The melanocortin receptors (MCR) are highly unusual, if not unique, among the extensive family of seven transmembrane domain G protein-coupled receptors (GPCR) because their activity is mediated both by endogenous peptide agonists and endogenous antagonists. Classical genetic studies of coat color in the mouse revealed two nonallelic recessive loci, extension (e) and agouti (a), whose loss-of-function mutations result in yellow or black fur, respectively. E encodes the MCR type 1 (MC1R) that is activated by the proopiomelanocortin (POMC)-derived agonist MSH to stimulate the production of black eumelanin by elevation of cAMP (1). A encodes the agouti signaling protein that antagonizes binding of MSH to MC1R and results in the production of yellow/red pheomelanin by reduction of cAMP (2). Experiments using mouse B16 melanoma cells, which express high levels of the MC1R, showed that agouti could inhibit melanogenesis and cell growth even in the absence of MSH in the culture medium, suggesting that agouti can function as a competitive antagonist or an inverse agonist at the MC1R (3) (Fig. 1).

There has been increasing acceptance of the concepts of GPCR constitutive activity and inverse agonism in the past decade (4), almost to the point of dogma for the melanocortin system in particular (5). Indeed, studies from our laboratory using POMC-deficient mice crossed onto an a/a genetic background confirmed that the wild-type mouse MC1R has sufficient constitutive activity to result in normal black eumelanin pigmentation and, therefore, indicate that agouti can act as an inverse agonist in vivo to cause a shift from black to yellow pigmentation (6).

Analogous to the peripheral MC1R/agouti system and its effects on pigmentation, agouti-related peptide (AgRP), which is expressed predominantly in the brain, can function as a competitive antagonist at central MC4R to produce hyperphagia and obesity (7, 8). Subsequently, two laboratories independently reported that AgRP exhibits inverse agonism at MC4R in transfected heterologous cellular expression systems (9, 10). However, for this latter pharmacological property to be clinically relevant, it is necessary that the MC4R have a physiologically significant degree of constitutive activity in the neural circuits that regulate energy homeostasis.

Srinivasan and colleagues (11) in the Vaisse laboratory postulated that this might indeed be the case based on their analysis of a subset of mutations in the human MC4R (hMC4R) associated with the development of severe obesity. These particular mutations were located in the amino-terminal extracellular domain of hMC4R and did not affect either receptor trafficking to the plasma membrane or the agonist or inverse agonist actions of MSH and AgRP, respectively, in cell-based expression systems. However, the mutations did reduce constitutive receptor activity in vitro, and further studies showed that the amino-terminal peptide of hMC4R acts as a tethered partial agonist that is essential for constitutive receptor activity (11, 12). Therefore, Vaisse predicted that mutant mice deficient in both POMC and AgRP would be less obese than mice deficient only in POMC, providing proof for the hypothesis that constitutive MC4R activity in the brain normally mitigates excessive food intake and weight gain (11). We addressed the same question by the pharmacological administration of AgRP to neuronal-specific POMC-deficient mice and showed a delayed but persistent action on energy balance consisting of small decreases in oxygen consumption and...
Researchers found no differences between corticosterone replacement supplied in the drinking water. The mutant mice with null alleles for the endogenous mouse POMC and agouti. A normal black coat color was established on the background and normal agouti banding on the background of wild-type or null Pomc alleles. The human MC1R was expressed from its own regulatory elements at levels approximately 10-fold less than that of mouse MC1R, and as a result, the mice were significantly phaeomelanic in the absence of POMC and agouti. A normal black coat color was established on the POMC+/+, ala background and normal agouti banding on the POMC+/+, Ala background, whereas the mice were yellow on the POMC+/+, AY/Jkn/ala background. Therefore, differences in available levels of human or mouse MC1R, despite identical in vitro constitutive activity and differing intrinsic sensitivity of the receptors to MSH, produces functional consequences that are species specific in the physiological context of hair follicles (16). A similar situation may apply to the human and mouse MC4R in their natural cellular environments.

The authors conclude that AgRP functions in vivo predominantly as a competitive antagonist to MSH peptides and not as an inverse agonist. Furthermore, Coll and his colleagues (14) provide a number of well-reasoned explanations for the apparent contradictions between the results of their elegantly performed experiments and past predictions based on less direct methods (14). These possibilities include a ceiling effect due to the already extreme hyperphagia exhibited by Pomc−/− mice after corticosterone replacement that precludes any further increase by exogenous AgRP, background strain differences in mutant mice used by different laboratories, developmental adaptations in response to the life-long AgRP deficiency, and region-specific effects of AgRP on MCR or possibly other unrecognized targets.

How can the data and theories be reconciled? Examination of another mouse model focused on the peripheral melanocortin system and pigmentation may be instructive. As noted earlier in this narrative, loss of POMC function on an ala genetic background does not result in yellow fur pigmentation, consistent with constitutive activity of the mouse MC1R (6). However, this finding is at odds with the reports of red hair pigmentation in humans with complete POMC deficiency (15). To explain this discrepancy, Jackson et al. (16) systematically studied a series of mutant mice that carried a humanized MC1R BAC transgene in combination with null alleles for the endogenous mouse Mcr1, varying gene dosage at the A and a alleles of the agouti locus and wild-type or null Pomc alleles. The human MC1R was expressed from its own regulatory elements at levels approximately 10-fold less than that of mouse MC1R, and as a result, the mice were significantly phaeomelanic in the absence of POMC and agouti. A normal black coat color was established on the POMC+/+, ala background and normal agouti banding on the POMC+/+, Ala background, whereas the mice were yellow on the POMC+/+, AY/Jkn/ala background. Therefore, differences in available levels of human or mouse MC1R, despite identical in vitro constitutive activity and differing intrinsic sensitivity of the receptors to MSH, produces functional consequences that are species specific in the physiological context of hair follicles (16). A similar situation may apply to the human and mouse MC4R in their natural cellular environments.
Contemporary biophysical models of the interaction between a GPCR, its ligand, and a G protein emphasize a dynamic equilibrium between at least two thermodynamic receptor states, inactive (R) and active (R*) (17). All GPCRs have a finite probability of entering the R* state, and agonist binding at its orthosteric site increases this probability. Constitutive activity of a GPCR simply is an expression of the R* state in the absence of agonist ligand binding. The efficacy of different ligands, whether to increase or decrease GPCR activity around this basal level depends on numerous other factors including receptor number, the presence of allosteric modulators, the local accessibility of different G proteins, and factors controlling individual GPCR translocation and internalization (18). AgRP, for example, can modulate MC4R signaling by the inducement of receptor endocytosis as well as by altering the R to R* equilibrium (19). In addition, different intracellular signaling pathways may be engaged in a ligand-specific manner to determine whether a compound acts as a neutral antagonist or an inverse agonist (20). A monoclonal antibody directed at an epitope in the amino-terminal domain of the hMC4R was recently shown to function as a noncompetitive antagonist for MSH but to synergize with AgRP in mediating inverse agonism (21). The intact antibody or its recombinantly produced single-chain variable fragment reduced food intake in rats, suggesting that it may serve as a new lead compound in the development of compounds with high specificity to the MC4R for the treatment of cachexia (21). However, until it is possible to study more directly the human MC4R in its native neuronal context, it seems wise to remain agnostic on the questions of whether AgRP is a physiologically relevant inverse agonist in humans or whether novel synthetic MC4R ligands, such as the monoclonal antibody just described, will demonstrate clinical efficacy by antagonism, inverse agonism, or some combination of the two mechanisms.

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