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PERSPECTIVE

New Insights into G-Protein-Coupled Receptor Signaling from the Melanocortin Receptor System

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For decades, geneticists, as well as breeders of "fancy" pets, have been interested in the interaction of the melanocortin 1 receptor locus (*Mc1r*; also known as melanocyte-stimulating hormone receptor, *Mshr*) with the *Agouti* locus because of the array of coat colors that alterations at these loci generate. In the simplest case, a mouse with two wild-type *Mc1r* alleles and two recessive *Agouti* alleles (Fig. 1b, $Mc1r^{+}/Mc1r^{+}$, *a/a*) is expectedly darker than the wild-type mouse (Fig. 1a, $Mc1r^{+}/Mc1r^{+}$, *A/A*) of "agouti" coloring, and a mouse with a pair of recessive alleles at both the *Mc1r* and *Agouti* loci is yellow (Fig. 1c, $Mc1r^{-}/Mc1r^{-}$, *a/a*). Geneticists have taken advantage of this unique system in model organisms to serve as readily visible markers for such experiments as gene targeting (Simpson et al., 1997). However, it is a century-old enigmatic observation that a mouse with wild-type *Mc1r* alleles and one of several dominant *Agouti* alleles (Fig. 1d, $Mc1r^{+}/Mc1r^{+}$, A^{Y}/a) is not only yellow, but obese. Furthermore, a pair of recessive alleles at yet another locus, *mahogany* (also known as *Attractin*, *Atrn*), ablates the affects of the dominant *Agouti* allele (Fig. 1e). These observations have led more recently to a series of investigations identifying new receptors and mediators of the melanocortin receptor (MCR) pathway making this signal transduction pathway an important model in the study of G-protein coupled receptor (GPCR) pathways in complex disease and for pharmacological insights as well.

The MCR pathway includes five known differentially expressed GPCRs: MC1R, corticotropin receptor (ACTHR), MC3R, MC4R, and MC5R (Mountjoy et al., 1992). Although all five receptors are known to be Gs-coupled, MC3R has also been shown to function through phospholipase C-mediated hydrolysis of phosphoinositides (Konda et al., 1994). MC1R is predominantly expressed in melanocytes, where it is known for its classic role in skin and hair pigmentation in many species. MC1R is also expressed in other tissues and cells, such as the pituitary and leukocytes (Chhajlani 1996), indicating putative physiological roles yet to be unveiled. MC3R and MC4R are found in the central nervous system but are notably absent from the melanocytes (Gantz et al., 1993); both are highly expressed in the hypothalamus, where they are involved in energy homeostasis. ACTHR is expressed in the adrenal cortex and MC5R in peripheral cells such as adipocytes. Melanocortins are the endogenous agonists to which the MCRs have differential affinities. These are small peptides derived from proopiomelanocortin: ACTH, α-MSH, β-MSH, and γ-MSH. α-MSH is the predominant melanocortin of action in the hypothalamus and skin and potently activates all MCRs except ACTHR. The effects of α-MSH in vivo are modulated by two endogenous paracrine peptides, agouti (or agouti signaling protein) and agouti-related protein (AGRP). Specifically, AGRP potently antagonizes MC3R, MC4R, and MC5R, whereas agouti potently antagonizes MC1R, ACTHR, and MC4R (Yang et al., 1997). Agouti and AGRP are normally expressed in the skin and brain, respectively.

In mice, agouti is a 131-amino acid peptide synthesized in the skin that causes the mouse melanocytes to produce yellow pigment instead of the brown/black pigment by competitively inhibiting the action of α-MSH on MC1R (Lu et al., 1994). The "agouti" coloring of wild-type animals is produced by a brown/black pigmented hair with a subapical yellow pigmented band resulting from transient expression of *Agouti* during hair development (Fig. 1a). Dominant mutations of *Mc1r* in which the receptor is constitutively active or has enhanced affinity for α-MSH result in dark, nonagouti mice, even in the presence of agouti, implicating agouti's role upstream of the receptor; recessive mutations of murine *Mc1r* rendering a nonfunctional MC1R produces yellow mice (Robbins et al., 1993). The role of agouti in human pigmentation or other physiological roles is not understood; however, agouti is found in human tissue and has been shown to antag-

ABBREVIATIONS: MCR, melanocortin receptor; GPCR, G protein-coupled receptor; ACTHR, corticotropin receptor; MSH, melanocyte-stimulating hormone; AGRP, agouti-related protein.

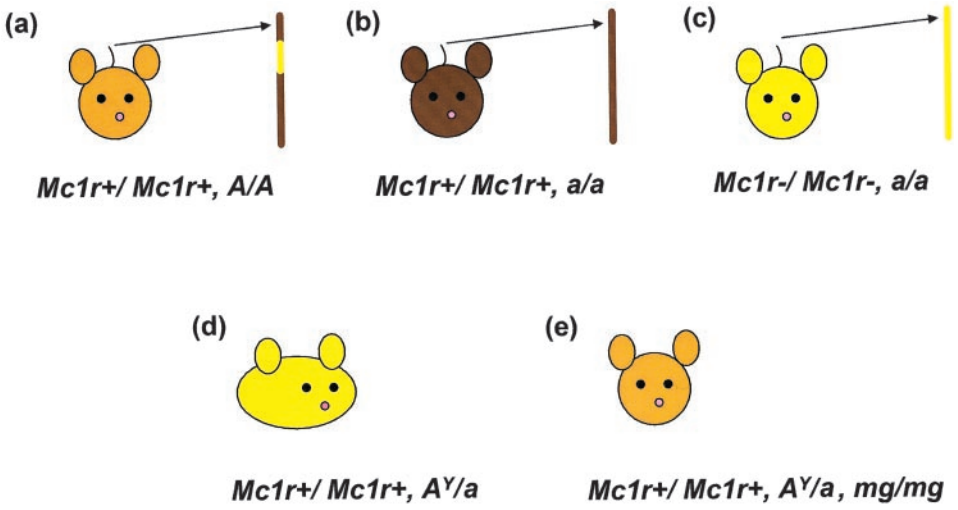

Fig. 1. Phenotype of (a) wild-type "agouti" mouse with a yellow subapical band pattern in hair caused by transient expression of *Agouti* during hair development; (b) mouse with genotype consisting of two wild-type *Mc1r* alleles and two recessive *Agouti* alleles ($Mc1r^{+}/Mc1r^{+}$, *a*/*a*) producing hair without subapical yellow band; (c) mouse with a pair of recessive alleles at both the *Mc1r* and *agouti* loci ($Mc1r^{-}/Mc1r^{-}$, *a*/*a*) is yellow; (d) mouse with genotype consisting of two wild-type *Mc1r* alleles and one dominant *Agouti* allele ($Mc1r^{+}/Mc1r^{+}$, A^{Y}/a) is yellow and obese; (e) mouse with two recessive *mahogany* alleles on a dominant *Agouti* background is similar to wild-type mouse in pigmentation and body mass.

onize human MCRs in vitro (Yang et al., 1997). Unlike agouti's elusive role in human physiology, AGRP is thought to regulate energy homeostasis. The exact mechanism of action on the MCRs by agouti and AGRP is still not well understood. Several potential explanations for AGRP action exist, including competitive antagonism, inverse agonism, or action on an effector other than adenylyl cyclase (Siegrist et al., 1997, Yang et al., 1999). In this issue of *Molecular Pharmacology*, Yang et al. (2003) use a series of MC1R/MC4R chimeric constructs to study AGRP's role in competitive inhibition of α-MSH to elucidate the role of MCR antagonism in energy homeostasis as well as to assess the significance of specific GPCR domains and residues in receptor binding and antagonism.

Naturally occurring variants with associated phenotypes of the MCR signal transduction (Table 1) have contributed to our understanding of the role of components of the MCR pathway, particularly the physiological role of MC4R and AGRP. The pleiotropic effects of altered expression of *Agouti* in four inherited dominant mouse *Agouti* mutations (A^{y}, A^{iy}, A^{sy}, A^{vy}) revealed an important physiological role for MC4R. As summarized above, mice that are wild-type for *Mc1r* have an expected yellow coat color with the expression of a dominant *Agouti* allele. However, some dominant *Agouti* polymorphisms are associated with obesity as well as increased tumor susceptibility and insulin resistance. These mutations facilitated the cloning of the *Agouti* gene, whose gene product provides a model for in vivo competitive inhibition of GPCRs. A^{y} results from a large deletion that fuses the promoter of the *Raly* gene with the *Agouti* gene. The *Raly* gene is constitutively expressed in all somatic cells, and its promoter overrides the regulation of the agouti gene, causing ectopic overexpression of *Agouti* (Bultman et al., 1992). A^{iy} and A^{vy} ectopic overexpression results from the insertion of a retrotransposon and A^{sy} from a novel DNA sequence that ultimately deregulates the *Agouti* promoter by altering *Agouti* transcripts via molecular mechanisms such as altered splicing. (Duhl et al., 1994). It was after the cloning of *AGRP* that the obesity phenotype associated with ubiquitous expression of the *Agouti* gene was understood. In these dominant *Agouti* mutant mice, agouti mimics AGRP's competitive inhibition of α-MSH action on MC4R (Yang et al., 1997) in the central nervous system, thereby affecting energy homeostasis analogous to its action on MC1R in skin and pigmentation (Fig. 2).

AGRP was cloned through sequence similarity with *Agouti* and found to be expressed primarily in the hypothalamus, where it serves as a selective antagonist for MC3R and MC4R; in contrast to the ubiquitously expressing *Agouti* mouse model that implicated agouti as an antagonist for MC4R as confirmed by in vitro studies, ubiquitous expression of *AGRP* in transgenic mice did not affect pigmentation (Oll-

TABLE 1

Phenotypes associated with variants of the MCR system

Coding region single nucleotide polymorphisms (cSNPs) alter the protein coding sequence. Other types of variants (e.g., deletions, promoter polymorphisms) exist at these loci, and many contribute to altered phenotype as well. Not all cSNPs counted have been associated with an altered phenotype.

	Human Phenotypes	Mouse Phenotypes	Human cSNPs
MC1R	Skin and hair pigmentation (Flanagan et al., 2000) Melanoma susceptibility (Palmer et al., 2000) Pain modulation (Mogil et al., 2003)	Hair pigmentation (Robbins et al., 1993) Pain modulation (Mogil et al., 2003)	21
ACTHR	Familial glucocorticoid deficiency (Clark et al. 1993; Tsigos et al, 1993)		13
MC3R		Feed efficiency (Chen et al, 2000)	
MC4R	Dominantly inherited obesity (Vaisse et al. 1998; Yeo et al., 1998)		10
Agouti/ASIP		Hair pigmentation and obesity (Duhl et al. 1994)	1
AGRP	Fatness and abdominal adiposity (Argyropoulos et al., 2002)		1
POMC	Red hair, severe early-onset obesity, and adrenal insufficiency (Krude et al., 1998)		1

ASIP, agouti signaling protein; POMC, proopiomelanocortin.

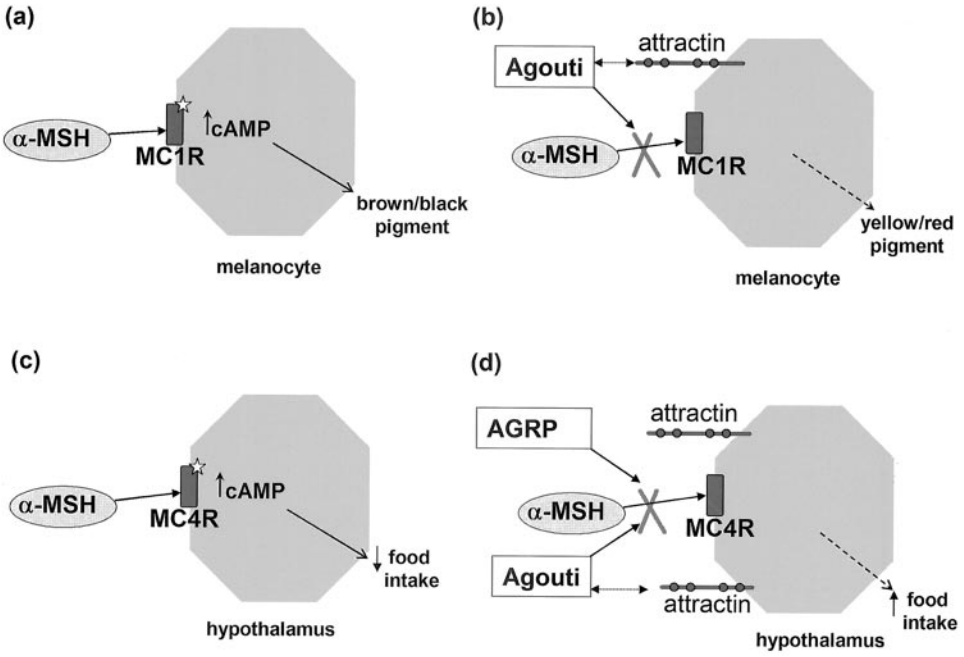

Fig. 2. Mechanism of agouti and AGRP inhibition of α-MSH action on MCRs in pigmentation and energy homeostasis. (a) α-MSH stimulates MC1R in skin increasing brown/black pigment. (b) Agouti inhibits the action of α-MSH on MC1R, producing yellow/red pigments. (c) α-MSH stimulates MC4R in the hypothalamus decreasing food intake. (d) AGRP and ectopically expressed agouti inhibits the action of α-MSH on MC4R increasing food intake. Attractin, the product of the *mahogany* locus may act as a low affinity receptor for agouti, increasing the local cell surface concentration of agouti.

mann et al., 1997). This indicated that AGRP lacks affinity for MC1R, as later confirmed by in vitro studies (Yang et al., 1999). In their article in this issue of *Molecular Pharmacology*, Yang et al. (2003) have used this AGRP binding selectivity for MC4R over MC1R in their chimeric receptors to begin to unravel the GPCR domains involved in differential binding properties of agonists and antagonists. For example, Yang et al. (2003) show that although the third and fourth transmembrane domains of human MC4R play a significant role in C-terminal AGRP binding in vitro, the fourth transmembrane domain does not affect agonist [(Nle^4,D-Phe^7)α-MSH, an analog of α-MSH] binding. These and further such pharmacological studies of the domains of MC1R, MC4R, agouti, and AGRP should help determine the differential agonist and antagonist binding properties of GPCRs.

Through these studies, a model is emerging on the action of AGRP and MC4R on feeding and obesity phenotype in mouse (Fig. 2). Neurons producing α-MSH and AGRP respond to energy balance changes to regulate food intake via the MC3 and MC4 receptors of downstream neurons. AGRP antagonist increases food intake by inhibiting the action of α-MSH on MC4R, as agouti antagonizes the effect of α-MSH on MC1R to regulate pigmentation (reviewed in Barsh and Schwartz, 2002).

The MCR pathway has also introduced new regulators of the G-protein-coupled receptor pathway. The pleiotropic effects of the dominant *Agouti* mutations described above were not seen in mice carrying two recessive alleles at the *mahogany* locus. The product of the murine *mahogany* locus was cloned and found to be expressed in many cells and tissues, including melanocytes and the hypothalamus (Gunn et al., 1999). It is orthologous to a transmembrane domain-coding splice variant of the human attractin molecule (Tang et al., 2000). The mechanism of action of this membrane-bound protein is also not fully understood; however, He et al. (2001) provided biochemical evidence of a mechanism in which the *mahogany* product, attractin, functions as a low-affinity receptor for agouti, but not for AGRP, that increases its local cell-surface concentration, enabling agouti to antagonize the action of α-MSH on MC1R and, when ectopically expressed, on MC4R (Fig. 2, b and d).

As described here, the plethora of naturally occurring polymorphisms and mutations of the MCR signal transduction pathway in human and mouse, coupled with their visibly altered phenotypes, provide for a unique model for understanding the mechanisms by which components of the GPCR signal transduction pathway interact and contribute to complex disease and traits. This system introduces new components that modify activation of the GPCR signal transduction pathway, provides numerous phenotype-associated human and mouse single nucleotide polymorphisms in GPCRs for "natural" site-directed mutagenesis studies into the roles of specific amino acid residues in GPCR structure-function, and provides naturally occurring animal variant models to study the action of endogenous antagonists on GPCRs and their modifiers. In addition to their traditional roles in pigmentation and adrenal function and the now established role in energy homeostasis, melanocortins have been observed to have numerous other actions such as on anti-inflammatory, analgesic, learning and memory, and sexual function (reviewed in Wikberg et al., 2000; Gantz and Fong, 2003). Further pharmacological studies on the interactions of these small peptides with their MCR pathway components have the potential to lead to new therapeutic approaches in disease involving GPCRs.

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