For example, γ2-MSH may potentiate the melanogenic activity of α- or β-MSH, whereas γ3-MSH, acting as a partial agonist on MSH receptors, can largely inhibit the melanogenic activity of α- or β-MSH (358).

A) Melanogenic effects in cell cultures. Studies on rodent malignant and normal melanocytes have uncovered the role of MSH receptors in the regulation of melanocyte functions via cAMP-dependent pathways (101, 141, 155, 201, 244, 257, 278–286, 366). Melanogenesis is a highly regulated process modified by posttranslational, translational, or transcriptional mechanisms (101, 141, 244, 257, 279). In melanoma cells, both α- and β-MSH stimulate melanogenesis activating tyrosinase as well as post-dopa oxidase steps (101, 141, 257, 281, 283). In rodent malignant melanocytes, α-MSH and β-MSH are more potent than ACTH in stimulation of melanogenesis (101, 244, 279). The effect of MSH on cell proliferation is variable and depends on cellular genotype (101, 161, 244, 257, 277–279, 284, 285, 287, 288, 352). The finding of inhibition of proliferation in amelanotic cells indicates that this effect is unrelated to production of toxic intermediates of melanogenesis (244, 279, 366). MSH stimulates dendrite production through a pathway independent from that regulating melanogenesis, although cAMP mediated (257, 366, 367). Similar MSH effects, e.g., stimulation of melanogenesis, dendrite formation, and stimulation of cell proliferation, are seen in normal cultured mouse melanocytes (141, 155, 257).

In cultured human melanocytes, α-MSH, β-MSH, and ACTH at concentrations in the nanomolar range or lower stimulate melanogenesis, cell proliferation, dendrite formation, and cAMP production (1, 161, 400). In some studies ACTH was more potent than MSH (161), whereas in...
other reports both peptides were equipotent (1, 400). γ-MSH, which stimulates cAMP production, has no significant effect on melanogenesis or proliferation when added alone at concentrations equal to or lower than 100 nM in human or rodent melanocytes (400). Among the effects of α-MSH and ACTH on melanogenesis are increases in concentration of tyrosinase and tyrosinase-related proteins (1, 400, 401) and in the eumelanin-to-pheomelanin ratio (161). α-MSH can also stimulate attachment of human melanocytes to laminin and fibronectin (161) and inhibit TNF-α-stimulated intercellular adhesion molecule-1 expression in normal and malignant human melanocytes (149, 246). Mutations in MC1 receptor that produce unresponsiveness of epidermal melanocytes to MSH have resulted in the red hair phenotype (257, 428).

b) Melanogenic Effects in Vivo. In rodents, application of α-MSH stimulates melanogenesis or switches pheomelanogenesis to eumelanogenesis (cf. Refs. 88, 101, 126, 140, 141, 257). In several strains of adult mice as well as in Siberian hamsters, α-MSH specifically stimulates follicular melanogenesis, depending on genotype and hair cycle (53, 54, 88, 126, 140, 141, 203, 213, 257). For example, e/e mice are not responsive to MSH progamipigmentary activity (405). α-MSH stimulates tyrosinase activity at the transcriptional, translational, or posttranslational level depending on the phase of hair cycle (53, 54, 101, 126, 141, 257). This is consistent with the hair cycle restricted expression of melanogenesis-related genes, protein concentration, and enzymatic activity (368, 369, 373). β-MSH also stimulates tyrosinase activity, as observed in skin of newborn Syrian golden hamsters as well as black and brown mice (306), and has actual melanogenic activity in adult guinea pigs and hairless mice (38, 39). Overall, the promelanogenic effect of MSH peptides has been documented in a number of species (88, 101, 126, 141, 257).

In humans, the systemic administration of α- and β-MSH or ACTH stimulates skin pigmentation (202). Subcutaneous application of α-MSH enhances tanning in sun-exposed areas of the skin (204), whereas the skin and hair pigmentation of patients with Addison disease or Nelson syndrome is related to increased concentration of circulating ACTH (101, 113, 137, 257). Furthermore, increased skin pigmentation has been described in a patient having increased α-MSH hypersecretion without adrenal failure (289). In most patients with ACTH/α-MSH excess, hyperpigmentation is generalized but most prominent in sun-exposed areas.

2. Epidermis

Since the detection of MSH binding sites in cultured human squamous cell carcinoma and immortalized keratinocytes (31, 65), several groups, with a single exception (400), have shown that human keratinocytes express both the MC1-R gene and MSH receptors (35, 47, 48, 64–66, 220, 264, 363). Moreover, the MSH receptor protein expressed on the cell surface of keratinocyte is similar to that characterized in pigment cells (65). Experiments in HaCaT keratinocytes showed that α-MSH stimulates cell proliferation and downregulates 70-kDa heat shock protein (HSP70) expression (264). α-MSH inhibition of HSP70 expression was only observed at high calcium concentrations when keratinocytes were induced to differentiate, and it was accompanied by increased sensitivity to oxidative stress and decreased survival (264). In agreement with that finding is the enhanced MC1-R gene expression associated with calcium-induced epidermal keratinocyte differentiation (64). Further studies in keratinocytes showed that α-MSH can modulate cytokine production (47, 48, 220, 314). Specifically, α-MSH stimulates production of the immunoinhibitory IL-10 and inhibits the IL-1-induced production and secretion of IL-8 and GROα. It has been proposed that α-MSH inhibition of IL-1 effects is mediated by downregulation of NFκB (219). In human adult skin, epidermal keratinocytes are negative for MC1-R antigen, with the exception of perilesional keratinocytes of the skin involved by melanoma (35). In contrast, epidermal keratinocytes in fetal skin are positive for the MC1-R (35). A role for ACTH on epidermal keratinocyte function is unclear, although clinically ACTH has been implicated in the pathogenesis of acanthosis nigricans (113, 137).

γ1, γ2, and β-MSH at the high concentration of 10−6 M stimulate mouse epidermal keratinocyte proliferation in organ culture of telogen skin, but not in anagen skin (370, 375). β-MSH inhibits epidermal keratinocyte proliferation in anagen IV (370, 375), whereas α-MSH shows the same inhibitory effect on telogen skin (273). ACTH at high concentrations inhibits DNA synthesis in epidermal keratinocytes (356).

The finding of increased β-endorphin levels in sera of patients with atopic dermatitis and psoriasis (125, 135, 136) has suggested a pathogenic role for the peptide in those conditions. Actual μ-opiate receptors, downregulated by β-endorphin, have been detected in epidermal and follicular keratinocytes (30). Also the μ-opiate receptors antagonist naloxone can relieve itching in patients with chronic liver diseases, but it is unclear whether the effect is exerted at the cutaneous or systemic levels (24).

3. Dermis

Human dermal fibroblasts have been recently recognized as a target for α-MSH (36, 191). Thus α-MSH stimulates synthesis and activity of collagenease/matrix metalloproteinase-1 in a dose-dependent manner (191). α-MSH alone stimulates IL-8 release; however, when added together with IL-1, it inhibits the IL-1-induced IL-8 secretion from fibroblasts (36). These effects may be mediated by
its effects on AP-1 and NFκB function. Thus α-MSH directly activates AP-1, but it inhibits IL-1-induced AP-1 activation and stimulates NFκB, without affecting IL-1-induced DNA binding (36). These effects may be mediated by MC1-R, because this receptor is expressed on skin fibroblasts (36).

HDMEC also express functional MC1-R as detected by molecular probing for gene expression, by binding of 125I-labeled α-MSH, and by the stimulation of cAMP production by α-MSH (341, 342). α-MSH also stimulates in a dose-dependent manner the production and secretion of IL-8 and GROα, being synergistic with TNF-α and IL-1 (342). These effects appear to be dependent on cAMP production and activation of PKA. Thus α-MSH may play a crucial role on endothelial cell function, decreasing the adherence and transmigration of inflammatory cells, a prerequisite for immune and inflammatory reactions. There is evidence on HDMEC that α-MSH, which by itself upregulates the expression of E-selectin and vascular cell adhesion molecule (VCAM) mRNA, inhibits the LPS-mediated upregulation of these adhesion molecules (172–174, 220, 221). These findings were confirmed at the protein level and the functional level by adhesion assays using T lymphocytes and cultured human dermal microvascular endothelial cells (172, 174). α-MSH, although capable of activating NFκB by itself on endothelial cells, blocks completely the LPS- or IL-1-mediated NFκB activation (172–174). These findings suggest that α-MSH modulates the activity/availability of a transcription factor required for VCAM and E-selectin activation.

4. Immune system

The POMC peptides β-endorphin, ACTH, and α-MSH have strong immunomodulating potential resulting in an overall immunosuppressive effect (32, 33, 111, 209, 269, 346). The melanocortin α-MSH seems to have the widest spectrum of immunomodulating capacities. Human and murine monocytes as well as macrophages express MC1-R, and LPS and mitogens enhance its expression on monocytes, whereas several cytokines are without effect (28, 312, 390). When cultured in the presence of granulocyte-macrophage colony-stimulating factor, IL-4, and monocyte conditioned medium, human peripheral blood-derived dendritic cells express MC-1R with the highest levels in the fully mature cells (21, 22). It must also be noted that whereas POMC peptides affect the function of T, B, and NK cells, it is not yet clear whether these cells express any of the known MC receptors. MC1-R is nevertheless expressed on cells of the monocytic lineage and on cells involved in the modulation of immune and inflammatory responses such as neutrophils, mast cells, endothelial cells, keratinocytes, and endothelial cells (34, 36, 47, 63, 147, 304). The MC1-R ligands α-MSH and ACTH affect the function of cells involved in immunoregulation, whereas melanocortins such as β-MSH and γ-MSH, which require the expression of other MC receptors, exhibit less pronounced immunomodulatory activity. It is not yet clear whether MC-1R expressing immunocytes are also affected by agouti protein.

Acting on vascular endothelial cells α-MSH directly stimulates the adherence and transmigration of inflammatory cells, a prerequisite for immune and inflammatory reactions. This effect could be mediated through the expression of adhesion molecules such as E-selectin and VCAM (see above). E-selectin is required for the rolling of cutaneous lymphocyte-associated antigen (CLA)-expressing lymphocytes and VCAM, which mediates lymphocyte flattening and transmigration, is the ligand for VLA-4 expressed on lymphocytes (133, 150). Therefore, α-MSH could influence the outcome of a local immune response by shifting the expression of adhesion molecules. However, in ongoing inflammatory reactions characterized by the upregulation of proinflammatory cytokines, α-MSH seems to function as a downregulatory signal.

An important example of the immunosuppressive capacity of α-MSH is demonstrated by its effect on the outcome of the contact hypersensitivity (CHS) reaction (Fig. 8). Thus administration of α-MSH, systemic or topi-
clinical, suppresses CHS at both the elicitation and sensitization phases (138, 153, 209, 315, 349). In addition, when α-MSH is applied before epicutaneous sensitization with hapten, it induces tolerance, which is hapten specific, since animals can be still sensitized with other antigens. The induction of hapten-specific tolerance by α-MSH may be mediated by IL-10, because it is blocked by administration of anti-IL-10 antibodies (138). Furthermore, in the mouse, a single intravenous α-MSH injection results in significant elevation of IL-10 plasma levels lasting for more than 2 wk (220). Another mechanism involved in α-MSH tolerance induction may be its downregulatory effect on the expression of accessory molecules such as CD86 and CD40 on antigen-presenting cells (21, 22, 28). Therefore, α-MSH may impair the function of antigen presenting cells (APC) and shift the outcome of an immune response toward tolerance acting directly as well as via IL-10 induction. The notion that α-MSH interferes with the function of APC has received support from experiments with Langerhans cells prepared from normal murine skin. The cells were treated in vitro with α-MSH derivatized with dinitrofluorobenzene (DNFB) and then used to induce CHS in naive syngeneic mice. Treatment with α-MSH impaired Langerhans cell ability to induce CHS but failed to induce tolerance, suggesting that additional pathways are responsible for antigen tolerance induction (89); thus a second application of the allergen induces production of proinflammatory cytokine IL-1 which upregulates the expression of adhesion molecules on endothelial cells. Pretreatment with α-MSH before challenge downregulates the expression of adhesion molecules on endothelial cells (172). Therefore, inflammatory cells required for the CHS reaction are unable to adhere and penetrate through the vessel wall, and CHS is suppressed. These findings suggest a therapeutic role for α-MSH in cutaneous immune responses.

There is evidence from several studies indicating that most of the anti-inflammatory effects of α-MSH are exerted by its NT tripeptide (209, 210). This tripeptide can actually compete with α-MSH for MC-1R binding and, by itself, induces IL-10 production by monocytes as well as downregulates CD86 expression (29). In vivo the NT MSH tripeptide is capable of blocking the induction as well as the elicitation of CHS, similar to the intact α-MSH molecule. In addition, epicutaneous application of the tripeptide at the site of sensitization suppresses the elicitation of CHS and induces tolerance (220). Therefore, topical application of these tripeptides, of ~350 Da molecular mass, and thus capable of penetrating the epidermis, may represent a novel approach for the treatment of allergic and inflammatory skin diseases.

α-MSH as well as its NT tripeptide can apparently serve as antagonist of proinflammatory signals and thereby downregulate immune and inflammatory responses. Therefore, its major therapeutical potential would be in allergic diseases such as contact dermatitis (see Fig. 8), atop dermatitis, asthma, autoimmune diseases, and organ transplantation. In contrast, cutaneous tumors may be associated with overproduction of POMC peptides, which may represent a mechanism for escape from immune surveillance (127–129, 223, 353, 363, 367). In these cases, neutralization of α-MSH could represent a useful approach.

In addition to causing skin damage, UVR is associated with cutaneous and systemic immunosuppression (220, 378). Thus UVR impairs the function of epidermal immunocompetent cells such as Langerhans cells and induces the production of immunosuppressing cytokines. In the preceding sections we reviewed the stimulatory effect of UVR on the cutaneous production of POMC peptides and on expression of MC1-R by skin cells. These data in conjunction with expression of functional MC1-R on immune cells implicate POMC-MC1-R axis as an important coordinator in the response to UVR. Such effect addressed probably at attenuating the UVR-induced inflammatory reaction could be exerted directly or indirectly. In the latter, UVR-stimulated α-MSH expression would induce production of immunosuppressive factors, resulting in local (skin) and systemic immunosuppression (220, 378).

5. Hair

Hypertrichosis is a process whereby a minute, nonpigmented vellus hair follicle (HF) is converted into a large terminal HF that produces strong, pigmented hair shafts. To some extent this process may, at least in part, reflect a change in HF cycling, since hypertrichotic HF have a substantially longer anagen phase than the original vellus HF (cf. Ref. 272). In humans, overproduction of ACTH, e.g., by pituitary tumors or therapeutic administration of ACTH, is a well-recognized cause of acquired hypertrichosis (272, 444). This induction of hypertrichosis by ACTH provides circumstantial evidence that the neuropeptide may stimulate and/or prolong anagen. α-MSH may also have a role in the regulation of hair growth, since α-MSH receptors have been detected in the human HF (273).

In minks and weasels, the effect of POMC peptides on hair growth has been the subject of detailed studies (cf. Refs. 126, 272). Thus it has been observed that hypophysectomy prevents the spontaneous onset of molting, an effect corrected by exogenous α-MSH or ACTH (325, 326). Furthermore, in the mink, bilateral adrenalectomy causes a sharp rise in pituitary ACTH release and premature onset of anagen (322, 324). In fact, hair growth in mink can be induced directly by intracutaneous injection of ACTH, but not α-MSH (323) (Fig. 9). In the C57BL/6 mouse, ACTH has a bimodal hair cycle-dependent effect on hair growth, e.g., intracutaneous injection of ACTH in...
telogen skin induces anagen development (275), whereas in anagen skin it induces the premature onset of catagen (228). The C57BL6 mouse has been also studied as a model for the effect of POMC peptides on hair growth (228, 273, 275, 356, 370, 371, 375). In mice, hair growth is synchronized along three developmental stages: resting (telogen), growth (anagen), and regression (catagen) (272, 368). The anagen follicle produces the hair shaft formed by pigmented, keratinized epithelial cells. Hair growth and the cyclic activity of the hair follicle are timed by a "biological clock" of unknown nature through as yet ill-understood tissue interactions. In rodents, the cyclic activity of hair follicles is accompanied by periodic changes in the physiology of the entire skin (cf. Refs. 272, 368). These are expressed morphologically as skin thickening, increased vascularization, and skin pigmentation. The cyclic activity of hair is coupled to cyclic changes in local immune system (272, 273); in the pigmented system (368, 369, 373); in POMC expression, production of POMC peptides, and expression of MC1-R (104, 122, 356, 360, 370, 371); and in hair follicle innervation (276). It has been proposed that the hair cycle-dependent local production of POMC and expression of the corresponding receptors are integral effectors of the biological clock that regulates cyclic activity of hair follicles, skin, and subcutis. In this context POMC peptides imported from extracutaneous sources may have limited effects because of competition for local receptors with endogenous peptides; therefore, any effect(s) of systemic supplies would be restricted to the phases when local production is low or absent. For example, γ- and β-MSH at pharmacological doses stimulate DNA synthesis in the epidermal compartment of mouse skin (370, 375). However, this effect is seen only in telogen but not during anagen development. Furthermore, pharmacological doses of α-MSH inhibit proliferation of epidermal and follicular keratinocytes, but again only in telogen and not anagen skin (273). ACTH also acts in telogen skin, where it has a dose-dependent dual effect, e.g., at physiological doses it stimulates DNA synthesis in dermal but not epidermal compartments, whereas at pharmacological doses ACTH inhibits DNA synthesis in both dermal and epidermal compartments (356).

6. Sebaceous glands

ACTH, α-MSH, and β-LPH influence sebaceous gland function (412). In the rat, ACTH has sebotropic activity that has been explained by its stimulation of adrenal androgens and progesterone secretion, since adrenalectomy completely inhibits this sebotropic effect. Also in rodents α-MSH stimulates sebum secretion and lipogenesis in cutaneous sebaceous glands and in specialized pheromone-producing preputial glands acting as trophic factor for those glands. α-MSH action on the preputial glands of rodents stimulates production and release of female sex attractant odors and of male aggression-promoting pheromones (412). α-MSH specifically stimulates wax and sterol ester biosynthesis (79, 412). It has been postulated that the sebotropic activity of α-MSH and ACTH is mediated by a peptide sequence different from the melanotropic one (412). In rodents, α-MSH and perhaps ACTH may be important for skin thermoregulation, preventing overwetting of hairs, and by behavior regulation through their action on nonspecialized and specialized sebaceous glands (412).

MC5-R that are fully functional are actually expressed in cutaneous sebaceous and preputial glands and in Harderian and lacrimal glands (79, 431). In the latter, melanocortin binding sites have been detected and ACTH and α-MSH stimulate lacrimal protein production and peroxidase secretion (79, 431). ACTH also stimulates porphyrins synthesis by Harderian glands (79). MC5-R-deficient mice have severely defective cutaneous water repulsion and thermoregulation, from impaired sebaceous
lipids production (79). In the MC5-R knock-out mice, Harderian gland function is also impaired (79), suggesting a role for MC5-R in the protection of the eye from environmental stresses.

In humans, it is likely that MSH and ACTH peptides act on sebaceous glands functions, since hypopituitarism is associated with decreased sebum production (137).

VIII. REGULATION OF THE CUTANEOUS CORTICOTROPIN RELEASING HORMONE-PROOPIOMELANOCORTIN SYSTEM

A. UVR

UVR also has a dominant stimulatory role on CRH and POMC peptide production as well as in the expression of the corresponding receptors amplifying the phenotypic effects of MSH. Thus UVR stimulates CRH peptide production in human melanocytes in a dose-dependent manner with increased production at higher levels of radiation (355). UVR also stimulates POMC gene expression with enhanced production and secretion of POMC peptides such as ACTH, α-MSH, β-LPH, and β-endorphin in a number of rodent and human cutaneous cell systems (48, 64, 67, 71, 96, 219–221, 337, 341, 342, 446). Also stimulated by UVR is the expression of prohormone convertase PC1, responsible for the processing of both pro-CRH and POMC (341). Recent data suggest that UVR-stimulated CRH and POMC peptide production may be mediated by UVR-triggered oxidative stress (64, 67, 378).

Thus the powerful cutaneous stressor represented by UVR stimulates all of the components of the regulatory arm (production of CRH and POMC peptides) in the local CRH-POMC system. In addition, UVR activates the functional arm of the system by inducing expression of the corresponding receptors. The latter effect is illustrated by the stimulation of MC1-R mRNA with increased expression of functional MSH receptors that follows, in a dose-dependent manner; UVR exposure (38, 64, 68, 71, 119, 120, 378). The UVR stimulatory effect on functional MSH receptors has been correlated with amplification of the melanogenic effect of MSH peptides in number of cell culture systems (31, 38–40, 64, 68, 71, 283); it is also seen in vivo, where it is associated with melanocyte proliferation (38, 39, 378).

B. Cytokines

These secretory products of the skin immune system (SIS) also regulate cutaneous CRH-POMC system activity. The cytokine IL-1 has significant stimulatory effects on POMC gene expression and on the production of POMC-derived ACTH, α-MSH, and β-LPH peptides by resident skin cells and circulating immune cells (27, 32, 33, 67, 219–221, 337, 341, 342, 445, 446). There is parallel stimulation of PC1 gene expression (340, 341), and IL-1 also stimulates MC1-R expression (31, 64, 119, 220, 341, 342). Another cytokine, TNF-α, stimulates POMC gene expression in dermal fibroblasts (408); TGF-β has the opposite effect, inhibiting POMC gene expression in the same cell system and in keratinocytes (408). Other related products such as endothelin-1, ADF/TRX, INF-α, INF-β, and INF-γ can also stimulate the expression of functional MSH receptors on melanocytes (31, 64, 70, 71, 95, 119, 120, 175, 176, 400). Thus, similar to UVR, the SIS also participates, through the opposing action of selected cytokines, in the regulation of local expression of POMC and its corresponding receptors.

C. Hair Cycle

In rodents, this cyclic process is characterized by dramatic changes in skin physiology, with variations in CRH content, expression of the POMC gene, production of POMC peptides, expression of CRH-R1 and MC1-R receptors, and expression of PC1 and PC2 convertases (104, 122, 230, 273, 321, 356, 360, 368). Thus the local pacemaker that determines the hair cycle also determines, directly or indirectly, the expression of the CRH-POMC system in rodent skin (104, 272, 273, 276, 356, 360, 368, 371, 372, 376). The effector messengers regulating cyclic follicular activity remain to be defined (104, 272, 273, 276, 368, 376).

D. Disease States

In normal corporal human skin (whole body except scalp and face), expression of the POMC gene is low or below the level of detectability (214, 381). In contrast, gene expression becomes readily detectable in pathological conditions such as keloids, psoriasis, basal and squamous cell carcinomas, vitiligo, and melanomas (127, 131, 135, 211, 212, 255, 363, 381). Most interestingly, malignant progression of pigmented lesions is accompanied by increased expression of POMC peptides in the atypical melanocytes of dysplastic nevi or melanoma (211, 255, 381). Concordantly, increased production of POMC peptides is found in the more advanced and aggressive forms of melanoma (127, 128, 131, 250, 353, 367, 457). Thus disease process can stimulate cutaneous POMC gene expression.

E. Cellular Metabolism Mediators

Factors that raise intracellular cAMP levels such as dibutylryl cAMP, forskolin, and MSH stimulate production of...
CRH and of POMC-derived peptides in skin cells (64, 67, 341, 361). In addition, dibutyryl cAMP and the hormones α-MSH, β-MSH, and ACTH stimulate expression of the functional cell surface MSH receptors in melanocytes, keratinocytes, and dermal microvascular endothelial cells (31, 64, 70, 71, 95, 119, 120, 175, 176, 341, 342, 400); in the latter, phorbol 12-myristate 13-acetate and TPA enhance POMC gene expression (219, 220, 337, 446). Together these observations suggest that PKA and PKC mediate significant regulatory pathways in cutaneous CRH-POMC system.

F. Glucocorticoids

Dexamethasone inhibits CRH production in cultured human skin cells and attenuates POMC gene expression in rodent skin (104, 361). Dexamethasone also inhibits expression of the MC1-R gene in rodent skin (104), and it terminates anagen-associated melanogenesis by inducing expression of the MC1-R gene in rodent skin (104, 361). Dexamethasone also inhibits human skin cells and attenuates POMC gene expression (95, 119, 120, 175, 176, 341, 342, 400); in the latter, phorbol 12-myristate 13-acetate and TPA enhance POMC gene expression (219, 220, 337, 446). Together these observations suggest that PKA and PKC mediate significant regulatory pathways in cutaneous CRH-POMC system.

IX. PHYSIOLOGICAL SIGNIFICANCE OF CUTANEOUS PRODUCTION OF CORTICOTROPIN RELEASEING HORMONE AND PROOPiomelanocortin

As noted above, local production of CRH and POMC and corresponding receptors expression can occur in response to environmental stimuli that do not reach the brain (e.g., UVR). This evidence strongly implies that cutaneous production of these peptides, and their effect on skin function may be regulated independently from their systemic counterpart. The effects of the CRH/POMC responses are cell-compartment and function specific. For example, CRH and related peptides stimulate intracellular calcium accumulation in malignant melanocytes and in normal and malignant keratinocytes and affect cell proliferation (109, 186–188, 357, 379). CRH action is nevertheless modified by a timing component, since implantation of slow-releasing CRH capsules in the vicinity of HF with the telogen phase will effectively arrest hair cycle progression, producing an extended telogen phase (273). Overall, peripheral CRH acts as a growth factor for the epidermis and HF, has antiedema effects, and is a vasodilator agent potentially enhancing blood flow through dermis acting on the vascular smooth muscle and endothelium (19, 86, 114, 116, 134, 316, 320, 338, 422, 440). CRH could be important for local hemostasis, since it inhibits IL-1-induced prostacyclin and prostaglandin synthesis (114, 116). It is also considered an immunomodulatory factor (9, 134, 142, 237, 268a, 305, 334, 355, 418, 430, 440), consistent with its ability to induce mast cell degranulation (409).

With regard to the POMC peptides MSH and ACTH, these stimulate epidermal and follicular melanogenesis, melanocyte proliferation, and modify immune and secretory functions of pigment cells (cf. Refs. 64, 101, 126, 141, 149, 161, 202, 220, 365, 374, 376, 445). ACTH, β-endorphin, and especially α-MSH are strong immunomodulators exerting an overall immunosuppressive effect (32, 33, 111, 209, 220, 269). For example, α-MSH can function as an antagonist of IL-1 and induce production of immunosuppressive cytokines while suppressing the expression of accessory molecules on APC (22, 28, 58, 138, 147, 219, 220, 254, 314). Of clinical significance, α-MSH and its NH₂-terminal tripeptide can prevent the induction of the contact hypersensitivity reaction and even block its elicitation (138, 153, 209, 220, 314, 315, 370, 375). ACTH stimulates hair growth by inducing anagen development (Fig. 8) (273, 275, 322, 323). The MC5-R knock-out mice model, which has defective sebaceous gland function and thermoregulation (79), provides further evidence for the role of ACTH/MSH in those functions (79, 412, 431). α-MSH has a regulatory role in rodent nonspecialized and specialized sebaceous gland function; it stimulates lipogenesis and sebum secretion as well as production and release of female sex attractant odors and male aggression-promoting pheromones (79, 412, 431). Finally, MSH stimulates dermal fibroblast collagenase production and secretion as well as cytokine production (36, 191, 220). In summary, cutaneous POMC peptides have marked effects on the pigmenteary, immune, epidermal, adnexal, and dermal functions of the skin, enhancing melanogenesis, stimulating cellular differentiation, stimulating hair growth and sebaceous gland functions, and attenuating inflammatory reactions.

X. HYPOTHESIS ON AN ORGANIZED SYSTEM MEDIATING SKIN RESPONSES TO STRESS

A. Hypothesis

The findings in the skin of CRH/CRH-like peptides, POMC peptides, and their corresponding receptors strongly advocate against random occurrence (Fig. 10). Rather, similar to the central levels, expression of these elements is highly organized. The functional purpose of this CRH/POMC system would be to respond to external and internal stresses through local pigmenteary, immune, epidermal, adnexal (HF and sebaceous, eccrine, and apocrine glands), and vascular structures to stabilize skin function and prevent disruption of internal homoeostasis. This skin stress response system (SSRS) has
the capacity to regulate its level of expression independent of the CNS. The SSRS operates at the cellular, tissue, and organ level via a combination of intra-, auto-, and paracrine mechanisms (Fig. 10). Because of the differences in skin structure between furry animals and humans, as well as the nature of the predominant stress reaching the viable portion of the skin, the operational organization of the local CRH-POMC system has characteristic species specificity. In humans, the SSRS is most reactive to solar radiation and thus linked to the pigmentation system; in rodents (mostly nocturnal animals), the SSRS is more reactive to chemical insults and coupled to the activity of adnexal structures.

**B. Background to the Organization and Function of the SSRS**

Physiologically, the skin is the body organ with the most extensive exposure to the environment; as such, it
has a critical role in the protection of global homeostasis. Thus the continuous barrier represented by skin is exposed to extreme temperatures, radiation, biological agents, and trauma. The most important cutaneous stress is the solar radiation, particularly the ultraviolet spectrum which interacts with epidermal components and leads, when excessive, to the local accumulation of toxic products. Exposure of humans to high-intensity solar radiation results in a highly circumscribed immediate reaction, i.e., typical erythematous eruption restricted to the exposure site; more chronically, it results in pigmentation. The reactive accumulation of the pigment melanin, which acts as a light filter and scavenger of cellular toxins and free radicals, prevents further skin damage.

CRH, ACTH, β-endorphin, and MSH peptides, together with receptors for CRH and the POMC peptides, are all present in the skin, while the corresponding genes are also expressed locally (Fig. 10). That the components of the highly organized system involved in the systemic response to stress would be expressed in cells whose normal destiny is to differentiate and die strongly support functional relevance for this energy-requiring function. Indeed, testing the functional modulation of the skin CRH-POMC system has shown strong linkages to UVR, cytokines, hair cycle, disease states, and metabolic modifiers.

C. Functional Organization of SSRS

When the normal integrity of the skin is disrupted, it is most important to restrict maximally the consequent biological reaction, to preserve its functional properties. The actions of the CRH-POMC system at the cellular level prevent spreading of the field of stress. Within this context, the space-restricted paracrine, autocrine, and intracrine mechanisms of action are critical for limiting tissue damage. Expression of truncated products lacking the signal peptide that would allow easy export from the cell would also ensure that the effect would be localized to structures in the immediate vicinity of manufacturing sites. The SSRS immunosuppressive activity decreases recruitment of local immunocytes; local immunoglobulin and cytokine production would abate, and local dermal capillary permeability would return to basal levels. Most important in this conception is the timing of the functional sequence, since CRH-POMC activation must follow the primary reactive mechanisms. It is possible that the full expression of CRH-POMC effects may require the presence of stress-dependent products acting as potential cofactors. An effect of stress-released molecules acting as cofactor for CRH-POMC phenotypic effects is supported by, for example, the finding of pigmentation restricted to the solar radiation-exposed areas in patients with adrenal destruction (Addison disease) or with tumoral pituitary ACTH hypersecretion (Nelson syndrome).

CRH/POMC molecules similar to those produced in the skin are being released continuously by the HPA axis and are likely to influence skin function. However, some degree of cutaneous insensitivity to CRH-POMC peptides of central origin may result from local impermeability or in situ degradation of peptides. In contrast, an opposite endocrine central effect from the low constitutive activity of the CRH-POMC skin system would seem less likely. Thus, when the intensity of skin damage is major, by the time the extent of local involvement might release a large amount of POMC peptides that could spill into systemic circulation, the systemic reaction to stress via the central HPA axis would have been already activated. Therefore, peripheral contribution to the acute systemic response to stress would be insignificant. Nevertheless, we cannot exclude the possibility that in this setting, as well as in pathological conditions resulting in chronic, sustained CRH/POMC skin overproduction, the same molecules could serve a different role as messengers of cutaneous distress. The latter function might be accomplished directly by the hormonal products themselves, or indirectly by stimulating ascending neural pathways.

We thank Drs. Z. Abdel-Malek, T. Kishimoto, E. Linton, J. Pawelek, J. Roberts, and E. Wei for their valuable comments. The excellent secretarial work of T. Hermann is acknowledged.

This work was supported by National Science Foundation Grants IBN-9890600, IBN-9604364, and IBN-9405242; American Cancer Society, II. Division, Grant gg-51; Bane Charitable Trust Grant LR9178 (to A. Slominski); Deutsche Forschungsgemeinschaft Grant Pa 345/6–1 (to R. Paus); a grant from Welia, Darmstadt (to R. Paus); and a grant from Deutsche Forschungsgemeinschaft (to T. Luger).

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