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Review

Melanocortin receptors: their functions and regulation by physiological agonists and antagonists

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Abstract. The melanocortins are a family of bioactive peptides derived from proopiomelanocortin, and share significant structural similarity. Those peptides are best known for their stimulatory effects on pigmentation and steroidogenesis. Melanocortins are synthesized in various sites in the central nervous system and in peripheral tissues, and participate in regulating multiple physiological functions. Research during the past decade has provided evidence that melanocortins elicit their diverse biological effects by binding to a distinct family of G protein-coupled receptors with seven transmembrane domains. To date, five melanocortin receptor genes have been cloned and characterized. Those receptors differ in their tissue distribution and in their ability to recognize the various melanocortins and the physiological antagonists, agouti signaling protein and agouti-related protein. These advances have opened new horizons for exploring the significance of melanocortins, their antagonists, and their receptors in a variety of important physiological functions.

Key words. Melanocortin receptor; proopiomelanocortin, melanocortin; agouti signaling protein; agouti-related protein

The melanocortins

The past decade has witnessed a tremendous advancement in the understanding of the diverse physiological effects of melanocortins and their mechanisms of action. The melanocortins are a group of structurally related peptides that derived their name from their melanotropic and corticotropic activities, and are comprised of adrenocorticotropic hormone (ACTH), α-melanocyte stimulating hormone (α-MSH), β-MSH, and γ-MSH, all of which are derived from a 31- to 36-kDa precursor peptide, proopiomelanocortin (POMC) [1, 2]. Post-translational modification of POMC by proteolytic cleavage generates many bioactive peptides, including the melanocortins, β-lipotropic hormone (β-LPH), and β-endorphin [3]. The processing of POMC into its various derivatives occurs in a tissue-specific fashion, and results from the action of two main proteolytic enzymes, proconvertases 1 and 2 [4]. Proconvertase 1 generates ACTH and β-LPH, while proconvertase 2 generates α-MSH by the cleavage of ACTH, and β-endorphin. Further translational modification of α-MSH occurs by amidation of the peptide at the C terminus and acetylation at the N terminus of the peptide. The entire 13-amino acid sequence of α-MSH is contained within the N-terminal region of ACTH, a 39-amino acid peptide. All melanocortins share a conserved tetrapeptide sequence, His-Phe-Arg-Trp, and α-MSH, β-MSH, and ACTH share a heptapeptide sequence, Met-Glu-His-Phe-Arg-Trp-Gly, which is important for the melanogenic effects of melanocortins.

Melanocortins are best known for their ability to stimulate eumelanin synthesis in mammalian melanocytes and steroidogenesis in adrenocortical cells [5, 6]. The two are by no means the exclusive effects of these hormones.

Other effects include regulation of food intake and energy metabolism, and neuronal regeneration [7–9]. Melanocortins act as endogenous antipyretic agents, and have systemic as well as peripheral antiinflammatory effects [10, 11]. Melanocortins have sebotrophic effects [12], induce lipolysis [5], regulate exocrine glands, cardiac and testicular functions [13, 14], and natriuresis [15]. Direct evidence for a role of melanocortins in at least some of the above effects in humans comes from the observation that mutations in the *POMC* gene that disrupt its expression result in adrenal insufficiency, obesity, and red hair [7].

The melanocortin receptors, a distinct family of G protein coupled receptors

The effects of melanocortins are mediated by activation of a family of melanocortin receptors (MCRs). The cloning of the *MCR* genes has led to a tremendous progress in understanding the biological effects of melanocortins. So far, five *MCR* (*MC1R*, *MC2R*, *MC3R*, *MC4R*, *and MC5R*) genes have been cloned and the receptors pharmacologically characterized [16–22]. Each receptor is the product of a separate gene, and collectively they represent a distinct family of G protein-coupled receptors with seven transmembrane domains. They are the smallest G protein-coupled receptors, with short amino-terminal and carboxyl-terminal ends, and a very small second extracellular loop. All five forms of MCR are functionally coupled to adenylate cyclase and mediate their effects primarily by activating the cAMP-dependent signaling pathway. The five human MCRs share significant amino acid identity: 60% identity between the MC4R and MC5R, about 45% identity between MC3R and MC1R and between MC3R and MC2R, and 38% identity between MC2R and MC4R. Activation of the MCR by binding of their respective agonists stimulates cAMP formation. The five MCRs differ from each other in their tissue distribution and in their binding affinity for the various melanocortins and their antagonists, agouti signaling protein (ASP) and agouti-related protein (AGRP).

The melanocortin 1 receptor(s)

The first member of the *MCR* gene family to be cloned was *MC1R*, from a mouse melanoma cell line and from a primary culture of normal human melanocytes by Mountjoy et al. [16], and from human melanoma cells by Chhajlani and Wikberg [17]. MCR1 is the receptor for α-MSH on melanocytes. Among the five MCRs so far identified, MC1R has the highest affinity for α-MSH [23]. Human MC1R has highest and almost equal affinity for α-MSH and ACTH, less affinity for β-MSH, and least affinity for γ-MSH [17, 24]. This correlates with the relative potency of those hormones in stimulating melanogenesis in normal human melanocytes, and explains the lack of melanogenic activity of γ-MSH [24]. The human and mouse MCR share 76% identity in amino acid sequence [23]. Unlike human MC1R, mouse MC1R has a markedly lower affinity for ACTH than α-MSH. Additionally, activation of mouse MC1R requires a higher concentration of α-MSH than does human MC1R.

Regulation of integumental pigmentation is the most known effect of α-MSH. This hormone is the physiologic regulator of rapid color change in lower vertebrate species, including fish, amphibians, and reptiles, and a stimulator of melanogenesis in mammalian melanocytes [25, 26]. In mouse follicular melanocytes, α-MSH induces eumelanin synthesis [6]. In the recessive (*e/e*) yellow mouse, a genetic mutation that uncouples the receptor from adenylate cyclase results in a yellow coat color due to an inability to respond to α-MSH by stimulation of eumelanin synthesis [27, 28].

In humans, the physiological relevance of melanocortins in regulating pigmentation remained questionable despite the demonstration that injection of melanocortins increased cutaneous pigmentation of human subjects [29]. The finding that cultured human melanocytes respond to α-MSH or ACTH with increased melanogenesis, as determined by stimulation of the activity of tyrosinase (the rate-limiting enzyme of the melanin synthetic pathway) and proliferation, put an end to a long-standing controversy about the role of melanocortins in the regulation of human pigmentation [30–32]. Additionally, the observation that α-MSH and ACTH are synthesized in the skin suggested a paracrine, rather than an endocrine regulatory role for those peptides [33, 34]. Human melanocytes express a low number of MC1Rs [35]. The response to α-MSH or ACTH is sustained, sence MC1R does not undergo desensitization upon prolonged exposure of melanocytes to either hormone. This may be attributed to the ability of α-MSH or ACTH to increase MC1R mRNA levels in human melanocytes [24]. The observation that α-MSH and ACTH are equipotent on human melanocytes and that ACTH is produced at a higher level in the skin than α-MSH suggest a potentially significant role for ACTH in regulating human pigmentation [24, 36].

The human MC1R seems to play a similar role as its mouse counterpart, since treatment of human melanocytes with the potent α-MSH analog, [Nle^4, D-Phe^7]-α-MSH, increases the relative eumelanin to pheomelanin content [37]. Additionally, mutations that cause loss of function of the human receptor, namely Arg142His, Arg151Cys, Arg160Trp, and Asp294His substitutions, are strongly associated with a red-hair phenotype [38, 39]. The human *MC1R* is highly polymorphic, and many allelic variants of the gene have been identified in Northern European populations and in Australia, but not in African populations [38, 40, 41]. Some of these variants have a

Table 1. Sites of expression of the melanocortin receptors, their activation by agonists and inhibition by ASP or AGRP.

Type of melano cortin receptor	Major sites of expression	Agonists	Antagonists
MC1R	melanocytes; immune/inflammatory cells (*e.g.*, neutrophils, monocytes); endothelial cells; Sertoli cells	α-MSH = ACTH > β-MSH > γ-MSH	ASP
MC2R	zona fasciculata and zona glomerulosa; adipocytes	ACTH	
MC3R	hypothalamus, thalamus, hippocampus, anterior amygdala, cortex, placenta, stomach, duodenum, pancreas	α-MSH = β-MSH = γ-MSH = ACTH	AGRP
MC4R	cortex, thalamus, hypothalamus, brain stem	α-MSH = ACTH > β-MSH $\gg$ γ-MSH	AGRP, ASP
MC5R	exocrine glands, sebaceous glands, skeletal muscle adipocytes	α-MSH > ACTH = β-MSH $\gg$ γ-MSH	AGRP

high association with the red-hair phenotype, poor tanning ability, and melanoma. A recent study comparing control and melanoma patients in Australia concluded that expression of Arg151Cys, Arg160Trp, or Asp294His MC1R alleles increased the risk for melanoma more than twofold [42]. Expression of variants in the homozygous or compound heterozygous state is thought to be necessary but not always sufficient for the red-hair phenotype [40]. These findings point to the significance of the MC1R in determining constitutive pigmentation, the response of individuals to sun exposure and the risk for melanoma.

The melanogenic response to ultraviolet radiation (UV) is mediated to a large extent by melanocortins. This proposal was based on the findings that UV irradiation of mouse melanoma cells increased α-MSH-binding and the number of α-MSH binding sites [43]. Further evidence came from the observation that stimulation of tyrosinase activity in UV-irradiated human melanocytes is only achieved when the cAMP pathway, the main signaling pathway for melanocortins, is stimulated [44]. Among the known melanogenic factors, α-MSH and ACTH stimulate this signaling pathway [24]. Irradiation of human skin increases the synthesis and release of both melanocortins by epidermal cells, a mechanism that up regulates MC1R expression and maintains the responsiveness of melanocytes to those hormones [24, 34]. The levels of MC1R mRNA in human melanocytes are also increased by endothelin-1, a paracrine mitogen and melanogenic modulator for human melanocytes [45, 46]. Endothelin-1 production by keratinocytes is up-regulated by UV exposure [46]. These findings suggest the existence of a paracrine network that is stimulated by UV exposure, and increases MC1R expression to mediate the UV-induced melanogenic response. Thus, the ability of human melanocytes to respond to melanocortins is crucial for their response to UV. Stimulation of the synthesis of eumelanin is thought to be photoprotective, since eumelanin quenches reactive oxygen species that are generated upon exposure to UV [47]. Additionally, eumelanin-containing melanosomes form supranuclear caps in keratinocytes, thus limiting the amount of UV rays that can reach nuclear DNA and generate DNA damage [48]. Recently, α-MSH itself was reported to have antioxidant effects, as shown by its ability to limit the generation of H_2O_2 in UV-irradiated keratinocytes and melanoma cells [49].

Recently, a new human MC1R isoform generated by alternative mRNA splicing was characterized [50]. It differs from the originally described MC1R in containing an additional 65 amino acids at its C-terminal end, and thus is 382 amino acids in length. It is similar to the original MC1R in its binding affinity for the various melanocortins, and is mainly expressed in melanoma cells.

A subtype of MC1R has been identified in inflammatory cells, such as monocytes and neutrophils, and in brain tissues, which may mediate the peripheral and central antiinflammatory effects of α-MSH [51, 52]. Human dermal microvascular endothelial cells also express a form of MC1R, and are a target as well as a source of POMC [53]. α-MSH and its carboxy terminus tripeptide sequence, α-MSH (11–13), have potent antiinflammatory properties [54]. The immunomodulatory and antiinflammatory effects of α-MSH are mainly due to its capacity to alter the function of antigen-presenting and endothelial cells [52]. Human dermal microvascular endothelial cells respond to α-MSH by significantly increasing the synthesis and release of interleukin-8 and Gro-α [53]. Human microvascular endothelial cells exhibit a reduction in lipopolysaccharide-induced expression of the adhesion molecules E-selectin and VCAM-1 after treatment with α-MSH [55].

α-MSH reduces acute inflammation induced by peripheral administration of inflammatory stimuli, delayed-type hypersensitivity, chronic inflammatory responses, and systemic inflammation. It inhibits neutrophil migration, an early event in inflammation, reduces macrophage activity, and inhibits the synthesis of nitric oxide by both neutrophils and macrophages [10]. It also inhibits the production of various cytokines, such as interferon-, tumor necrosis factor-α, interleukin-1, interleukin-6, and

monocyte chemotactic protein 1. α-Melanotropin stimulates the synthesis of interleukin-10, and reduces the expression of the co-stimulatory molecule B7-2 by monocytes [10]. The mechanism for the antiinflammatory effects of α-MSH involves inhibition of NF-κB activation by lipopolysaccharides and inflammatory cytokines, such as tumor necrosis factor-α [55]. An interesting negative feedback loop seems to be generated by pro-inflammatory stimuli. This is supported by the findings that MC1R expression on monocytes is up-regulated by endotoxin or mitogen treatment, and that tumor necrosis factor-α increases the synthesis of α-MSH by macrophages [10]. Both events contribute to the antiinflammatory effects of α-MSH. Furthermore, UV irradiation of skin induces the synthesis and release of melanocortins, which contribute to the UV-induced immunosuppression [52].

The receptors that mediate the antiinflammatory effects of α-MSH differ drastically in their pharmacologic properties from the MC1R expressed on melanocytes. First, the melanocortin receptors on immune and endothelial cells are activated by picomolar concentrations of α-MSH, unlike the melanocyte MC1R that requires nanomolar concentrations [24, 53, 55]. Second, the pentapeptide Glu-His-Phe-Arg-Trp sequence of α-MSH is required for activation of the MC1R on melanocytes and for inducing the melanogenic response, while the carboxy-terminal tripeptide sequence of α-MSH is required for the antiinflammatory effects of the hormone [54]. These differences suggest that immune cells express a receptor that is distinct from the MC1R that was originally identified on melanocytes.

MC1R is also the receptor for ASP, the product of the *agouti* gene [56, 57]. ASP is the physiological antagonist of α-MSH. In mouse hair follicles, temporal expression of ASP in dermal papilla cells results in an abrupt switch from eumelanin synthesis (due to the influence of α-MSH) to pheomelanin synthesis by melanocytes. In human melanocytes, ASP acts as a competitive inhibitor of α-MSH binding to MC1R, and thus abrogates the stimulatory effects of α-MSH on melanogenesis and proliferation [58]. MC1R is the predominant, and possibly the only, receptor for ASP. B16 mouse melanoma cells that lack the expression of MC1R fail to respond to ASP [59]. Furthermore, mouse melanocytes cultured from the skin of *e/e* mice with a deficient MC1R that is uncoupled from adenylate cyclase are refractory to ASP (Z. A. Abdel-Malek et al. unpublished data). A role for ASP in the control of inflammation has been proposed. A^y mice, characterized by ectopic expression of ASP, have a stronger acute inflammatory response than wild-type mice [60]. Hypersecretion of ASP promotes interleukin-6 production following injection of lipopolysaccharides, possibly by counteracting the endogenous effects of α-MSH. Since the agouti phenotype does not exist in humans, the exact role of ASP in human pigmentation and its physiological significance remain speculative.

The melanocortin 2 receptor

Shortly after the cloning of *MC1R*, *MC2R* was cloned from the adrenal gland [16, 18]. MC2R is the ACTH receptor, and is mainly expressed in the zona fasciculata, the site of glucocorticoid production, and in the zona glomerulosa, the site of mineralocorticoid production in the adrenal cortex [16]. MC2R is expressed in adipocytes of various mammals, and mediates most of the lipolytic activity of ACTH [61, 62]. Other melanocortins, mainly α-MSH, and β-lipotropic hormone have lipolytic activities. The relative lipolytic activities of ACTH and α-MSH differ drastically among species. For example, hamster adipocytes respond exclusively to ACTH, while guinea pig adipocytes are responsive to both ACTH and α-MSH [63]. In humans and other primates, however, melanocortins do not appear to regulate adipocyte function, and it is not known if human adipocytes express any melanocortin receptor [64].

MC2R differs from the remaining four MCRs in its specificity for ACTH. Binding of ^{125}I-ACTH to MC2R can only be competed by cold ACTH, but not by α-, β-, or γ-MSH. Exposure to ACTH results in up-regulation of MC2R mRNA and an increase in receptor number [65]. MC2R is similar to MC1R in that its expression is up-regulated by its ligand ACTH [24]. Pretreatment of bovine adrenal fasciculata cells with ACTH results in increased ACTH binding due to a significant increase in the number of ACTH receptors, with no change in the binding affinity [66]. The increase in the number of MC2Rs is due to up-regulation of MC2R-specific mRNA by ACTH treatment [65]. This effect is cAMP dependent, since it can be induced by forskolin or dibutyryl cAMP. In contrast, treatment of bovine adrenocortical cells with transforming growth factor-β diminishes MC2R expression and reduces ACTH binding [67].

The melanocortin 3 receptor

The third MCR gene to be cloned was *MC3R*. Human MC3R is 361 amino acids in length, and is the receptor for α-MSH [19, 21]. It differs from the other MCRs in that it has no specificity for any of the melanocortins, and shows similar binding affinity for α-, β-, and γ-MSH, and ACTH. MC3R is highly expressed in the hypothalamus, thalamus, hippocampus, anterior amygdala, and cortex. Its distribution sites suggest a role in regulating cardiovascular functions and thermoregulation, as well as in control of feeding behavior [68]. MC3R is also expressed in the placenta, stomach, duodenum, and pancreas, and is detectable in the testis, ovary, mammary gland, skeletal muscle, and kidney [19, 69].

MC3R is unique in being the only MCR that is activated by γ-MSH to the same extent as by any other melanocortin. γ-MSH has opposing effects on cardiovascular functions depending on its route of administration. Centrally

administered γ-MSH reduces blood pressure and heart rate [70]. This effect can be blocked by SHU 9119, the antagonist for MC3R and MC4R. Injection of γ-MSH into the renal artery induces natriuresis, i. e., increased sodium secretion, an effect that seems to be mediated by activation of MC3R.

The melanocortin 4 receptor

Human *MC4R* was the second neural MCR to be cloned [20]. It is 333 amino acids in length. It is widely expressed in the central nervous system, including the cortex, thalamus, hypothalamus and brain stem. The distribution of the receptor suggests its involvement in autonomic and neuroendocrine functions. Pharmacologic characterization of the MC4R expressed in heterologous cells indicated that it is very similar to the MC1R in its relative affinities for the various melanocortins. The order of potency for activation of the receptor by the physiologic melanocortins is α-MSH = ACTH > β-MSH ≫ γ-MSH. A property that distinguishes MC4R from MC3R is that α-MSH is 100-fold more potent than γ-MSH in activating MC4R, but the two hormones are equipotent in activating MC3R. However, γ-MSH still acts as a full agonist for MC4R, and if present at sufficiently high concentrations, can serve as a true ligand for this receptor.

Dominant mutations in the mouse *agouti* gene that cause its ectopic over-expression result in a mouse that is yellow in color, obese, and with increased longitudinal growth [71]. The yellow coat color is attributed to the ability of ASP to act as a competitive inhibitor of α-MSH binding to MC1R. However, the obesity phenotype remained an enigma until recently, when it was recognized that ASP binds to a neural MCR, MC4R, resulting in hyperphagia. As with MC1R, ASP acts as an antagonist of MC4R by competitively inhibiting the binding of α-MSH [72]. Direct evidence for MC4R as a regulator of food intake came from the observation that MC4R knockout mice are obese [73]. In humans, missense mutations in *MC4R* have been identified in obese adolescents [74]. One missense mutation, Ile137Thr, was identified in an obese adult and found to reduce significantly the binding of NDP-α-MSH, a potent analog of α-MSH, to MC4R [75]. Identification and pharmacological characterization of *MC4R* mutations is an area of intense investigation that should help determine the significance of MC4R in human obesity.

ASP is normally synthesized in dermal papilla cells within the mouse hair follicle [71]. Thus, under normal physiological conditions, ASP antagonizes MC1R, not MC4R. Only when ASP is expressed ectopically and in abnormally high concentrations does it act as an antagonist of MC4R. Therefore, ASP is not considered a regulator of food intake under normal physiological conditions. Alternatively, AGRP is thought to be the physiologic antagonist for the neural MC3R and MC4R [76, 77]. In humans, AGRP is expressed primarily in the adrenal gland, subthalamic nucleus, and hypothalamus [76]. It is expressed at low levels in testis, lung, and kidney. The levels of AGRP are increased significantly in two strains of obese mice, *ob*/*ob* and *db*/*db* [78]. Transgenic mice ubiquitously expressing AGRP are obese, but show no alteration in coat color. The interaction of AGRP with the MCRs was examined by comparing its ability to block α-MSH-induced stimulation of cAMP formation in cells transfected with each of the five human MCR subtypes [76]. No effect of AGRP was detected on MC1R or MC2R. A slight inhibitory effect was found on MC5R, and a potent dose-dependent inhibition of α-MSH action was observed on MC3R and MC4R. The ability to be antagonized by ASP and/or AGRP is a criterion that distinguishes the five different MCR subtypes. Overexpression of AGRP in genetically obese mice and its specific and potent antagonism of MC3R and MC4R suggest that it is a physiological regulator of feeding behavior. MC4R differs from the other MCRs in its ability to bind both antagonists, ASP and AGRP. MC3R binds AGRP but not ASP, while MC1R binds only ASP. AGRP has limited antagonism for MC5R, and does not antagonize MC2R.

The melanocortin 5 receptor

The *MC5R* gene was the last of the melanocortin receptor gene family to be cloned [22]. This receptor has the most sequence homology to MC4R and the least homology to MC2R. It is similar to MC1R and MC4R in its ability to respond to all melanocortins, except γ-MSH. The order of potency of the melanocortins in activating MC5R is α-MSH > ACTH = β-MSH ≫ γ-MSH. MC5R mRNA is expressed at high levels in the exocrine glands, such as lacrimal and Harderian glands [79]. It is also expressed in skin tissues, particularly in sebaceous glands, and in skeletal muscles. Expression of MC5R mRNA has been detected in mouse adipocytes but at a lower level than MC2R mRNA. The functions of MC5R are still not very well understood; they are speculated to include neuro/myotropic, gastric, and antiinflammatory effects, and regulation of aldosterone secretion [22, 80–82]. MC5R may mediate the weak lipolytic activity of α-MSH on the adipocytes of several rodent species [83]. The best-described functions of MC5R are regulation of hair lipid production, water repulsion, and thermal regulation [79, 84]. Evidence for these functions comes from the observation that MC5R knockout mice demonstrate impaired water repulsion and a reduced core body temperature after swimming. MC5R is the mediator of the sebotropic activity of α-MSH [12]. A role of MC5R in the stress response is suggested by its ability to regulate protein secretion by lacrimal glands and to stimulate tear secretion [85]. MC5R is a prime candidate for mediating the

secretion of stress-induced alarm substances, or stress pheromones [84].

Conclusions

The melanocortins are bioactive peptides that are widely expressed in the central nervous system and in various peripheral tissues where they regulate numerous physiologic functions. The specificity of the effects of melanocortins is determined mainly by the differential processing of POMC which determines the availability of the various melanocortins, expression of the different forms of MCR, as well as by the ability of the various MCRs to bind their respective physiologic agonists and antagonists. Melanocortins do not necessarily function as endocrine effectors. In many instances, they elicit their biological effects by acting locally as paracrine or autocrine factors. For example, in the testis, Leydig cells synthesize POMC-derived peptides and Sertoli cells respond to ACTH and α-MSH in particular by stimulation of aromatase activity and inhibition of plasminogen activator activity [13, 14]. Those effects seem to be mediated by activation of MC1R and MC2R on Sertoli cells [69]. Similarly, in the skin, various POMC peptides are synthesized by melanocytes, keratinocytes, and Langerhans cells [33, 34]. The synthesis of these peptides is up-regulated by UV exposure, and in turn, they mediate the tanning and immunosuppressive effects of UV [10, 34, 44, 52]. Another site that seems to be regulated in a paracrine fashion by melanocortins is the hair follicle. The melanocortins ACTH and α-MSH are produced within the hair follicle and interact with their cognate receptors to preserve the anagen hair bulb as an immune-privileged site, and to contribute to the development of the follicle pigmentary unit [86].

The cloning and characterization of the MCRs have provided solutions to old dilemmas. The identification of many allelic variants of MC1R has provided a genetic basis for the red-hair phenotype and poor tanning ability and confirmed the role of melanocortins as regulators of human pigmentation [38, 87]. In addition characterization of MC4R and its ability to bind ASP offered an explanation for the obesity observed in mice with mutations in the *agouti* gene that cause its ectopic and over-expression [71, 72]. Association of certain mutations in the MC4R gene with obesity in humans suggests that this receptor is significant for regulating food intake in human beings [74, 75]. Identification of MC5R explained the sebotrophic effect of α-MSH [12, 79]. Future challenges lie ahead in elucidating fully the role of MCRs, their physiologic agonists and antagonists in human health as well as in disease processes.

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