

The melanocortin system

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Gantz, Ira, and Tung M. Fong. The melanocortin system. *Am J Physiol Endocrinol Metab* 284: E468–E474, 2003; 10.1152/ajpendo.00434.2002.—The melanocortin system consists of melanocortin peptides derived from the proopiomelanocortin gene, five melanocortin receptors, two endogenous antagonists, and two ancillary proteins. This review provides an abbreviated account of the basic biochemistry, pharmacology, and physiology of the melanocortin system and highlights progress made in four areas. In particular, recent pharmacological and genetic studies have affirmed the role of melanocortins in pigmentation, inflammation, energy homeostasis, and sexual function. Development of selective agonists and antagonists is expected to further facilitate the investigation of these complex physiological functions and provide an experimental basis for new pharmacotherapies.

inflammation; obesity; pigmentation; sexual function; receptor

THE MELANOCORTIN SYSTEM consists of 1) the melanocortin peptides α -, β -, and γ -melanocyte-stimulating hormone (α -, β -, γ -MSH) and adrenocorticotrophic hormone (ACTH), 2) a family of five seven-transmembrane G protein-coupled melanocortin receptors, and 3) the endogenous melanocortin antagonists agouti and agouti-related protein (AGRP). In addition, two ancillary proteins, mahogany and syndecan-3, have been found that modulate the activity of the melanocortin peptides (melanocortins). This minireview is meant to introduce the melanocortin system to the unacquainted reader.

The melanocortins are involved in an extraordinarily diverse number of physiological functions, including pigmentation, steroidogenesis, energy homeostasis, exocrine secretion, sexual function, analgesia, inflammation, immunomodulation, temperature control, cardiovascular regulation, and neuromuscular regeneration. On the basis of their prominent regulatory role in many of these functions, the development of melanocortin-based drugs is currently being considered, or is presently in the developmental phase, for the treatment of skin cancer and other cutaneous disorders, obesity, anorexia nervosa and cachexia, erectile dysfunction, inflammatory diseases, pain, and nerve injury. The physiological basis for considering melano-

cortins as central participants in some of the aforementioned processes will be discussed.

The first portion of this minireview will present, largely in isolation, the various elements of the melanocortin system. These elements will then be discussed in the context of four physiological functions: pigmentation, inflammation, energy homeostasis, and sexual behavior. With the use of this approach, however, there is an inevitable overlap of organization.

COMPONENTS OF THE MELANOCORTIN SYSTEM

Proopiomelanocortin Prohormone

The melanocortins are posttranslational products of the proopiomelanocortin (POMC) prohormone. This prohormone also gives rise to the opiate peptide β -endorphin, hence the name pro-opio-melanocortin. Among the peptide products of that prohormone, the melanocortins are unified by the fact they contain the amino acid sequence His-Phe-Arg-Trp, which is a key pharmacophore that is necessary for the biological activity of these peptides. Posttranslational processing of the POMC prohormone is tissue specific (35). This results in the production of different POMC peptides by different cell types and, therefore, provides latitude for the control of multiple physiological functions by the same prohormone. Processing is performed at dibasic cleavage sites by the prohormone convertases PC1 and PC2. Carboxypeptidases and aminopepti-

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dases subsequently remove dibasic residues, and enzymatic modifications such as *N*- α -acetylation and COOH-terminal amidation may occur. The pharmacological significance of these changes is evidenced by the diminished potency of desacetyl α -MSH compared with acetylated α -MSH. Rare mutations in the POMC hormone and PC1 have been found in humans and are associated with adrenal insufficiency and early-onset obesity and, in the case of POMC mutations, altered pigmentation (22, 26).

The POMC gene is expressed primarily in the central nervous system (CNS), where it is expressed in the pituitary, arcuate nucleus of the hypothalamus, and nucleus of the solitary tract in the brain stem. The POMC gene is also expressed by cutaneous keratinocytes and melanocytes. In addition, POMC mRNA and immunoreactivity have been reported in a number of peripheral human tissues, including genitourinary tract, gastrointestinal tract, adrenal, spleen, lung, and thyroid and in cells of the immune system (52).

Melanocortin Receptors

There are five G protein-coupled melanocortin receptors (MCRs), which are all linked to cAMP generation via the stimulatory G protein G_s and adenylate cyclase. However, MCR signaling has also been associated with increases in intracellular Ca^{2+} concentration secondary to activation of inositol trisphosphate (25), influx of extracellular Ca^{2+} (24), and activation of the MAP kinase (15), janus kinase/signal transducer and activator of transcription (7), and PKC pathways (23). Importantly, the five MCRs have differing affinities for the melanocortins and the endogenous antagonists agouti and AGRP (Table 1).

MC1R is the "classical" melanocyte α -MSH receptor. It is expressed by cutaneous melanocytes, where it has a key role in determining skin and hair pigmentation. However, other cell types in the skin also express MC1R, including keratinocytes, fibroblasts, endothelial cells, and antigen-presenting cells (31). Other tissues and cell types have also been found to express MC1R (9). In this respect, it is notable that MC1R is expressed by leukocytes, where it mediates the anti-inflammatory and immunomodulatory properties of melanocortins.

MC2R is the classical adrenocortical ACTH receptor. It is expressed in the adrenal cortex zona reticularis and zona fasciculata, where it mediates the effects of ACTH on steroid secretion. Notably, it is distinguished

pharmacologically from the other MCR subtypes in that it is activated only by ACTH and has no affinity for α -, β -, or γ -MSH (Table 1). A rare human autosomal recessive disorder, hereditary isolated glucocorticoid deficiency, is caused by mutations in MC2R (44). Attention has been paid to the fact that MC2R is also expressed by adipose tissue in mice and humans (52). Although ACTH is lypolytic in mice, it is not so in humans, and the function of MC2R in human adipose tissue is presently unclear.

MC3R is expressed in many areas of the CNS and in several peripheral tissues, including the gastrointestinal tract and placenta (9). All of the melanocortins are roughly equipotent at MC3R (Table 1). Notably, among the MCR subtypes, γ -MSH has its greatest affinity at MC3R, an observation that is assumed to be of physiological significance. Of importance, MC3R is involved in energy homeostasis (see *The Melanocortin System and Energy Homeostasis*).

MC4R is expressed predominantly in the CNS. As is the case with MC3R, it is involved in energy homeostasis. More recently, MC4R has been shown to be involved in sexual function (see *Melanocortins and Sexual Function*).

MC5R is expressed in numerous human peripheral tissues, including adrenal gland, adipocytes, leukocytes, and many others (9). It also has a very limited distribution in the CNS. The only firmly established function of MC5R, which was discovered by targeted deletion of that receptor, is its participation in exocrine function, particularly sebaceous gland secretion (11). Although the role of melanocortins in sebaceous gland function had been reported some 20 years earlier (42), their role in that process received little attention until this recent discovery. The role of MC5R in exocrine secretion has the potential to be exploited for the treatment of skin disorders such as acne and dermatitis.

Endogenous Antagonists

Perhaps one of the most interesting aspects of the melanocortin system is that it has two endogenous antagonists, agouti and AGRP. These proteins are unique in that no inhibitory proteins have been identified for any of the seven-transmembrane receptor family. Agouti and AGRP are paracrine signaling molecules, which are endogenous antagonists of the MCRs (13). Of physiological significance, agouti and AGRP have MCR subtype selectivity (Table 1). Interestingly, agouti and AGRP both have a cysteine-rich COOH-

Table 1. *The melanocortin system*

	Potency of Ligands	Antagonists	Primary Functions
MC1R	α -MSH = ACTH > β -MSH > γ -MSH	Agouti	Pigmentation, inflammation
MC2R	ACTH	Agouti	Steroidogenesis
MC3R	α -MSH = β -MSH = γ -MSH = ACTH	Agouti, AGRP	Energy homeostasis
MC4R	α -MSH = ACTH > β -MSH > γ -MSH	Agouti, AGRP	Energy homeostasis, erectile function
MC5R	α -MSH > ACTH > β -MSH > γ -MSH		Sebaceous gland secretion

MC1R, melanocortin receptor-1; MSH, melanocyte-stimulating hormone; ACTH, adrenocorticotrophic hormone; AGRP, agouti-related protein.

terminal domain. Although the structure of agouti has not been resolved, nuclear magnetic resonance studies demonstrate that the cysteine residues in AGRP adopt a structural motif called an inhibitor cystine knot (32). This motif is common to invertebrate toxins, but in mammals this structure is unique to AGRP and, presumably, agouti. Another commonality is that agouti and AGRP have both been shown *in vitro* to be inverse agonists (33). Thus they have the potential *in vivo* to regulate their respective MCRs, even in the absence of melanocortins.

The term agouti refers to a hair color pattern commonly seen in mammals, which is characterized by a subapical yellow band on an otherwise black or brown background. Historically, scientific interest in the *agouti* locus extended beyond its effect on coat color. Dominant mutations of the *agouti* gene cause mice to develop yellow fur, obesity, insulin resistance, increased somatic growth, and a predisposition to tumorigenesis. With the isolation of the gene encoding agouti, it was noted that these pleiotropic effects were associated with a deregulated expression of agouti in all tissues (6). Subsequent investigations have demonstrated that the obesity displayed by these mutant mice is secondary to the ectopic expression of agouti in the hypothalamus, where it acts as an antagonist of α -MSH at MC4R (30). In light of recent discoveries that hypothalamic α -MSH is a major satiety factor that transmits its message by activating MC4R, the hyperphagia and resultant obesity of those animals are readily understood.

The normal role of agouti, however, is to act in conjunction with α -MSH and MC1R to determine mammalian coat color. Agouti is produced by the dermal papillae cell and acts on the adjacent melanocyte to block melanocortin action at MC1R. This interaction has a major effect on pigmentation (see *Melanocortins and Pigmentation*). Pharmacologically, agouti is a high-affinity, competitive antagonist of the melanocortin peptides at MC1R and MC4R. In rodents, agouti is expressed only in skin. The human homolog of agouti, called agouti-signaling protein (ASP), has a wider pattern of expression, including adipose tissue, testis, ovary, and heart and lower levels of expression in foreskin, kidney, and liver (54). However, humans do not have a banded agouti-like hair pattern, and the role of ASP in hair and skin pigmentation in humans is doubtful. At the present time, the physiological function(s) of ASP in humans is unknown.

Subsequent to the discovery of agouti, AGRP was identified by database searches for molecules with homology to agouti (34). AGRP is a competitive antagonist of MC3R and MC4R that is equipotent at both of those receptors. AGRP has little activity at the other MCRs. AGRP is expressed primarily in the arcuate nucleus of the hypothalamus, the subthalamic region, and the adrenal cortex, with a small amount of expression observed in the lung and kidney. However, its major physiological function is in the hypothalamus, where AGRP acts as a potent orexigenic (appetite-stimulating) factor due to its ability to antagonize

melanocortins at MC3R and MC4R. Very low levels of circulating AGRP have been found in both rat and human (40). An interesting question that remains to be answered is the physiological role of AGRP in the adrenal. The adrenal is the tissue with the second highest concentration of AGRP. However, human and rat adrenals have been reported to express only the MC2R and MC5R receptors with no affinity for AGRP, and the adrenal is apparently not the origin of blood-borne AGRP in rats, since adrenalectomy does not affect blood levels.

Ancillary Proteins

Mahogany and syndecan-3 are proteins that modulate the activity of agouti and AGRP, respectively. Although both have convincingly been shown to interact with agouti and AGRP, important questions remain to be answered about those interactions.

Mahogany is a single-pass transmembrane protein that is expressed primarily in brain, including the hypothalamus, and skin (18). It is clear that mahogany is involved in mammalian coat coloration. In mice, there is an absolute requirement for functional mahogany protein for the action of agouti. The *mahogany* mutation completely suppresses the obesity and yellow hair coloration of dominant *agouti* mutations. Mahogany has been shown to be a low-affinity receptor for agouti but not AGRP (19). However, it is difficult to reconcile the dramatic effect that *mahogany* mutations have on dominant *agouti* mutations simply in terms of the loss of a low-affinity receptor. In addition, *mahogany* appears to have effects on metabolic rate independent of its suppression of *agouti* mutations (14). Therefore, it would seem that the convergence of mahogany with the melanocortin pathway is still incompletely understood.

Syndecan-3 is a heparan sulfate proteoglycan, a class of single-pass transmembrane molecules whose ectodomain is shed from the cell surface in response to defined stimuli. Importantly, syndecans are molecules that bind extracellular ligands. Awareness of the involvement of syndecan-3 with the melanocortin system arose from the observation that transgenic mice that overexpress the related molecule syndecan-1 display obesity similar to that of transgenic mice that overexpress AGRP or mice with dominant *agouti* mutants (37). It was hypothesized that misexpression of syndecan-1 in the hypothalamus mimicked a physiological modulator of feeding behavior. Because syndecan-1 is not normally found in the hypothalamus, attention was drawn to syndecan-3, which is. Indeed, syndecan-3 has been shown in pharmacological assays to augment AGRP antagonism of α -MSH at MC4R. The data suggest that syndecan-3 might act as an AGRP coreceptor. The affinity of this interaction is presently unknown. However, syndecan-3-null mice do not have a phenotype, and the only feeding abnormality that they display is decreased reflex hyperphagia after fasting. This raises some question about the importance of the syndecan-3-AGRP interaction, although compensatory

mechanisms could certainly be called into account. Nonetheless, it is noteworthy that food deprivation increases hypothalamic syndecan-3 more than four-fold. According to one model, in the food-deprived state, syndecan-3 is upregulated on the surface of hypothalamic neurons expressing MC3R and MC4R. This would increase local concentrations of AGRP and promote an orexigenic state. In the fed state, the ectodomain of syndecan-3 is shed and the local concentrations of AGRP fall. This allows increased activity of α -MSH at MC3R and MC4R and promotes a sated state. Of course, regulation of AGRP release occurs independently in those states, and the relative contribution of syndecan-3 to AGRP function is presently unknown.

SELECTED FUNCTIONS

The four functions of the melanocortins that are perhaps most heavily studied at the present time are their role in pigmentation, inflammation, energy homeostasis, and sexual function. These functions are briefly discussed below.

Melanocortins and Pigmentation

In mammals, skin, coat, and hair color are determined by the relative ratio of pheomelanin (yellow/red pigment) to eumelanin (brown/black pigment) produced by the melanocyte. In fur-bearing mammals, both MC1R and agouti affect this ratio. Activation of MC1R by α -MSH stimulates eumelanin synthesis. Conversely, antagonism of α -MSH action by agouti favors pheomelanin synthesis. Expression of *agouti* is temporally and spatially regulated (49). Temporal expression of *agouti* accounts for the agouti banding pattern; spatial regulation accounts for the differences in dorsal and ventral coat color seen in some mammals.

Mutations of MC1R also have profound effects on pigmentation. Both gain-of-function and loss-of-function mutations of MC1R have been shown to alter pigmentation in a range of species (38). MC1R is also highly polymorphic in humans (39). Certain allelic variants of the gene in humans are associated with red hair and pale skin (46). Although human pigmentation is genetically complex, to date only polymorphism at MC1R has been associated with phenotypic changes. The relationship of MC1R variants to melanoma and nonmelanoma skin cancer has been the subject of controversy.

In humans, α -MSH and ACTH produced locally in the skin have a major role in pigmentation (41). The production of both peptides is upregulated in the keratinocyte by UV radiation, and they act as paracrine factors that stimulate the melanocyte to produce eumelanin. α -MSH is also produced by the melanocyte and may act as an autocrine factor that affects eumelanin synthesis and melanocyte morphology and as a paracrine factor that protects the melanocyte against immune system damage. MC1R has also been reported to be upregulated by UV radiation. The contribution of centrally produced α -MSH, which circulates at an ex-

remely low level in humans, and serum ACTH to pigmentation in humans in nonpathological states has yet to be determined.

Melanocortins and Inflammation

The melanocortins have significant anti-inflammatory properties (8, 31). The administration of α -MSH or its COOH-terminal tripeptide Lys-Pro-Val (α -MSH-11–13) has been shown to inhibit the production or action of proinflammatory factors (nitric oxide, IL-1, IL-6, TNF- α , INF γ , monocyte chemoattractant protein-1), upregulate the production of immunosuppressive IL-10, and downregulate endothelial adhesion molecules. In the models in which it has been studied, these effects involve modulation of the transcription factor NF- κ B. α -MSH may also be secreted by cells involved in the inflammatory and immune response and presumably acts as an autocrine and paracrine factor. The anti-inflammatory effects of α -MSH have been extensively studied in UV-induced cutaneous inflammation (31). Many cells involved in the anti-inflammatory and immunomodulatory actions of melanocortins express MC1R. Of note, the tripeptide Lys-Pro-Val lacks the melanocortin pharmacophore His-Phe-Arg-Trp, and studies examining the affinity of Lys-Pro-Val at the known MCRs have not been published.

The Melanocortin System and Energy Homeostasis

Although earlier publications had firmly implicated melanocortins in the inhibition of food intake on the basis of the observation that injection of ACTH (1–24) into the lateral ventricle or ventromedial hypothalamic nucleus inhibited food intake in rats (48) and that POMC mRNA levels were regulated by metabolic state (3), it was not until 1994 that researchers took greater notice of the melanocortin system as a mediator of feeding behavior. By that time, the MCRs had been cloned, and it was known that MC3R and MC4R were expressed in the hypothalamus, a CNS region that controls many physiological functions, including feeding behavior. Importantly, that year it was discovered that agouti was a potent antagonist of MC4R (30). It was hypothesized that the obesity of mice with dominant mutations of the *agouti* gene was due to overexpression of agouti in the hypothalamus and its antagonism of MC4R. Several publications in 1997 (16, 21, 34) solidified these observations into a coherent framework. First, it was demonstrated that the newly developed MC4R antagonist SHU-9119 could block the inhibition of food intake induced by the nonspecific melanocortin agonist MT-II (16). Second, it was reported that targeted deletion of MC4R resulted in obesity associated with hyperphagia (21). Finally, the endogenous agouti-like orexigenic factor AGRP was discovered (34).

These observations set the stage for a multitude of studies that have continued up to the present, which have established the hypothalamic melanocortin system (MC4R, POMC peptides, and AGRP) as one of the convergence points for peripheral and central factors

that regulate feeding behavior and metabolism. Although α -MSH is presumed to be the most relevant melanocortin involved in energy regulation within the hypothalamus, POMC neurons probably release a complex soup of POMC peptides (35). More recently, it has been demonstrated that MC3R is also involved in energy homeostasis. MC3R-null mice have a loss of lean body mass and an increase in subcutaneous fat while maintaining a relatively normal body weight (10).

Notably, the aforementioned observations extend to humans. It has been estimated that MC4R mutations occur in 4% of severely obese French individuals (45). Not only is the hypothalamic melanocortin system involved in obesity, it has also been implicated in cachexia (27) and anorexia (5) in rodents.

Whole animal, neuroanatomical, and electrophysiological studies continue to confirm the importance of the melanocortin system in feeding behavior and metabolism. POMC-containing neurons have been shown to be the site of convergence of a variety of peripheral and central hormones, neurotransmitters, and nutrients involved in feeding behavior. By use of an electrophysiological slice preparation, it has been shown that the activity of POMC neurons can be affected either directly or indirectly by leptin, insulin, glucose, ghrelin, peptide YY, neuropeptide Y, β -endorphin, serotonin, GABA, melanin-concentrating hormone, and orexins (12, 17, 20). In turn, projections of POMC and AGRP neurons project to other hypothalamic centers that modulate feeding and metabolism (4). In this respect, it is notable that the dopaminergic system, which has been implicated in both energy homeostasis and sexual function (see *Melanocortins and Sexual Function*), is modulated by melanocortin receptor agonists (28, 29).

Although the aforesaid describes a hypothalamic centric melanocortin feeding model, the hindbrain is also an important site of melanocortin action (53). POMC peptides and MC3R and MC4R are expressed in the hindbrain, and it has been shown that subnanomolar concentrations of MT-II or SHU-9119 administered into that region have effects on feeding behavior similar to those observed in the hypothalamus.

Although in vivo experimental evidence indicates the importance of AGRP in energy homeostasis, recently it was shown that AGRP-null mice have no phenotype and display normal feeding behavior (36). The majority of obesity researchers at the present time think that this observation is due to compensatory mechanisms and that it highlights the redundancy of orexigenic pathways.

The central role of the melanocortin system in feeding behavior has made it an attractive target for the development of antiobesity agents. This is particularly true for MC4R.

Melanocortins and Sexual Function

The involvement of the melanocortin system in sexual function has been known since the 1960s, when it was observed that injection of ACTH or α -MSH intra-

cerebroventricularly in laboratory animals caused penile erection and ejaculation (Ref. 1 and references therein). More recently, it has been shown that microinjection of α -MSH and ACTH into discrete periventricular nuclei surrounding the third ventricle induces penile erection in rats (2). The MCR(s) involved in the central effects of melanocortin-mediated sexual function has not as yet been conclusively defined.

Importantly, in small (10 subjects), double-blind, placebo-controlled crossover studies, subcutaneous administration of the nonselective MCR agonist MT-II evoked spontaneous penile erections in men with either psychogenic or organic erectile dysfunction (50, 51). The percentage of responders who had erections of sufficient rigidity for sexual intercourse (as determined by penile tumescence monitoring and patient self-reporting) was 94% (psychogenic) and 70% (organic), although subjects did not necessarily respond to both of the injections administered.

α -MSH has been reported to influence female sexual behavior in rats (43). However, its influence on female sexual behavior is less clear, because, depending on receptivity levels, ACTH-MSH peptides increased or decreased sexual behavior. There is presently no information on the effects of melanocortins on human female sexual response.

Recently, the role of MC4R in mediating the peripheral actions of melanocortin effects on erectile function and copulatory behavior in male rodents was elucidated in studies using a highly selective tetrahydroisoquinoline (THIQ) MC4R agonist and MC4R-null mice (47). THIQ was shown to augment electrically evoked intracavernosal pressure in mice, an effect that was absent in MC4R-null mice and independent of direct action on cavernosal smooth muscle. The efficacy of this effect is comparable to that of sildenafil (Viagra) in certain rodent models. MC4R-null mice were also found to have impaired copulatory behavior, although a breeding colony of these animals could be established. Of significance, it was demonstrated that MC4R mRNA was expressed in tissues that modulate erectile function, including the spinal cord and pelvic ganglion of rats and the penis of both rats and humans, providing an anatomical basis for melanocortin effects on sexual function. The cellular source of melanocortins mediating these effects is still unknown. In addition, these studies do not prove that MC4R is the only MCR involved in the peripheral action of melanocortins on sexual function. Nonetheless, the studies do demonstrate that administration of an MC4R agonist is sufficient to elicit melanocortin effects on sexual function.

Presently, it is thought that melanocortin modulation of sexual function is due to both central and peripheral actions. Because it appears that the full complement of melanocortin-mediated sexual responses can be elicited by peripheral administration of a selective MC4R agonist, this may become one of the therapeutic uses for such an agent. On the other hand, it may also represent an undesirable side effect to the use of such agonists for the treatment of obesity.

SUMMARY

In the past 10 years, substantial progress has been made in understanding the physiological functions of the melanocortin system. In that time, cloning of the various components of the melanocortin system, the application of gene targeting technology, and the development of selective pharmacological agents have provided insight into the biological basis for the pro-lean effects of the melanocortins.

The role of melanocortins MC3R, MC4R, and AGRP in metabolic regulation and the role of α -MSH and the MC4R in sexual function have received a great deal of attention and have provided a framework to explore the melanocortin system for the treatment of obesity, other metabolic abnormalities, and sexual dysfunction. The role of the melanocortins in pigmentation can now be understood in the context of MC1R variants and the competition among ACTH-MSH peptides and agouti. Expression of MC1R by leukocytes begins to unravel the role of the melanocortins in inflammation and immunomodulation. The role of MC5R in exocrine function has opened new avenues for dermatological research. Although development of highly selective agonists and antagonists for MC1R and MC5R has lagged behind the pace of drug development directed at MC3R and MC4R, future identification of such compounds promises to lead to an even greater understanding of the roles of melanocortins in normal and pathological physiology.

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REFERENCES

- Argiolas A. Neuropeptides and sexual behavior. *Neurosci Biobehav Rev* 8: 1127–1142, 1999.
- Argiolas A, Melis MR, Murgia S, and Schiöth HB. ACTH- and α -MSH-induced grooming, stretching, yawning and penile erection in male rats: site of action in the brain and role of melanocortin receptors. *Brain Res Bull* 51: 425–434, 2000.
- Brady LS, Smith MA, Gold PW, and Herkenham H. Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. *Neuroendocrinology* 52: 441–447, 1990.
- Brent WD, Didier B, Kaelin CB, Ollmann MM, Gantz I, Watson SJ, and Barsh GS. Physiological and anatomical circuitry between agouti-related protein and leptin signaling. *Endocrinology* 140: 2387–2397, 1999.
- Broberger C, Johansen J, Brismar H, Johansson C, Schalling M, and Hökfelt T. Changes in neuropeptide Y receptors and pro-opiomelanocortin in the anorexia (anx/anx) mouse hypothalamus. *J Neurosci* 19: 7130–7139, 1999.
- Bultman SJ, Michaud EJ, and Woychik RP. Molecular characterization of the mouse agouti locus. *Cell* 71: 1195–1204, 1992.
- Buggy JJ. Binding of α -melanocyte-stimulating hormone to its G-protein-coupled receptor on B-lymphocytes activates the Jak/Stat pathway. *Biochem J* 331: 211–216, 1998.
- Catania A, Airaghi L, Colombo G, and Lipton JM. α -Melanocyte-stimulating hormone in normal human physiology and disease states. *Trends Endocrinol Metab* 11: 304–308, 2000.
- Chhajlani V. Distribution of cDNA for melanocortin receptor subtypes in human tissues. *Biochem Mol Biol Int* 38: 73–80, 1996.
- Chen AS, Marsh DJ, Trumbauer ME, Frazier EG, Guan X-M, Yu H, Rosenblum CI, Vongs A, Feng Y, Cao L, Metzger JM, Strack AM, Camacho RE, Mellin TN, Nunes CN, Min W, Fisher J, Gopal-Truter S, MacIntyre DE, Chen HY, and Van der Ploeg LHT. Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. *Nat Genet* 26: 97–102, 2000.
- Chen W, Kelly MA, Opitz-Araya X, Thomas RE, Low MJ, and Cone RD. Exocrine gland dysfunction in MC5R-deficient mice: evidence for coordinated regulation of exocrine gland function by melanocortin peptides. *Cell* 91: 789–798, 1997.
- Cowley MA, Smart JL, Rubinstein M, Cerdan MG, Diano S, Horvath TL, Cone RD, and Low MJ. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature* 411: 480–484, 2001.
- Dinulescu DM and Cone RD. Agouti and agouti-related protein: analogies and contrasts. *J Biol Chem* 275: 6695–6698, 2000.
- Dinulescu DM, Fan W, Boston BA, McCall K, Lamoreux ML, Moore KJ, Montagno J, and Cone RD. Mahogany (mg) stimulates feeding and increases basal metabolic rate independent of its suppression of agouti. *Proc Natl Acad Sci USA* 95: 12707–12712, 1998.
- Englaro W, Rezzonico R, Durand-Clément M, Lallemand D, Ortonne J-P, and Ballotti R. Mitogen-activated protein kinase pathway and AP-1 are activated during cAMP induced melanogenesis in B-16 melanoma cells. *J Biol Chem* 270: 24315–24320, 1995.
- Fan W, Boston BA, Kesterson RA, Hruby VJ, and Cone RD. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* 385: 165–168, 1997.
- Fan W, Marks DL, Haqq A, Weissman A, Rene P, Cowley M, Takahashi K, Simerly R, Low M, Chagnon Y, and Cone RD. Mechanisms of melanocortin action in the CNS (Abstract). *Proc. Int. Melanocortin Mtg. 5th Sun River CO 2002*.
- Gunn TM, Miller KA, He L, Hyman RW, Davis RW, Azarani A, Schlossman SF, Duke-Cohan JS, and Barsh GS. The mouse mahogany locus encodes a transmembrane form of human attractin. *Nature* 398: 152–156, 1999.
- He L, Gunn TM, Bouley DM, Lu XY, Watson SJ, Schlossman SF, Duke-Cohan JS, and Barsh GS. A biochemical function for attractin in agouti-induced pigmentation and obesity. *Nat Genet* 27: 40–7, 2001.
- Heisler LK, Cowley MA, Tecott LH, Fan W, Low MJ, Smart JL, Rubinstein M, Tatro JB, Marcus JN, Holstege H, Lee CE, Cone RD, and Elmquist JK. Activation of central melanocortin pathways by fenfluramine. *Science* 297: 609–611, 2002.
- Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, Smith FJ, Campfield LA, Burn P, and Lee F. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 88: 131–141, 1997.
- Jackson RS, Creemers JWM, Ohagi S, Raffin-Sanson M-L, Sanders L, Montague CT, Hutton JC, and O'Rahilly S. Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet* 16: 303–306, 1997.
- Kapas S, Purbrick A, and Hinson JP. Role of tyrosine kinase and protein kinase C in the steroidogenic actions of angiotensin II, alpha-melanocyte-stimulating hormone and corticotropin in the rat adrenal cortex. *Biochem J* 305: 433–438, 1995.
- Kojima I, Kojima K, and Rasmussen H. Role of calcium and cAMP in the action of adrenocorticotropin on aldosterone secretion. *J Biol Chem* 260: 4248–4256, 1985.
- Konda Y, Gantz I, DeValle J, Shimoto Y, Miwa H, and Yamada T. Interaction of dual intracellular signaling pathways activated by the melanocortin-3 receptor. *J Biol Chem* 269: 13162–13166, 1994.
- Krude H, Biebermann H, Werner L, Horn R, Brabant G, and Grüters A. Severe early-onset obesity, adrenal insufficiency

- ciency and red hair pigmentation caused by POMC mutations in humans. *Nat Genet* 19: 155–157, 1998.
27. **Lechan RM and Tatro JB.** Hypothalamic melanocortin signaling in cachexia. *Endocrinology* 142: 3288–3291, 2001.
 28. **Lindblom J, Kask A, Hagg E, Harmark L, Bergstrom L, and Wikberg J.** Chronic infusion of a melanocortin receptor agonist modulates dopamine receptor binding in the rat brain. *Pharmacol Res* 45: 119–124, 2002.
 29. **Lindblom J, Opmane B, Mutulis F, Mutule I, Petrovska R, Klusa R, Bergstrom L, and Wikberg JE.** The MC4 receptor mediates α -MSH induced release of nucleus accumbens dopamine. *Neuroreport* 12: 2155–2158, 2001.
 30. **Lu D, Willard D, Patel IR, Kadwell S, Overton L, Kost T, Luther M, Chen W, Woychik RP, Wilkison WO, and Cone RD.** Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. *Nature* 371: 799–802, 1994.
 31. **Luger TA, Schwarz H, Scholzen KT, Schwarz A, and Brzoska T.** Role of epidermal cell-derived α -melanocyte stimulating hormone in ultraviolet light mediated local immunosuppression. *Ann NY Acad Sci* 885: 209–216, 1999.
 32. **McNulty JC, Thompson DA, Bolin KA, Wilken J, Barsh GS, and Millhauser GL.** High-resolution NMR structure of the chemically-synthesized melanocortin receptor binding domain AGRP(87–132) of the agouti-related protein. *Biochemistry* 40: 15520–15527, 2001.
 33. **Nijenhuis WA, Oosterom J, and Adan RAH.** AGRP(87–132) acts as an inverse agonist on the human-melanocortin-4 receptor. *Mol Endocrinol* 15: 164–171, 2001.
 34. **Ollmann MM, Wilson BD, Yang Y-K, Kerns JA, Chen Y, Gantz I, and Barsh GS.** Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science* 278: 135–138, 1997.
 35. **Pritchard LE, Turnbull AV, and White A.** Pro-opiomelanocortin processing in the hypothalamus: impact on melanocortin signaling and obesity. *J Endocrinol* 172: 411–421, 2002.
 36. **Qian S, Chen H, Weingarh D, Trumbauer ME, Novi DE, Guan X, Yu H, Shen Z, Feng Y, Frazier E, Chen A, Camacho RE, Shearman LP, Gopal-Truter S, MacNeil DJ, Van der Ploeg LH, and Marsh DJ.** Neither agouti-related protein nor neuropeptide Y is critically required for the regulation of energy homeostasis in mice. *Mol Cell Biol* 22: 5027–5035, 2002.
 37. **Reizes O, Lincecum J, Wang Z, Goldberger O, Huang L, Kaksonen M, Ahima R, Hinkes MT, Barsh GS, Rauvala H, and Bernfield M.** Transgenic expression of syndecan-1 uncovers a physiological control of feeding behavior by syndecan-3. *Cell* 106: 105–116, 2001.
 38. **Robbins LS, Nadeau JH, Johnson KR, Kelly MA, Roselli-Rehfuss L, Baack E, Mountjoy KG, and Cone RD.** Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. *Cell* 72: 827–834, 1993.
 39. **Schaffer JV and Bolognia JL.** The melanocortin-1 receptor: red hair and beyond. *Arch Dermatol* 137: 1477–1485, 2001.
 40. **Shen CP, Wu KK, Shearman LP, Camacho R, Tota MR, Fong TM, and Van der Ploeg LH.** Plasma agouti-related protein level: a possible correlation with fasted and fed states in humans and rats. *J Neuroendocrinol* 14: 607–610, 2002.
 41. **Thody AJ.** α -MSH and the regulation of melanocyte function. *Ann NY Acad Sci* 885: 217–229, 1999.
 42. **Thody AJ, Cooper MF, Bowden PE, Meddis D, and Shuster S.** Effect of α -melanocyte-stimulating hormone and testosterone on cutaneous and modified sebaceous glands in the rat. *J Endocrinol* 71: 279–288, 1976.
 43. **Thody AJ, Wilson CA, and Everard D.** Facilitation and inhibition of sexual receptivity in the female rat by α -MSH. *Physiol Behav* 22: 447–450, 1979.
 44. **Tsigo C, Arai K, Hung W, and Chrousos GP.** Hereditary isolated glucocorticoid deficiency is associated with abnormalities of the adrenocorticotropin receptor gene. *J Clin Invest* 92: 2458–2461, 1993.
 45. **Vaisse C, Clement K, Durand E, Herberg S, Guy-Grand B, and Froguel P.** Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. *J Clin Invest* 106: 253–62, 2000.
 46. **Valverde P, Healy E, Jackson I, Rees JL, and Thody AJ.** Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. *Nat Genet* 11: 328–330, 1995.
 47. **Van der Ploeg LHT, Martin WJ, Howard AD, Nargund RP, Austin CP, Guan X, Drisko J, Cashen D, Sebhat I, Patchett AA, Figueroa DJ, DiLella AG, Connolly BM, Weinberg DH, Tan CP, Palyha OC, Pong S-S, MacNeil T, Rosenblum C, Vongs A, Tang R, Yu H, Sailer AW, Fong TM, Huang C, Tota MR, Chang RS, Stearns R, Tamvakopoulos C, Christ G, Drazen DL, Spar DB, Nelson J, and MacIntyre DE.** A role for the melanocortin 4 receptor in sexual function. *Proc Natl Acad Sci USA* 99: 11381–11386, 2002.
 48. **Vergoni AV, Poggioli R, and Bertonlini A.** Corticotropin inhibits food intake in rats. *Neuropeptides* 7: 153–158, 1986.
 49. **Vrieling H, Duhl DM, Miller SE, Miller KA, and Barsh GS.** Differences in dorsal and ventral pigmentation result from regional expression of the mouse agouti gene. *Proc Natl Acad Sci USA* 91: 5667–5671, 1994.
 50. **Wessells H, Fuciarelli K, Hansen J, Hadley ME, Hruby VJ, Dorr R, and Levine N.** Synthetic melanotropic peptide initiates erections in men with psychogenic erectile dysfunction: double-blind placebo controlled crossover study. *J Urol* 160: 389–393, 1998.
 51. **Wessells H, Gralnek D, Dorr R, Hruby VJ, Hadley ME, and Levin N.** Effect of an alpha-melanocyte stimulating hormone analog on penile erection and sexual desire in men with organic erectile dysfunction. *Urology* 56: 641–646, 2002.
 52. **Wikberg JES.** Melanocortin receptors: perspectives for novel drugs. *Eur J Pharmacol* 375: 295–310, 1999.
 53. **Williams DL, Kaplan JM, and Grill HJ.** The role of the dorsal vagal complex and the vagus nerve in feeding effects of melanocortin-3/4 receptor stimulation. *Endocrinology* 141: 1332–1337, 2000.
 54. **Wilson BD, Ollmann MM, Kang L, Stoffel M, Bell GI, and Barsh GS.** Structure and function of ASP, the human homolog of the mouse agouti gene. *Hum Mol Genet* 4: 223–230, 1995.