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The central melanocortin system and the regulation of energy balance

Michelle Lee, Sharon L. Wardlaw

Department of Medicine, Columbia University College of Physicians & Surgeons, New York, NY 10032

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1. ABSRTACT

The central melanocortin system is comprised of discrete populations of neurons and circuits that play a key role in maintaining energy balance. This system can sense levels of peripheral energy stores and can integrate a variety of nutrient, neuronal and hormonal signals to regulate food intake, energy expenditure and nutrient metabolism. Disruption of this system at multiple levels causes obesity in humans and animals. This article reviews the normal physiology and regulation of the central melanocortin system, the abnormalities of this system that cause impaired energy balance in humans and in rodents and the potential to target this system for the treatment of obesity and cachexia.

2. INTRODUCTION

The central melanocortin system plays a critical role in regulating energy balance in humans and animals (1,2). This system consists of the proopiomelanocortin (POMC)-derived MSH peptides, including α- and γ_3-MSH, the MSH antagonist, agouti-related protein (AGRP), and the brain melanocortin receptors (MC-Rs) (Figure 1). α-MSH inhibits feeding and stimulates energy expenditure while AGRP is orexigenic and decreases energy expenditure. α-MSH and AGRP are synthesized in distinct neuronal populations in the arcuate nucleus of the hypothalamus but their fiber tracts project to the same brain regions where their peptide products interact at the MC3-R and MC4-R to regulate both feeding behavior and energy expenditure (1,3,4). Projections of POMC and AGRP neurons to other hypothalamic regions, including the paraventricular nucleus (PVN) and lateral hypothalamus (LH), and to the brainstem are particularly important in regulating energy balance (5,6). Some POMC is also synthesized in brainstem neurons. POMC and AGRP neurons can act as sensors of peripheral energy stores and respond to a variety of nutrient, neuronal and hormonal signals. Both sets of neurons are important targets for leptin and for insulin. In rodents, genetic or pharmacological inactivation of POMC or the MC4-R results in hyperphagia and obesity as does overexpression of AGRP (7-11). Targeted deletion of the MC3-R also causes an obesity phenotype (12,13). This system is highly relevant to human energy balance as defects in POMC synthesis and processing and in the MC4-R have all been reported in human obesity syndromes (2). This article will review the anatomy, regulation and physiology of the

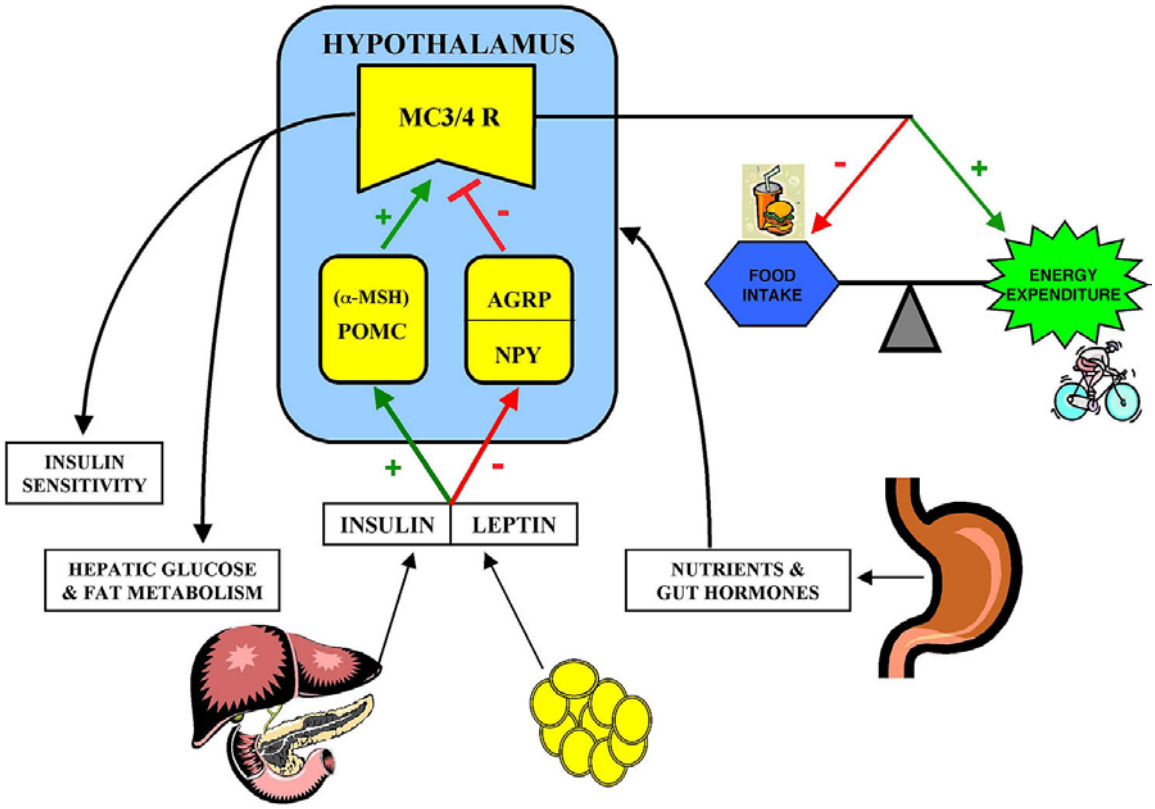

Figure 1. Diagram depicting the regulation of hypothalamic POMC and AGRP neurons and their interaction with hypothalamic melanocortin receptors. MC3/4-R signaling is stimulated by a-MSH and inhibited by AGRP. Leptin, which is secreted by fat cells, stimulates POMC and inhibits AGRP, as does insulin. This leads to an increase in MC4-R signaling resulting in decreased food intake and increased energy expenditure. Gut hormones and nutrients also regulate the activity of the central melanocortin system which can in turn modulate peripheral glucose and fat metabolism.

central melanocortin system and will describe how alterations in POMC, AGRP and MC-R function can lead to impaired energy balance. The potential to target this system for the treatment of disorders of human energy balance such as obesity and cachexia will also be discussed.

3. CENTRAL MELANOCORTIN SYSTEM: ANATOMY, REGULATION AND PHYSIOLOGY

3.1. Proopiomelanocortin

3.1.1. Anatomy, synthesis and processing

POMC is a 30-32-kDa precursor protein that is synthesized in the pituitary, in the arcuate nucleus of the hypothalamus, the nucleus of the solitary tract (NTS) in the medulla and in several peripheral tissues (1). Arcuate POMC neurons have dense fiber tracts that project widely throughout the brain including other hypothalamic and brainstem regions known to be important in regulating energy balance (5, 6). Projections to the paraventricular nucleus (PVN) and lateral hypothalamus (LH) are particularly important in this respect. POMC peptides in the brainstem are derived form both arcuate and NTS neurons (14).

The posttranslational processing of POMC is tissue specific and results in the production of a number of peptides with very different biological activities (15-16,17) (Figure 2). Functionally active peptides are produced by endoproteolytic cleavage at adjacent pairs of basic amino acids by the prohormone convertases, PC1 and PC2 (18). In the anterior pituitary, POMC is processed predominantly to ACTH, ß-lipotropin (LPH) and a 16K N-terminal fragment. ACTH is critical for the maintenance of adrenocortical function. In the hypothalamus and in the intermediate lobe of the pituitary (which is prominent in the rodent), POMC is more extensively processed: ACTH is further processed to produce α-MSH and corticotropin-like-intermediate lobe peptide (CLIP); ß-LPH is processed to ß-EP and γ-LPH; N-terminal POMC is processed to γ3-MSH (17,19). In the human, γ-LPH can be further processed to ß-MSH. It is now clear that α-MSH regulates feeding behavior and energy balance via interaction with brain melanocortin receptors. There is also evidence that other POMC-derived MSH peptides, including ß-MSH and perhaps γ–MSH, may play a role in this process. In addition, the POMC-derived opioid peptide, ß-EP, can affect energy balance. Regulation of POMC processing is particularly important because a number of peptides are produced with very different (and even opposing) biological activities. For example, α-MSH can attenuate the effects of ß-EP on gonadotropin and prolactin release (20, 21) and can also attenuate ß-EP and morphine-induced analgesia (22,23). With respect to feeding, α-MSH is

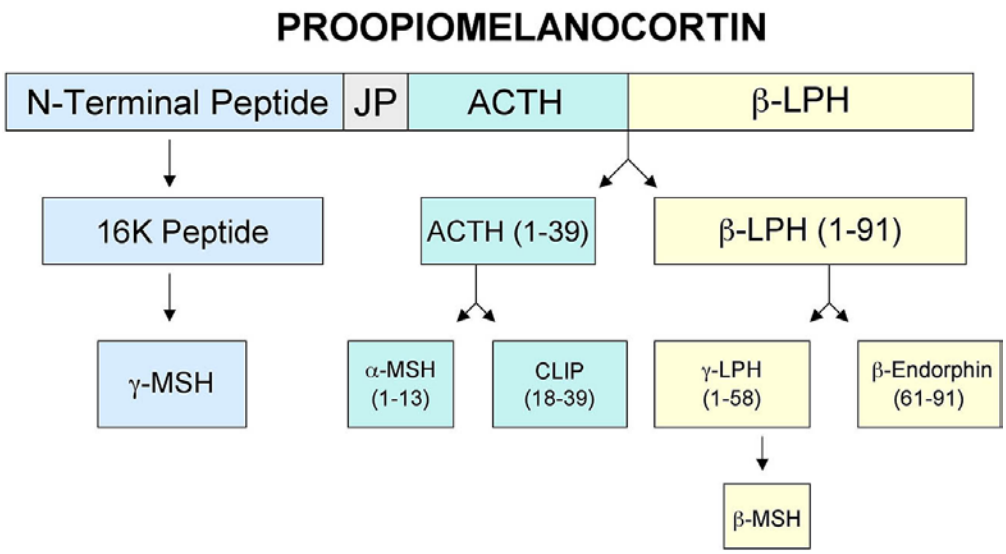

Figure 2. Schematic diagram of the POMC precursor molecule and the major peptide products which are derived from this precursor by endoproteolytic cleavage. (JP = Joining peptide; LPH= Lipotropin; CLIP= corticotropin-like-intermediate lobe peptide).

inhibitory while ß-EP and other opioids have well documented stimulatory effects (24). The role of opioids with respect to energy balance is, however, complex as demonstrated by recent studies in ß-EP null mice (25,26). Opioid receptors, like MC-Rs, are G-protein coupled but modulate inhibition rather than stimulation of cAMP. Several studies have reported interactions between the melanocortin and opioid pathways with respect to feeding and weight gain (27,28).

3.1.2. Regulation and physiology

The regulation of POMC gene expression and peptide release is tissue specific and is quite different in the hypothalamus as compared to the pituitary. In the hypothalamus, POMC is regulated by a variety of hormones, neuropeptides and neurotransmitters, many of which are known to affect energy balance. These include leptin, insulin, glucocorticoids, sex steroids, opioids, dopamine, serotonin, GABA and neuropeptide Y (NPY) (29-41). The effects of leptin, a hormone secreted by fat cells, on POMC neurons in the hypothalamus have been well documented (42) (Figure 1) and there is accumulating evidence that α-MSH mediates some of the downstream effects of leptin on energy balance. POMC expression in arcuate neurons is suppressed during fasting and stimulated when energy stores are increased. Levels of peripheral energy stores are sensed by leptin receptors on POMC neurons (5). There is extensive evidence documenting the activation of POMC neurons by leptin as shown by the induction of Fos, SOCS-3, STAT3 phosphorylation, an increase in POMC heteronuclear RNA and mRNA levels and by an increased frequency of action potentials in electrophysiological studies (29,43-47). In addition, mice with selective deletion of leptin receptors on POMC neurons are obese (48). Leptin can also affect the development of POMC neuronal projections and can modulate the number of excitatory and inhibitory synapses on POMC neurons (49,50). Stimulatory effects of insulin on POMC gene expression have also been demonstrated (30). Although leptin and insulin can act by distinct signaling pathways, there is evidence for some shared intracellular signaling pathways with respect to POMC regulation (51).

Orexigenic AGRP/NPY neurons synapse on POMC neurons and can inhibit their activity through the release of both NPY and GABA (41). POMC neurons also express mu opioid and melanocortin-3 receptors which can both function as inhibitory autoreceptors in response to the release of ß-endorphin and MSH (52,53). There is evidence that CNS circuits can sense nutrient levels including glucose as well as specific fatty acids and amino acids and that POMC neurons in the arcuate may be nutrient responsive (54-56). POMC neurons express ATP-sensitive potassium channels that can couple membrane excitability to cellular metabolism and electrophysiology studies have demonstrated that they are glucose responsive (57). Thus POMC neurons may integrate both hormonal and nutrient signals that reflect levels of energy stores.

A number of studies have shown that intracerebroventricular injection of α-MSH and other synthetic MSH agonists can suppress food intake in the rodent and that this effect can be blocked by specific α-MSH antagonists (58-60). Peripheral injection of MSH agonists has also been shown to suppress food intake in some rodent models (61). Icv injection of an MSH agonist was also effective in decreasing food intake in a monkey model (62). Furthermore, injection of synthetic α-MSH antagonists, increases food intake, indicating a role for endogenous α-MSH in appetite control (63,64). In addition to suppressing food intake, α-MSH can affect energy expenditure, oxygen consumption and fuel oxidation, all of which contribute to overall changes in

Table 1. Melanocortin receptor family

Receptor	Localization	Activity	Primary Ligand
MC1-R	Melanocytes, inflammatory cells (monocytes, neutrophils)	Pigmentation, anti-inflammatory	α-MSH, ACTH
MC2-R	Adrenal cortex	Adrenal steroidogenesis	ACTH
MC3-R	Hypothalamus, limbic system, pancreas, stomach, duodenum, placenta, monoctyes, heart	Energy homeostasis, anti-inflammatory	α-MSH, γ-MSH
MC-4-R	Hypothalamus, cerebral cortex, brainstem	Energy homeostasis	α-MSH
MC5-R	Exocrine glands, skeletal muscle, brain, lymphocytes, adipocytes	Control of exocrine gland secretion	α-MSH, ACTH

energy balance. Icv injection of the α-MSH agonist, MTII, increased resting oxygen consumption in lean and diet-induced obese (DIO) mice and in lean and obese Zucker rats compared to pair-fed controls (61,65). MTII also increased the proportion of expended energy derived from fat as evidenced by a reduction in the respiratory quotient (RQ) in lean and obese Zucker rats (65). Peripheral injection of an MSH analog increased FFA levels in normal and *ob/ob* mice (66). There is evidence that MTII can also modulate the expression of liver enzymes involved with both the synthesis and oxidation of fat. MTII decreased the hepatic expression of stearoyl-CoA desaturase-1 (SCD1), a lipogenic enzyme involved in the synthesis of monounsaturated fatty acids (67) and increased the hepatic expression of carnitine palmitoyltransferase-1 (CPT-1) which is involved in lipid oxidation (68). At least one mechanism by which α-MSH might increase energy expenditure is via increasing thermogenesis in brown adipose tissue (BAT). There is evidence that the stimulatory effects of leptin on the sympathetic nervous system and on the UCPs in BAT are mediated by the melanocortin system (69-71). Infusion of MTII has also been shown to increase UCP1 expression in BAT (68). Another mechanism by which MSH may affect energy balance is via modulation of the hypothalamic-pituitary-thyroid (HPT) axis. α-MSH containing nerve terminals have been shown to innervate TRH neurons in the PVN and these neurons can be stimulated or suppressed by α-MSH (72,73). It has also been shown that some of the effects of fasting and leptin on the HPT axis are mediated by the melanocortin pathway.

Transgenic neuronal overexpression of *Pomc* has been shown to attenuate obesity in *ob/ob* mice (74). Central *Pomc* gene delivery via recombinant adeno-associated virus has also been shown to reduce food intake and adiposity in obese Zucker rats (75). Overexpression of an N-terminal POMC transgene, that includes both α-MSH and γ□-MSH, reduced weight gain and adiposity in male mice on a normal diet and attenuated obesity in male and female db^{3J}/db^{3J} mice (76). This transgene also protected male and female mice from weight gain and increased adiposity when exposed to a high fat diet. The importance of POMC in regulating energy balance is demonstrated by the fact that disruption of the melanocortin system via POMC deficiency results in severe obesity. Mice with targeted deletion of the *Pomc* gene and humans with *POMC* null mutations are obese despite having profound adrenal insufficiency (section 4.1.1). Increased susceptibility to obesity has been noted even with partial deficiency in both *Pomc* +/- mice and in humans heterozygous for *POMC* null mutations (8,77).

α-MSH has long been known to have potent anti-inflammatory activity and it is possible that this may impact on the metabolic phenotype. For example, α-MSH can antagonize many of the biological effects of endotoxin and the pro-inflammatory cytokines, including effects on body temperature, immune function, endocrine function and behavior (78-80). There is also evidence that α-MSH and the central MC-Rs may play a role in endotoxin-induced anorexia and cachexia (85,86). α-MSH can act directly on MC-Rs on peripheral immune cells to downregulate the production of pro-inflammatory cytokines and can also act within the brain to inhibit peripheral immune responses. At least one mechanism by which α-MSH antagonizes the effects of the inflammatory cytokines is by blocking the activation of the nuclear transcription factor NF-κB by thesecytokines (81). α-MSH has also been shown to block toll-like receptor (TRL4) signaling on macrophages (82). In addition, α-MSH has been shown to induce production of the anti-inflammatory cytokine, IL-10, in human monocytes (83). However, it is at present unknown if α-MSH plays any role in modulating the pro-inflammatory state that is characteristic of obesity (84).

3.2. Melanocortin Receptors

The biological effects of melanocortins are mediated by interaction with a family of G protein-coupled, seven-transmembrane melanocortin receptors, of which thus far five have been identified (87,88) (Table 1). All the melanocortin receptors can be activated by both α-MSH and ACTH, with the exception of MC2-R which is activated primarily by ACTH alone. In addition, MC3-R differs from the other melanocortin receptors in that it is also potently activated by γ-MSH.

MC1-R is expressed predominantly on melanocytes and mediates melanocortin effects on skin pigmentation and coat color (89). Mutations of the *Mc1-r* gene are associated with alterations in coat color in animals. In humans, *MC1-R* gene variants have been associated with a phenotype of red hair and/or fair skin that tans poorly (90), with some variants linked to an increased risk of melanoma as well as other skin cancers (91). Although primarily localized to melanocytes, MC1-R expression has been detected in inflammatory cells (92), and the periaqueductal gray matter of rodents and humans (93), as well. It has been postulated that MC1-R may play a role in melanocortin mediated immunomodulatory and anti-inflammatory effects (94).

Expressed almost exclusively in the adrenal cortex, MC2-R mediates the effects of ACTH on glucocorticoid synthesis and release, and mutations in this receptor account for a number of cases of familial glucocorticoid deficiency (95). Among rodents, but not in humans, MC2-R as well as MC5-R expression has also

been found in adipocytes (96,97). Thus, it has been postulated that MC2- and MC5-R may both play a role in mediating melanocortin induced lipolysis in some animals. MC5-R is found in multiple peripheral tissues, but is most predominantly expressed in exocrine glands and skeletal muscle (98). Its most well characterized role is the regulation of exocrine gland function, and *Mc5-r* knockout mice exhibit exocrine gland dysfunction with decreased production of sebaceous lipids (99). The finding that MC5-R is also expressed in lymphocytes has suggested a role for this receptor in modulating immune responses as well (100).

The melanocortin receptors MC3- and MC4-R are both highly expressed in the brain, particularly within key areas involved in regulating energy homeostasis. Centrally, the MC3-R localizes to the limbic system and hypothalamus, where it is expressed within the arcuate nucleus on both POMC and AGRP neurons and may mediate crosstalk between these neurons (4, 101). Activation of the MC3-R by a specific MC3-R agonist has been shown to inhibit POMC neuronal activity, consistent with an autoinhibitory function for MC3-R (47). Peripherally, MC3-R has also been found in the placenta, gut, heart, and monocytes (97,102). In contrast, expression of MC4-R has not been detected in peripheral tissues, but within the brain its distribution pattern is much more extensive than that of MC3-R. MC4-R expression sites include the hypothalamus, cortex, thalamus, brainstem, and spinal cord (103). Within the hypothalamus, the MC4-R is highly expressed in the paraventricular nucleus and the lateral hypothalamic area which are both important in regulating energy balance. As discussed in sections 4.2.1 and 4.2.2, targeted disruption of this receptor produces hyperphagia and obesity in mice and *MC4-R* mutations have been found in obese humans. Pharmacological studies with icv injection of selective MC4-R ligands have also demonstrated the importance of this receptor in regulating energy balance. Finally, it has beem shown that inactivation of the MC3-R also causes an obese phenotype.

3.3 Agouti-Related Protein

3.3.1 Anatomy, synthesis and processing

AGRP is a 132 amino acid peptide that is synthesized in the arcuate nucleus and is structurally homologous to agouti protein which normally controls coat pigmentation by antagonizing the effects of α-MSH at the MC1-R (104,105). The naturally occurring lethal yellow (A^y) mutation of the *agouti* locus causes widespread ectopic expression of agouti. These animals have a yellow coat color and develop hyperphagia, hyperinsulinemia and obesity due to ectopic expression of agouti within the brain (106). AGRP is a potent MC3-R and MC4-R antagonist which is normally expressed in brain and when overexpressed in transgenic mice, causes hyperphagia and obesity. There is almost complete coexpression of AGRP with NPY, another orexigenic peptide, in arcuate neurons (3). AGRP/NPY neurons are distinct from POMC neurons but their fiber tracts project to the same regions (4). The projections of AGRP neurons are, however, more limited than those of POMC neurons which project more widely throughout the brain. It is believed that the projections of POMC and AGRP/NPY neurons to the PVN and the lateral hypothalamic area (LHA) are particularly important in regulating feeding behavior (5,6). Interactions between AGRP and α-MSH at the MC4-R in these hypothalamic regions appears to be critical in maintaining energy homeostasis. AGRP/NPY neurons also synthesize GABA and strong inhibitory GABAergic fibers project to POMC neurons. Thus stimulation of AGRP/NPY neurons can result in subsequent inhibition of POMC neurons.

AGRP is processed in the hypothalamus to a C-terminal $AGRP_{83-132}$ fragment that is known to have full biological activity (107-109). The processing enzyme, PC1/3, is expressed in AGRP neurons in the rat hypothalamus and there is evidence that PC1/3 is primarily responsible for cleavage of AGRP *in vitro* (109). Recent studies have shown that syndecan-3, a cell surface heparin sulfate proteoglycan found in neurons in the hypothalamus, potentiates AGRP activity at the MC4-R and that modulation of cell surface expression of syndecan-3 can affect feeding behavior (110). Syndecan-3, however, interacts with the amino terminal domain of AGRP and not with the C-terminal fragment. Thus, if the majority of AGRP is cleaved, the mechanism by which syndecan-3 potentiates AGRP activity is at present unclear.

3.3.2. Regulation and physiology

Chronic icv infusion of AGRP causes hyperphagia and obesity but a metabolic phenotype, consisting of increased adiposity and increased leptin and insulin levels, persists even when the hyperphagia is prevented (111,112). In a rodent model, AGRP has been shown to decrease energy expenditure as reflected by a decrease in oxygen consumption and a decrease in the capacity of brown adipose tissue to expend energy (113). Transgenic overexpression of AGRP also leads to hyperphagia and obesity. On the other hand, reduction of hypothalamic AGRP by RNA interference has been shown to decrease body weight and increase metabolic rate (114). In contrast to POMC, mice with genetic AGRP ablation are reported to have a relatively normal phenotype with respect to energy balance (115) (section 4.3.1). There is, however, evidence for developmental compensation in *Agrp*-/- mice as recent studies have revealed a lean, hypophagic phenotype in mice with postembryonic ablation of AGRP neurons and the degree of compensation depends on the age at the time of neuronal ablation.

AGRP neurons, like POMC neurons, express leptin and insulin receptors but the regulation of AGRP by leptin and insulin is opposite to that of POMC (5,116). AGRP expression in arcuate neurons is increased during fasting when leptin and insulin levels are suppressed and AGRP expression declines when energy stores are repleted (117-119). Obese leptin deficient mice (*ob/ob*) and leptin receptor deficient mice (*db/db*) have increased AGRP mRNA levels in the hypothalamus which in *ob/ob* mice are restored to normal by leptin injection (118,120,121). Leptin has also been shown to suppress AGRP mRNA levels in food deprived rodents (119,120). Fasting can also stimulate AGRP peptide release from the rat hypothalamus when studied *in vitro* and this was attenuated

by leptin and insulin (107,108). The gut hormone ghrelin interacts with AGRP neurons and the stimulatory effect of ghrelin on feeding is mediated in part by the release of AGRP (122,123). There is also evidence that the gut hormone, PYY3-36, which has an inhibitory effect on feeding, inhibits the electrical activity of AGRP/NPY neurons (124). Glucocorticoids also regulate AGRP expression. AGRP is suppressed in adrenalectomized rats and this was reversed by glucocorticoid replacement (31). The importance of glucocorticoid regulation is underscored by the fact that that the decrease in AGRP in adrenalectomized animals occurred despite a fall in leptin and insulin levels, which in other situations would lead to an increase in AGRP. Recently, serotonin has been shown to inhibit AGRP neuronal activity (39); this is an important finding, given that central serotonergic pathways are known to regulate food intake. There is also evidence that some of the downstream effects of AGRP on food intake are mediated through opioid receptors (125).

AGRP exerts a number of neuroendocrine effects that are similar to the changes that occur with fasting. Icv AGRP has been shown to suppress the hypothalamic-pituitary-thyroid (HPT) axis in rodents (73,126) and to both stimulate the hypothalamic-pituitary-adrenal (HPA) axis and suppress the hypothalamic-pituitary-gonadal (HPG) axis in monkeys (127,128). Interactions with the HPT axis have been most extensively studied and it has been shown that α-MSH and AGRP containing nerve terminals innervate TRH neurons in the PVN; these neurons can be stimulated or suppressed by α-MSH and AGRP respectively. It has also been shown that some of the effects of fasting and leptin on the HPT axis are mediated by the melanocortin pathway.

4. ABNORMALITIES OF THE CENTRAL MELANOCORTIN SYSTEM CAUSING IMPAIRED ENERGY BALANCE

4.1. POMC Mutations

4.1.1. Pomc null mice

Two POMC-null mutant mouse models have been created and both have an obese phenotype despite profound adrenal insufficiency (7,8). In the first model, the entire third exon of *Pomc* was deleted, thus removing the coding region for the relevant POMC-derived peptides but the first 18 amino acids of POMC still remained (7). In the second model the entire POMC sequence was deleted (8). In both models, homozygous *Pomc* knockout mice have defective adrenal development, altered pigmentation and develop hyperphagia and obesity. Serum levels of corticosterone and aldosterone were undetectable in the mutant mice and plasma epinephrine levels were also markedly reduced compared to the wild-type mice. Thus POMC-derived peptides appear to be critical not only for steroidogenesis but for normal adrenal development. In both models, homozygous *Pomc* null mice were born at only a fraction of the expected frequency (8% rather than 25%) consistent with partial embryonic lethality. This was not reversed by administering glucocorticoids to the mother.

Yaswen *et al.* noted increased weight gain in *Pomc -/-* mice in the second postnatal month and by the third postnatal month, weights were about twice those of the wild-type mice. There was also a significant increase in body length as was reported for *Mc4-r* knockout mice. Serum leptin levels were markedly increased in homozygous *Pomc* mutant mice. Leptin levels were also increased in heterozygous mutant mice although body weight was normal. When homozygous *Pomc* mutant mice were treated with daily intraperitoneal injections of a synthetic α-MSH agonist, there was a significant decrease in food intake and substantial weight loss. After two weeks of treatment, the mutant mice had lost 46% of their excess body weight; there was no weight loss in the similarly treated wild-type littermates. When the α-MSH agonist injections were stopped, the mutant mice returned to their pretreatment weight.

Challis *et al.* noted an increase in body weight in the *Pomc -/-* mice after 8 weeks of age. Both fat and lean body mass were increased relative to wild-type mice and basal metabolic rate (as measured by oxygen consumption) was decreased by 23%. Plasma T4 levels were also significantly lower in *Pomc -/-* mice. Although *Pomc +/-* mice were not obese on a standard chow diet, they did become obese on a 45% high fat diet. Thus haploinsufficiency of this gene can cause obesity but only when exposed to a high fat diet. The obesity of *Pomc -/-* mice and its associated metabolic complications were markedly exacerbated by either replacement with glucocorticoids or by selective transgenic restoration of pituitary *Pom*c (129,130). In another model, POMC neurons were progressively ablated by deleting the mitochondrial transcription factor A (*Tfam*) gene using a Cre-lox approach. These mice developed an obesity syndrome similar to that described for *Pom*c null mice (131).

Mutations in the POMC processing enzyme, PC1, are associated with obesity in humans, but PC1-null mice are not obese. A recent study, however, does support a role for impaired regulation of POMC processing in the pathogenesis of obesity in mice. Deletion of the neuronal transcription factor, Nhlh2, which is expressed in POMC neurons and regulates PC1 and PC2 mRNA levels, results in adult onset obesity (132). The null mice have normal POMC mRNA levels in the arcuate but have reduced levels of α-MSH with relatively more ACTH and pro-ACTH.

4.1.2. Human *POMC* mutations and mutations in POMC processing enzymes

In 1998, Krude and colleagues reported two patients from Germany with genetic *POMC* deficiency characterized by adrenal insufficiency, red hair pigmentation and early-onset obesity (133). The first patient was found to be a compound heterozygote for two mutations in exon 3 that resulted in ACTH and α-MSH deficiency. She had a normal birth weight and was diagnosed with adrenal insufficiency when she developed cholestasis at 3 weeks of age and was treated with hydrocortisone replacement. Increased appetite and obesity were first noted at 4 months of age. The second patient

was homozygous for a mutation in exon 2 which abolishes *POMC* translation. His birth weight was normal and obesity was first noted at 5 months of age. Adrenal insufficiency was diagnosed at 12 months of age when he developed hypoglycemia and hyponatremia and was treated with hydrocortisone replacement. Subsequent development in both children was normal except for the abnormal eating behavior and obesity. Both children had pale skin and red to red-orange hair color. The heterozygous parents in both families had normal adrenal function and did not have obesity or red hair. Remarkably, the children were obese despite adrenal insufficiency which normally leads to anorexia and weight loss. The contrast between these patients with generalized POMC deficiency and with the more typical patients who have POMC deficiency limited to the pituitary, underscores the critical role that hypothalamic POMC plays in regulating energy balance. Two other pediatric patients were subsequently described with a similar POMC deficiency syndrome (134). A 4 year old boy from Slovenia was found to be a compound heterozygote for two new *POMC* mutations and a boy from the Netherlands was found to have the same previously described *POMC* exon 2 mutation. Recently a novel homozygous frameshift mutation in *POMC*, predicted to lead to the lose of all POMC-derived peptides, was found in a child of Turkish origin with adrenal insufficiency and severe obesity (77). In this family, of the 12 relatives that were heterozygous for the *POMC* mutation, 11 were obese. In contrast, of the 7 relatives that were wild-type only one was obese. Thus, in humans, as in mice, the loss of one copy of the *POMC* gene predisposes to obesity.

Recently two groups have reported POMC variants that implicate ß-MSH in the control of human body weight regulation (135,136). In one study, 538 patients with severe, early-onset obesity were screened for *POMC* mutations and 5 unrelated probands, who were heterozygous for a rare missense variant in the region coding for ß-MSH, were identified (135). In the other study, a similar mutation was found during a screen of 15 severely obese children (136). Compared to wild-type ß-MSH, the ability of the variant peptide to bind to and activate the MC4-R was impaired. A missense mutation that disrupts the dibasic amino acid cleavage site between ß-MSH and ß-EP has also been reported to occur more frequently in obese children (137).

Impaired processing of POMC has also been associated with human obesity in three instances. In two patients the processing abnormality was due to mutations in the prohormone convertase 1 gene (138, 139) but in the third patient the cause remains obscure (140). Further evidence that POMC may modulate weight level in humans is provided by a study in a population of Mexican Americans showing a linkage of serum leptin levels and fat mass to an interval on chromosome 2 which includes the POMC locus (141). Subsequent studies in a French and in an African-American population have reported similar associations (142,143).

4.2. Melanocortin Receptor Mutations

4.2.1. *Mc-r* null mice

Mice with targeted deletion of the melanocortin-4 receptor (MC4-R) display an obesity syndrome that parallels the obesity phenotype exhibited by mice that overexpress the endogenous melanocortin receptor antagonists, agouti protein and AGRP (9,10). With complete absence of MC4-R, mice develop a maturity onset obesity characterized by hyperphagia, hyperinsulinemia, hyperglycemia, hyperleptinemia, and increased body length. Mice heterozygous for the *Mc4-r* deletion display a phenotype intermediate to that of wild-type and homozygous littermates. Pair feeding studies have demonstrated that hyperphagia alone does not entirely account for the obesity developed by *Mc4-r* knockout mice. *Mc4-r-/-* mice still weighed significantly more than wild-type mice when their food intake was restricted to that of the wild-type mice (144). Pair-fed *Mc4-r-/-* mice also demonstrated increased adiposity consistent with effects of MC4-R on energy partitioning (144). Oxygen consumption was also reduced among *Mc4-r* knockout animals compared to wild-type (144). Normally, wild-type mice respond to increased fat in the diet with increased diet-induced thermogenesis and physical activity; neither response was observed in *Mc4r* -/- mice (145). *Mc4r* -/- mice also exhibit an attenuated increase in fatty acid oxidation after exposure to a HF diet, consistent with a role of the MC4-R in regulating fat metabolism in the liver (146). Selective restoration of the *Mc4r* to the PVN of *Mc4r* -/- mice has been shown to attenuate the obesity primarily by decreasing hyperphagia but the reduced energy expenditure was unaffected (147). Thus food intake and energy expenditure are regulated by distinct melanocortin pathways. Finally, α–MSH analogs fail to suppress feeding or stimulate metabolic rate in *Mc4r* -/- animals which implies that MC4-R is the receptor primarily responsible for mediating the effects of α–MSH on energy balance (148,149).

The importance of the MC4-R in modulating insulin sensitivity has also emerged from studies of *Mc4r* null mice and it is clear that their insulin resistance is not merely secondary to the obesity. Young *Mc4*-r deficient mice, maintained on a low fat diet, exhibited fasting hyperinsulinemia and impaired insulin tolerance even prior to the development of hyperphagia or obesity (150). Additional support for the MC4-R in modulating insulin sensitivity is provided by a study, using icv infusion of antisense oligonucleotide to reduce MC4-R expression in the hypothalamus, that resulted in decreased insulin sensitivity even in the absence of significant differences in feeding and adiposity (151).

While studies in both rodent models and obese humans have defined a critical role for MC4-R in mediating energy homeostasis, the exact contribution of MC3-R is not entirely clear, but is likely related to feed efficiency and energy partitioning of fat stores. *Mc3-r* deficient mice have a milder obesity phenotype which is distinct from that of the *Mc4-r* knockout. *Mc3-r* knockout mice have increased fat mass and reduced lean body mass but are not hyperphagic (12,13). In addition, *Mc3-r*

deficient mice are only mildly hyperinsulinemic and exhibit increased feed efficiency as well as a significantly higher respiratory quotient compared to wild types when placed on a high fat diet (12). Moreover, mice lacking both MC3 and MC4 receptors are significantly heavier than mice deficient in MC4-R alone (13). Thus both receptors regulate energy balance in a non-redundant manner. In addition, the MC3-R and MC4-R play very different roles in tumor-induced cachexia. While *Mc4-r* null animals are relatively protected from disease induced anorexia and cachexia, *Mc3-r* null animals demonstrate enhanced susceptibility with increased weight loss and reduction in lean body mass (86).

4.2.2. Human *MC3/4-R* mutations

MC4-R mutations are considered to be the most common monogenic form of severe obesity. In 1998, heterozygous frameshift mutations in the *MC4-R* gene were first reported in association with dominantly inherited obesity (152,153). Subsequently, many *MC4-R* gene mutations have been detected in multiple ethnic groups (154,155 -156). One large study of 500 patients reported that 5.8% of patients with severe early onset morbid obesity harbor pathogenic mutations of the *MC4-R* (157). A recent review of several large series noted the discovery of 91 mutation carriers (3%) in 3057 children and adolescents with severe early onset obesity and also reported for the first time an incidence of 2.8% in 769 obese adults with later onset obesity (158). Although the majority of *MC4-R* gene mutations altering MC4-R activity have been detected in patients with obesity, some subjects with these variants were not obese. Thus, it appears that haploinsufficiency mutations in the *MC4-R* gene promote obesity with variable expressivity. In light of the many potential factors contributing to the regulation of body weight, it is also reasonable to expect that a combination of genetics and environment may alter the phenotypic expression of these gene variants.

Obese individuals with *MC4-R* mutations demonstrate hyperphagia, hyperinsulinemia and increased linear growth in childhood (156,157). These subjects display a marked increase in bone mineral density and possess not only increased fat mass but increased lean mass as well (157). Thyroid, adrenal, and reproductive axes appear to be normal in these patients. Thus the human phenotype caused by impaired MC4-R function appears to resemble that of the *Mc4-r* knockout mouse.

Functional studies have also provided cogent supporting evidence for the pathogenic role of *MC4-R* mutations in causing obesity. *MC4-R* mutations leading to defective cell surface expression of the receptor or alterations in ligand binding affinity and impaired cAMP generation have been documented (159). *MC4*-R mutations that lead to impairments in the constitutive activity of MC4-R have also been described, and it has been suggested that this may contribute to the development of obesity in some carriers (160). There is a correlation between *in vitro* function of the mutant *MC4-R* and the clinical phenotype (157). An association between the severity of the functional alterations in the MC4-R and the age of onset of obesity has also been reported (158). These studies provide compelling data for the role of the MC4-R in controlling energy balance in humans.

Although several *MC3-R* mutations have been found in humans, the data regarding their relevance to the pathogenesis of human obesity is, thus far, inconsistent. One study of 355 overweight and non-overweight children found two partially inactivating polymorphisms of *MC3-R* associated with high body weight (161). However, another report examining the same polymorphism in an adult population found no such correlation (162). Similarly, a study of 252 morbidly obese adults and 312 controls detected 3 *MC3-R* gene variants with equal frequency in both the obese and control groups (163). Thus, convincing evidence that MC3-R mutations have a major impact on the pathogenesis of human obesity is lacking.

4.3. AGRP Mutations

4.3.1. *Agrp* null mice

Hyperphagia and obesity are produced by either chronic central infusion of AGRP or transgenic overexpression of AGRP. One might have then predicted that deletion of *Agrp* would generate a lean mouse resistant to diet induced obesity. Instead, a surprisingly normal phenotype was initially reported. In one study, *Agrp* -/- mice, studied on a mixed genetic background, demonstrated weight gain and feeding behavior that was no different from wild-type mice (115). Furthermore, changes in body weight and reflex hyperphagia in response to fasting as well as cumulative food intake during refeeding were comparable between *Agrp* -/- and wild-type mice (115). When challenged with a high fat diet, weight gain was similar in *Agrp* -/- and wild-type mice (115). In a more recent study, *Agrp*-/- mice were independently generated and studied on the C57BL/6J background and were found to have an age related lean phenotype that only became evident after 6 months (164). At 6 months the *Agrp*-/- mice demonstrated reduced body weight and adiposity that was associated with elevations in metabolic rate, body temperature, and locomotor activity (164). This was accompanied by increased circulating thyroid hormone levels and greater UCP-1 expression in brown adipose tissue which could both contribute to the genesis of this age related lean phenotype (164). Developmental compensatory changes are likely responsible for the relatively normal phenotype exhibited by *Agrp*-/- mice as recent studies have revealed a lean, hypophagic phenotype in mice with postembryonic ablation of AGRP neurons and the degree of compensation depends on the age at the time of neuronal ablation. In one study, the human diphtheria toxin receptor was targeted to the *Agrp* locus which allows temporally controlled ablation of AGRP neurons after injection of diphtheria toxin (165). Neonatal ablation of AGRP neurons had little effect on feeding while ablation in adults caused a profound decrease in food intake and body weight. Another group, using a similar approach, also reported that selective ablation of AGRP neurons in adults results in acute reduction of feeding (166). Finally a third group using a somewhat different neurotoxic approach, also noted a lean, hypophagic phenotype (167). It should be noted that the manipulations in these studies lead not just to

the loss of AGRP, but to the entire neuron, and it is at this point unclear how much of this phenotype is due to loss of AGRP versus GABA and NPY which are also synthesized by these neurons. Indeed, there is evidence for upregulation of POMC despite the lean phenotype that could result from the loss of GABA inhibitory inputs (165).

4.3.2. Human *AGRP* mutations

There is some evidence that polymorphisms in both the promoter and coding regions of AGRP are associated with body weight regulation and a propensity towards a lean phenotype. In one population study of 874 subjects in Quebec, investigators found that homozygosity for a substitution of alanine to threonine at codon 67 in exon 3 of the AGRP gene (Ala67Thr) was associated with significantly lower body weight, adiposity, and fat free mass compared to heterozygous patients (168). This same polymorphism in the heterozygous state was also found at a significantly higher frequency (11%) among patients with anorexia nervosa compared to 4.5% in Dutch and German control patients (169). Although the Quebec study did not reveal any correlation between body weight and heterozygosity for the Ala67Thr allele, Argyropoulos *et al.* did find an association between Ala67Thr and the presence of lower BMI, fat mass, percent body fat, and amount of abdominal visceral fat but only among a population with a mean age of 53 years (170). However, a Dutch study with subjects whose mean age was 29 found that male subjects with the Thr67 allelic variant actually had higher BMI values than those who were Ala/Ala homozygous (171). Thus, the data regarding the impact of these *AGRP* polymorphisms are somewhat inconsistent, and further study is needed before conclusions on their true effect in modulating human energy balance can be drawn.

Another polymorphism located in the promoter region of the *AGRP* gene was identified that was found to affect transcription factor binding affinity (172). The variant, conferring increased binding affinity, was significantly associated with high BMI and type 2 diabetes in an African population. Similar results were found among Black patients in the Heritage Family Study (173). This latter study also demonstrated that subjects homozygous for the allelic variant with reduced binding affinity had significantly lower BMI and adiposity than heterozygous patients (173). Thus, it is possible that this polymorphism may offer protection from development of obesity in certain populations.

5. MELANOCORTIN SYSTEM AS A POTENTIAL TARGET FOR THE TREATMENT OF OBESITY AND CACHEXIA

The studies described above clearly indicate an important role for the central melanocortin system in maintaining energy balance and have potential therapeutic implications for human obesity and cachexia. One therapeutic approach for the treatment of obesity would be to increase the activity of the central melanocortin pathway by administration of α-MSH analogues. Conversely, selective blockade of central MC-Rs could be used to decrease the cachexia associated with many chronic illnesses (174). An important consideration in developing effective α-MSH agonists and antagonists is that the potential compounds cross the blood-brain barrier and gain access to appropriate central melanocortin receptors. Numerous animal studies have demonstrated inhibitory effects of either native α-MSH or of α-MSH analogues on food intake, body weight gain and adiposity. In most of these studies, peptides were administered directly into the brain by the intracerebroventricular route. Peripheral administration of α-MSH has, however, been effective in a few studies with the largest effect on body weight demonstrated in *Pomc* knockout mice with lifelong α-MSH deficiency (7). Peripheral injection of the MSH analog MTII was also effective in suppressing body weight gain in leptin receptor deficient rats and in diet-induced obese mice (61,68).

Potent, stable MSH analogues have been administered to a small number of human subjects but to date published studies have focused on skin tanning and on erectile function rather than on energy balance. Both [Nle^4, $DPhe^7$] α-MSH, referred to as melanotan-I (MT-I), and Ac-Nle-c[Asp-His-DPhe-Arg-Trp-Lys]-NH_2, referred to as melanotan-II (MT-II), have been shown to be effective in promoting tanning and providing photoprotection when given subcutaneously for several weeks (175-177). Side effects noted in these studies included nausea, vomiting, anorexia, gastrointestinal discomfort, flushing and fatigue in a substantial proportion of subjects. MT-II was noted to produce penile erections in normal men during the skin tanning studies and has subsequently been evaluated for treatment in men with erectile dysfunction (178). A new MTII analog, PT-141 is currently being evaluated for the treatment of erectile dysfunction via the intranasal route (179). Although these analogs are likely acting on the MC1-R in the skin to produce tanning, the exact locus of action of the other reported effects is still unknown. No effects on energy balance were reported in the published MTI and MTII human studies but interpretation may have been complicated by the side effect profile. Effects on body weight and adiposity have been reported in humans treated via the intranasal route with the MSH fragment $ACTH_{4-10}$ which is much less potent than either MTI or MTII. An initial study reported that intranasal $ACTH_{4-10}$ reduced body fat in normal weight subjects (180). However, subsequent studies showed that intranasal $ACTH_{4-10}$ had no effect on body weight or adiposity in obese subjects or in two *POMC* deficient subjects (181,182).

The challenge for the treatment of obesity will be to develop stable, well-tolerated α-MSH analogues which gain access to and selectively activate the appropriate central melanocortin receptors that regulate food intake and energy expenditure. Another treatment approach would be to enhance melanocortin signaling by selectively antagonizing AGRP. Since AGRP neurons project less widely than POMC neurons and thus interact with only a subset of MC-Rs, antagonism of AGRP should produce more selective effects than administration of an MSH agonist. Such a strategy and might also avoid some of the side effects noted with MSH agonists. Additional

approaches might include treatment with compounds that stimulate hypothalamic POMC synthesis and increase α-MSH release or inhibit AGRP synthesis. In addition, downstream pathways that mediate effects of melanocortin receptor activation could also be targeted. There is still much to be learned about these downstream pathways and how the melanocortin pathway interacts with other central and peripheral regulators of energy homeostasis. Thus, while there has been considerable progress in elucidating the physiology of the central melanocortin system and its role in regulating energy balance, the science has yet to fully realize its therapeutic promise.

6. PERSPECTIVE

The central melanocortin system, which consists of POMC, AGRP and the brain melanocortin receptors, plays a key role in regulating feeding behavior and energy homeostasis. A growing number of studies in both the mouse and the human with genetic defects in the synthesis or processing of POMC, or with defects in melanocortin receptor signaling, clearly indicate the importance of this system. A genetic POMC deficiency syndrome characterized by adrenal insufficiency, red hair pigmentation and early-onset obesity has been described in the human. It is particularly striking that obesity occurs in patients with generalized POMC deficiency and in *Pomc* knockout mice despite the presence of adrenal insufficiency, which under other circumstances, would lead to weight loss. The contrast between these patients with generalized POMC deficiency and the more typical patients with POMC deficiency limited to the pituitary, underscores the critical role that hypothalamic POMC plays in regulating energy balance. The importance of the MC4-R in this process is demonstrated by the *Mc4-r* knockout mouse and by the growing number of obese patients reported with *MC4-R* mutations, making this the most common known monogenic cause of human obesity. The melanocortin regulatory system appears to be sensitive to variations in *MC4-R* and *POMC* expression as indicated by the fact that heterozygous mutations are associated with obesity in both mice and in humans. Thus, there is considerable evidence that the hypothalamic melanocortin pathway regulates human feeding behavior and energy homeostasis and that abnormalities in this pathway can lead to obesity. A more detailed understanding of the control of this pathway and its integration with a growing number of other hypothalamic signaling pathways involved in maintaining energy balance will hopefully lead to effective new therapies for human obesity.

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Send correspondence to: Dr. Sharon L. Wardlaw, Department of Medicine, Columbia University College of Physicians and Surgeons, 630 West 168th Street, New York, NY 10032, Tel: 212-305-3725, Fax: 212-305-2282, E-mail: sw22@columbia.edu

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