Annu. Rev. Nutr. 2004. 24:133–49
doi: 10.1146/annurev.nutr.24.012003.132428

First published online as a Review in Advance on January 16, 2004

THE CRITICAL ROLE OF THE MELANOCORTIN SYSTEM IN THE CONTROL OF ENERGY BALANCE

Randy J. Seeley, Deborah L. Drazen, and Deborah J. Clegg
Department of Psychiatry, University of Cincinnati, Cincinnati, Ohio 45267-0559; email: Randy.Seeley@uc.edu; Deborah.Drazen@uc.edu; Debbie.Clegg@uc.edu

Key Words obesity, food intake, energy expenditure, metabolism

■ **Abstract** Animals have developed highly adaptive and redundant mechanisms to maintain energy balance by matching caloric intake to caloric expenditure. Recent evidence has pointed to a variety of peripheral signals that inform specific central nervous system (CNS) circuits about the status of peripheral energy stores as critical to the maintenance of energy balance. A critical component of these CNS circuits is the melanocortin system. Regulation of signaling by melanocortin 3 and melanocortin 4 receptors in the CNS is controlled via neuronal cell bodies in the arcuate nucleus of the hypothalamus that synthesize melanocortin receptor agonists such as alpha-melanocyte-stimulating hormone (α-MSH) or antagonists such as agouti-related protein (AgRP). The activity of these two populations of neurons is reciprocally regulated by a number of peripheral and central systems that influence energy balance. Further, increased melanocortin signaling via pharmacological or genetic means in the CNS causes potent reductions in food intake and weight loss. Decreased melanocortin signaling via pharmacological or genetic means results in increased food intake and weight gain. Reviewed here is the wide range of evidence that points to the melanocortin system as a critical node in the diverse neurocircuitry that regulates food intake and body weight.

CONTENTS

INTRODUCTION

The amount of stored fuel in the body is regulated in a very accurate manner. To accomplish this, energy intake must be matched to energy expenditure over time. Over the past decade, our understanding of how this is accomplished has increased enormously. A major component of that increased understanding has been the identification of neural systems that can influence food intake and/or energy expenditure. Over the past decade, powerful molecular biological tools have identified dozens of neurotransmitters and receptor systems with a potential role in the maintenance of energy balance. This embarrassment of riches has led to an alternative dilemma, which is how to knit several dozen neurotransmitter systems into functional circuits whose activity might be altered so as to provide treatment strategies for the escalating problem of obesity. To this end, it is critical to identify primary neural systems that respond directly to changes in peripheral energy status. This review details the wealth of evidence that points to the melanocortins and their associated receptors as a vital component of the ongoing matching of caloric intake to caloric expenditure.

Many lines of evidence indicate the important role of circulating hormones in the regulation of energy balance (22, 35). In particular, hormones appear to provide information about the status of peripheral energy stores in the form of adipose tissue. Such adiposity signals act in the central nervous system (CNS) to alter subsequent food intake and energy expenditure in order to maintain adequate supplies of stored fuel. To do so, adiposity signals must have several properties (94). First, levels of these signals need to vary appropriately with energy status. Second, they must cross the blood-brain barrier, where they can act on appropriate receptors in CNS regions linked to the control of energy balance. Third, changes in signaling for adiposity signals must result in predictable changes in energy balance.

Several hormones have been hypothesized to act as such an adiposity signal. The one that has received the most attention is the adipocyte-derived hormone leptin. Leptin is made primarily in adipocytes and under most circumstances circulates in proportion to the amount of adipose tissue (25). It is capable of crossing the blood-brain barrier (2, 11, 76), where it interacts with specific receptors found on a variety of neuronal populations (77). Further, administration of leptin directly into the CNS results in reduced food intake and weight loss (81), whereas reduced leptin signaling through a variety of means results in increased food intake and weight gain (13, 48). Thus, leptin fulfills all of the criteria of being an adiposity signal.

Although it receives less attention, insulin also fulfills the criteria of being an adiposity signal (75, 92). Although insulin levels rise and fall in response to blood glucose levels, both basal levels and the 24-hour area under the curve are proportional to body fat (68, 69). Moreover, insulin crosses the blood-brain barrier, where it can interact with insulin receptors found in several regions in the CNS. Insulin administration directly into the CNS causes a reduction of food intake and body

weight in a number of species (92, 93, 95). Reductions in insulin signaling, via either administration of insulin antibodies or targeted deletion of insulin receptors in the CNS, result in increased food intake and body weight (10, 57). The totality of these data support the hypothesis that both leptin and insulin provide important negative feedback signals to the CNS concerning the status of peripheral energy stores.

MELANOCORTINS AND THEIR RECEPTORS

Identifying peripheral signals that inform the CNS is just one of several important steps in understanding how energy balance is regulated. Also critical is understanding the neural circuitry upon which these peripheral signals act. This has been made more complicated by the large number of neurotransmitters that has now been linked to the control of either food intake or energy expenditure. One strategy to begin building these functional circuits is to focus upon the systems that receive the most direct input from peripheral adiposity signals. One region with high levels of expression for both insulin and leptin receptors is the arcuate nucleus of the hypothalamus (4, 77). Thus, the systems contained in the arcuate nucleus are a logical starting point to begin unraveling the complicated neural underpinnings of the regulation of energy balance. Although the arcuate contains a number of neuropeptide systems that can influence energy balance, it is the primary site in which a variety of ligands for the receptors for the melanocortin system are produced.

As the name implies, the melanocortin system was first linked to the control of skin and hair pigmentation. Several important melanocortin receptor ligands come from the precursor peptide proopiomelanocortin (POMC). In particular, alpha-melanocyte-stimulating hormone (α-MSH) acts on the first of five identified melanocortin receptor subtypes (MC1) located primarily in the skin and hair to produce dark pigmentation (26). A second melanocortin ligand produced from POMC is adrenocorticotrophic hormone (ACTH). ACTH is produced in the pituitary as an essential part of the stress response. ACTH circulates in the blood and acts upon MC2 receptors found primarily in the adrenal gland to stimulate glucocorticoid secretion (26).

POMC is made not only in the periphery but also in the CNS and particularly within the arcuate nucleus of the hypothalamus. These neurons project to a number of other hypothalamic regions and project directly to sympathetic efferent projections in the spinal cord. Thus these neurons appear to be ideally situated to be critical regulators of both food intake and energy expenditure. α-MSH is not only a potent agonist for MC1 receptors but also for MC3 and MC4 receptors that are found primarily within the CNS. Although MC3 receptors have a rather narrow distribution within specific subregions of the hypothalamus, MC4 receptors are broadly distributed in the CNS, including in several regions of the hypothalamus, but also in the nucleus accumbens and the dorsal motor nucleus of the vagus (45, 54).

MELANOCORTINS AND ENERGY BALANCE

Several lines of evidence link α-MSH action on MC4 receptors to the control of energy balance. α-MSH is an agonist at both MC3 and MC4 receptors. Central administration of α-MSH, or synthetic analogues such as melanotan II (MTII), results in potent reductions in food intake and significant loss of body weight (20, 83, 85). Moreover, synthetic antagonists for MC3/MC4 receptors can block the effects of MTII or increase food intake on their own (8, 20). The hypothesis that these actions depend primarily on MC4 receptors is supported by the observation that mice with targeted genetic disruption of the MC4 receptor have elevated food intake and body weight and show no reductions in food intake after MTII administration (41, 56). Over the last several years, agonists that are relatively more selective for the MC4 over the MC3 receptor have been developed and these compounds also reduce food intake (8).

One of the unique aspects of the melanocortin system is the existence of endogenous antagonists that work to reduce melanocortin signaling under a variety of circumstances. In the periphery, pigmentation is not only controlled by the level of activity induced by α-MSH but is the result of a balance of action between α-MSH and an antagonist for peripheral MC1 receptors termed agouti signaling protein (ASP). Whereas α-MSH works to darken pigmentation, ASP works to lighten pigmentation. What makes ASP so interesting is that it does not act just as a competitive antagonist for α-MSH at MC1 receptors. Rather it acts as an "inverse agonist," meaning that ASP can actually decrease constitutive MC1 receptor activity even in the absence of α-MSH (90). A naturally occurring mutation of ASP results in inappropriate ubiquitous expression of ASP (59). These mice, typically termed agouti or A^y mice, have a yellow coat color as a result of increased ASP action on MC1 receptors. Interestingly, the phenotype of these mice also includes increased food intake and body weight. This is a result of ASP being expressed in the brain and acting as an antagonist/inverse agonist at MC4 receptors.

The phenotype of agouti mice was among the original observations that led to the hypothesis that the melanocortin system is a critical regulator of energy balance. However, because ASP is not normally expressed in the CNS, its relevance to the control of food intake and energy expenditure under normal conditions was suspect. Like the peripheral melanocortin system, however, the CNS melanocortin system also includes an endogenous antagonist/inverse agonist that was identified by its sequence homology to ASP and was termed agouti related protein (AgRP) (24). AgRP is an antagonist/inverse agonist at MC3 and MC4 receptors (65, 67) and is found in a discrete population of neurons located entirely within the arcuate nucleus adjacent to cells that express POMC (34). Overexpression of AgRP results in mice that are hyperphagic and obese in a manner similar to agouti mice but without the yellow coat color, presumably because AgRP has little affinity for MC1 receptors (67).

A biologically active c-terminal fragment of AgRP delivered into the ventricular system of either rats or mice produces a profound increase in food intake similar

to that produced by administration of synthetic MC4 receptor antagonists (72). In the first few hours after administration of AgRP, this effect is not quite as powerful as the most potent stimulator of food intake, neuropeptide Y (NPY). However, the effect of AgRP to increase food intake is not limited to a few hours. In fact, a single administration of AgRP can continue to increase food intake for a period of up to six days (33). This effect is quite unexpected because a single injection of NPY rarely influences food intake for more than a 24-hour period. So when compared at any periods longer than a few hours, AgRP is easily the most potent endogenous orexigen yet identified.

An obvious question is how a peptide that is subject to degradation in a matter of hours can have an effect that alters behavior for a period of days. If AgRP is administered immediately prior to the MC receptor agonist MTII, it can block the anorexic effects of MTII; however, AgRP cannot block the effects of MTII that is administered 24 hours later, even though AgRP continues to be able to stimulate further food intake (33). Either nonspecific or combined mu and kappa opioid receptor antagonists can block the immediate effect of AgRP, but not the long-term effect (32). Moreover, the pattern of neuronal activation induced by AgRP two hours after its administration looks quite different from the pattern observed 24 hours after its administration (31). These observations indicate that the ability of AgRP to potently change long-term aspects of food intake is not just the result of continued melanocortin receptor blockade, but likely represents a form of plasticity induced by acute fluctuations in melanocortin signaling. The open question here is whether such plasticity is a normal component of energy balance regulation that may contribute to alterations in the defended level of body weight as a result of environmental or dietary changes or whether it is an epiphenomenon produced by exogenous administration of AgRP.

Unfortunately the regulation of melanocortin signaling is even more complicated than the number of receptors, agonists, and antagonists described thus far. It turns out that the ability of AgRP to be a competitive antagonist/inverse agonist depends importantly on a cell surface molecule termed syndecan-3. Syndecan-3 is a heparin-sulfated proteoglycan that is heavily expressed in the hypothalamus and appears to act as a coreceptor for AgRP to aid in its binding to the MC4 receptor. Overexpression of the close family member syndecan-1 results in a highly unexpected and unusual pattern of upregulation localized to the paraventricular nucleus of the hypothalamus. It also results in an obese phenotype presumably due to increased ability of existing AgRP to inhibit CNS melanocortin signaling (71).

REGULATION OF THE CNS MELANOCORTIN SYSTEM

To play an important role in the control of energy balance, changes in the melanocortin system must result in changes in body weight, and changes in energy balance must alter the amount of melanocortin signaling in the CNS. Food restriction and the resulting weight loss increase AgRP gene expression in the arcuate nucleus

(61, 62) while simultaneously decreasing POMC gene expression (78). These two changes would be expected to result in a net decrease in melanocortin signaling because of decreased levels of the agonist and increased levels of the antagonist at the critical melanocortin receptors. Because melanocortin signaling is associated with decreased food intake, this response is consistent with the hypothesis that regulation of the CNS melanocortin system is an important contributor to the response to negative energy balance that maintains constant levels of adiposity. On the positive energy balance side, POMC expression is increased in the arcuate nucleus as a function of involuntary overfeeding, and the decreased food intake associated with involuntary overfeeding can be blocked by exogenous administration of melanocortin receptor antagonists (30). These results are consistent with the hypothesis that increased melanocortin receptor signaling is critical to the regulatory responses that occur during positive energy balance as well.

The potent responses of the melanocortin system to manipulations of energy balance raise the question of how the melanocortin system senses changes in energy balance. Several lines of evidence point to the actions of adiposity signals directly upon melanocortinergic neurons in the arcuate. First, both insulin and leptin receptors are found on POMC neurons, and central administration of insulin or leptin can stimulate POMC gene expression in fasting animals (7, 60, 78). Further, leptin-deficient mice show increased expression of AgRP and decreased expression of POMC that can be normalized by exogenous leptin administration (60, 78). Leptin also increases the neuronal firing rate in POMC neurons (16). Finally, the actions of central administration of both insulin and leptin to reduce food intake and body weight can be blocked by exogenous melanocortin antagonists (7, 80). Taken together, these data make a strong case that the melanocortin system is an important effector system that mediates the effects of adiposity signals in the CNS to regulate energy balance.

Adiposity signals do not represent the only input into the CNS melanocortin system. Other peripheral peptides have been hypothesized to have an action directly upon melanocortinergic neurons in the arcuate. For example, the stomach makes a peptide called ghrelin that stimulates food intake when given either peripherally or directly into the CNS (39, 42). Consistent with this action of ghrelin, circulating levels of ghrelin are increased after periods of fasting and decreased during feeding (the opposite of leptin) (39, 42). Moreover, ghrelin reduces activity in POMC neurons via actions on ghrelin receptors (also termed growth hormone secretagogue receptors) (15). Additionally, circulating levels of peptide YY(3–36) [PYY(3–36)] are increased after meals, and peripheral or interarcuate injections of PYY(3–36) have been reported to reduce food intake. PYY(3–36) also results in increased activity of POMC neurons (6). These data support the hypothesis that a variety of circulating peripheral factors may act upon melanocortin neurons in the arcuate to mediate their role in the control of energy balance.

It is not merely peripheral hormones that appear to have actions on the CNS melanocortin system. The serotonergic system has long been linked to the control of food intake, and the primary actions of one of only two currently FDA-approved

weight-loss medications are on the serotonergic system. In fact, such serotonergic agonists appear to act via the melanocortin system as well. Serotonin receptors are found on POMC neurons, dex-fenfluramine stimulates activity of POMC neurons, and its ability to reduce food intake is blocked by melanocortin antagonists (36). Taken together, these data indicate that the CNS melanocortin system is a nodal point that integrates a number of important inputs to influence food intake and energy expenditure in the service of energy balance regulation.

HUMAN GENETICS OF THE MELANOCORTIN SYSTEM

The growing epidemic of obesity and the clear indication that susceptibility to obesity includes an important genetic component have increased the urgency to understand the underlying genes that contribute to body weight regulation. Although they are exceedingly rare, several single gene mutations that cause human obesity syndromes in a Mendelian fashion have been instrumental in defining the role of key molecules in appetite control, such as leptin (63, 82) and the leptin receptor (12). Given the evidence summarized above, it is logical to extend this analysis to search for monogenic obesity syndromes associated with critical aspects of the melanocortin system that could provide conclusive evidence that this system is as critical in humans as it appears to be in animal models.

The first direct evidence for the involvement of melanocortin peptides in the control of human food intake came from the description of two patients with defects in the POMC gene (50). In these patients, obesity occurred in the first month of life and increased dramatically due to constant hyperphagia (50). One patient was compound heterozygous for two mutations, which prevented synthesis of ACTH and α-MSH. The second, unrelated patient was homozygous for a nucleotide transversion upstream of the start codon, which interferes with translational initiation by introducing an additional start site. As expected, these patients demonstrated other predicted effects of POMC deficiency including very low levels of glucocorticoids, very light skin color, and bright red hair. In addition to defects in the prohormone itself, disruptions of the melanocortin system can result from an inability to appropriately process POMC. Prohormone convertase 1 is a cleavage enzyme responsible for cleaving a number of prohormones, including POMC. Two patients with mutations in prohormone convertase 1 that resulted in undetectable levels of α-MSH also showed similar phenotypes, including early-onset obesity (43). These case studies provide compelling evidence for an important role of POMC in human body weight regulation.

Although complete POMC deficiency is rare, four studies have linked a region of chromosome 2 encompassing the POMC gene to obesity in broader populations. One study of a Mexican American population reported that this locus accounted for 47% of the variation in serum leptin levels (14). Similar associations were reported in subsequent studies of French families (37) and a population of African Americans (73). Thus, polymorphisms in the POMC gene and/or its promoter

may be linked to susceptibility to common obesity, particularly in conjunction with other genetic mutations that also increase susceptibility.

Another identified monogenic obesity syndrome is the result of MC4 receptor mutations. Several authors have suggested that MC4 receptor mutations are the most frequent monogenic obesity syndromes, with estimates of up to 4% of morbidly obese patients showing mutations of the MC4 receptor (97). Most compelling, Farooqi and colleagues sequenced the MC4 receptor gene in 500 patients with early onset obesity and showed that 5.8% had mutations in the MC4 receptor gene (22, 23, 97). Moreover, the severity of the obesity in these patients could be predicted based on the ability of the mutation to alter in vitro MC4 receptor function. Although considerable controversy still surrounds these genetic observations (3), such data provide a strong argument that MC4 receptor activity is an important determinant of body weight in humans even if mutations of this gene only account for a minority of the obesity now observed in developed countries.

The bottom line of the information summarized thus far is that evidence links the activity of the melanocortin system to the regulation of energy balance. The evidence is extremely diverse and includes pharmacological studies utilizing endogenous and synthetic ligands, spontaneous and directed gene mutations in mouse models, and human genetic data. Such multifactorial evidence continues to fuel considerable interest in this system as one potential contributor that separates obese from lean individuals, and as a source for therapeutic options to treat obese individuals.

OTHER FUNCTIONS ASSOCIATED WITH THE MELANOCORTIN SYSTEM

Peripheral Glucose Homeostasis

Obesity is highly correlated with hyperinsulinemia, insulin resistance, and hyperglycemia in humans (58). Most animal models of obesity are also associated with varying degrees of hyperinsulinemia and hyperglycemia. As discussed in the previous sections, the central melanocortin system plays a crucial role in energy homeostasis, integrating numerous inputs that influence food intake and energy expenditure. Recent evidence suggests that the melanocortin system's effects on glucose homeostasis are at least in part independent of changes in food intake.

The first evidence linking the melanocortin system to glucose homeostasis is from studies in which the peripheral administration of α-MSH increased plasma insulin, glucose, and glucagon levels (46, 47). Data from obese mouse models with genetic impairments of the melanocortin system also demonstrate a link between melanocortins and glucose homeostasis. Male MC4R$-/-$ mice, for example, display marked hyperinsulinemia in addition to hyperphagia, adult-onset obesity, and diabetes mellitus (41). A gene dosage effect has been observed for this phenotype, with late-onset hyperinsulinemia and glucose intolerance present in the

heterozygous mice (41). Impairments of glucose homeostasis have also been observed in MC3R−/− mice and agouti mice (64).

Recent data on POMC-deficient mice demonstrate that they maintain normal serum levels of insulin, normal fasting levels of glucose, and normal clearance of glucose during a glucose tolerance test (38). POMC−/− mice, however, have a severe impairment in the glucagon-mediated counter-regulatory response after insulin-induced hyperglycemia that can be reversed by peripheral application of α-MSH (38). Because POMC also encodes for ACTH that stimulates glucocorticoid secretion, POMC-deficient mice also are profoundly hypocortisolemic, which potentially confounds the role of α-MSH in peripheral glucose homeostasis in this model.

Recent data indicate that central melanocortin signaling may have a tonic inhibitory effect on insulin secretion, which suggests that this system may be involved in the control of glucose homeostasis independent of effects on food intake (21). Young, lean MC4R−/− mice have increased plasma insulin levels as well as reduced insulin tolerance prior to the onset of detectable hyperphagia, obesity, or changes in free fatty acid levels. In addition, the central administration of MTII to C57BL/6J mice dose-dependently inhibits basal plasma insulin and reduces glucose tolerance. In hyperinsulinemic ob/ob mice, MTII decreases insulin concentrations, which is not secondary to a reduction in blood glucose. The inhibitory effect of MTII on insulin release is completely blocked by the nonspecific α-adrenoreceptor agonist phentolamine, which suggests that the melanocortins may increase the sympathetic drive to the pancreas to exert a tonic inhibitory effect on insulin secretion (21).

Further support for the effects of the central melanocortin system on insulin action can be found in a study in which week-long infusions of either α-MSH or the high-affinity melanocortin receptor antagonist, SHU9119, were centrally administered to rats while a constant food intake was maintained (66). α-MSH markedly enhanced the action of insulin on glucose uptake and production from the liver, whereas an antagonist had the opposite effects on these parameters that are not secondary to changes in food intake (66).

Reproduction

The central melanocortin system also has a wide range of effects on both male and female reproductive functions (74). It is well established that α-MSH and ACTH cause potent erections in male mice, rats, and rabbits (1, 9), and it has been hypothesized that this effect is mediated by the MC3R (87). In addition, administration of MTII can evoke spontaneous erections in men (89), and in humans α-MSH stimulates gonadotropin secretion (51). Recent evidence suggests that the melanocortins also exert their effects on sexual function via the MC4R (86). For example, the MC4R has been implicated in the modulation of penile erection, and the MC4R−/− mouse has an impairment in copulatory behavior in both motivation and performance parameters (86). A heterozygous frameshift mutation in the

human MC4R gene, however, which results in obesity, does not result in reproductive incompetence (97). Humans with mutations in the POMC gene also appear to have normal reproductive function (50). In rodents, female sexual behavior is influenced by melanocortins as well. For example, α-MSH administration into the ventromedial nucleus of the hypothalamus of female rats stimulates both sexual receptivity and lordosis, and this may be mediated by the MC3R (17). There is also evidence that α-MSH is involved in the regulation of luteinizing hormone and prolactin (44, 79). More research is needed to determine the precise role of the melanocortins in reproductive physiology and behavior.

Cardiovascular Function

The melanocortins have multiple effects on cardiovascular function, and more than one melanocortin receptor, and possibly nonmelanocortin receptors, appears to mediate these effects (88). For example, the administration of δ-MSH and ACTH to rats causes an increase in blood pressure and heart rate (19). Members of the δ-MSH family also cause an increase in macro- and microcirculatory cerebral blood flow (18). These effects of δ-MSH appear to depend on the maintenance of sympathetic drive to the vascular system and the heart (88).

Inflammation

α-MSH has been shown to have anti-inflammatory effects in every animal model studied to date (53). The peptide reduces acute inflammation induced by peripheral administration of inflammatory stimuli or the injection of mediators of inflammation, such as cytokines and leukotrines. α-MSH acts both directly on peripheral inflammatory cells (i.e., neutrophils, macrophages) and within the brain to inhibit inflammation. A dose-dependent reduction in inflammation in peripheral tissues (e.g., ear, foot) is observed after central injection of α-MSH (52). Blockade of β2-adrenergic receptors, but not β1-adrenergic receptors, effectively reduces the anti-inflammatory effect of central α-MSH, though the precise location of β2 receptors in the pathway remains to be determined (55). There is also evidence that α-MSH acts within the brain to inhibit local production of tumor necrosis factor-alpha (TNF-α). The rapid anti-TNF-α effects of α-MSH within the brain appear to occur via direct action of α-MSH on brain cells that produce TNF-α (e.g., astrocytes and microglia) (53).

OPEN QUESTIONS ABOUT THE MELANOCORTIN SYSTEM

Although our understanding of the important role for the melanocortin system has grown rapidly in the last decade, a number of important questions have yet to be answered. In the CNS, the melanocortin system is not limited to the hypothalamus. In fact, MC4 receptors are widely distributed, including significant populations in

the nucleus accumbens and in the dorsal motor nucleus of the vagus (45, 54). In fact, administration of both MC4 receptor agonists and antagonists in the region of the caudal brainstem is as potent in reducing food intake as that in the region of the hypothalamus (27). Moreover, POMC is made not only in the hypothalamus but also in the caudal brain stem. A role for α-MSH derived from these neurons has yet to be clearly established, but remains a viable way in which melanocortin signaling in the CNS could be altered and contributes to the regulation of food intake and body weight under different conditions (28, 29). A complete understanding of the CNS melanocortin system will not emerge until the interplay between hypothalamic and brain stem melanocortins has been revealed.

A second open question concerns the role that peripheral melanocortin signaling plays in the regulation of energy balance. This review has largely focused on the evidence that links the central melanocortinergic system to the control of energy balance, but an important role for the peripheral system cannot be excluded. Mice with deletions of the POMC gene show profound obesity in the face of decreased melanocortin signaling in both peripheral and CNS tissues (38, 96). It is also the case that there are significant sources of α-MSH, AgRP, and ASP in peripheral tissues including adipocytes. Further, although both central and peripheral administration of melanocortin receptor ligands potently reduce food intake, recent evidence has led researchers to question if peripheral administration of these peptide ligands can activate CNS melanocortin receptors (84), and if the effects of peripheral administration are secondary to activation of peripheral melanocortin receptors. Thus, although it is clear that the CNS melanocortin system plays an important role in the regulation of energy balance, the relationship between the peripheral and central melanocortin systems remains largely undetermined.

A third and critical question is whether the melanocortin system can be exploited to provide therapeutic options for obese patients. The most obvious way to do this is to develop centrally acting small agonists specific to the MC4 receptor. The effort to produce these compounds is substantial and is ongoing in several major pharmaceutical houses. Several concerns remain about such ligands. In particular, there is a significant possibility that activation of MC4 receptors may result in unwanted side effects related to extrahypothalamic populations of MC4 receptors. For example, some reports have shown significant levels of MC4 receptors in the human penis (86), and peripheral administration of melanocortin receptor ligands results in unwanted and persistent erections. These sexual side effects may make the use of MC4 agonists much more difficult. Another problem is that the reduced body weight from melanocortin receptor agonists would be expected to increase AgRP action. An increase in this endogenous activity would then serve to potentially limit the actions of the exogenous agonist and thereby limit the ultimate efficacy of the drug.

There are several alternatives to using agonists for the MC4 receptor. One such alternative is to inhibit the secretion or action of AgRP to inhibit MC4 receptor signaling. The powerful effect of AgRP to increase food intake and rapidly produce obesity supports this particular strategy. However, mice with targeted deletion

of AgRP show little or no body weight phenotype (70). Thus, pharmacological inhibition of AgRP may not result in sustained weight loss because of compensation by some other aspect of the body weight regulatory system. Another possibility would be to alter one of the several identified inputs to the melanocortin system. One suggested possibility is to use peripheral PYY(3–36) to directly activate the CNS melanocortin system (5), but considerable controversy surrounds whether PYY(3–36) can actually activate the correct set of neurons in vivo.

Regardless of the hurdles, the enormous burden of obesity compels the scientific community to increase its understanding of how energy balance is regulated. We no longer have the luxury of studying these systems as a function of scientific curiosity, but rather it is now a medical necessity. All of the evidence indicates that the melanocortin system is an important component of the complex and redundantly controlled regulatory system that maintains energy balance. Thus, for the foreseeable future the melanocortin system likely will be an important part of understanding both the etiology and potential treatment of obesity.

ACKNOWLEDGMENTS

The writing of this review was supported by grants from the NIH (DK54080) and the Procter & Gamble Company.

The *Annual Review of Nutrition* is online at http://nutr.annualreviews.org

LITERATURE CITED

1. Argiolas A. 1999. Neuropeptides and sexual behaviour. *Neurosci. Biobehav. Rev.* 23:1127–42
2. Banks WA, Kastin AJ, Huang W, Jaspan JB, Maness LM. 1996. Leptin enters the brain by a saturable system independent of insulin. *Peptides* 17:305–11
3. Barsh GS, Farooqi IS, O'Rahilly S. 2000. Genetics of body-weight regulation. *Nature* 404:644–51
4. Baskin DG, Sipols AJ, Schwartz MW, White MF. 1993. Immunocytochemical detection of insulin receptor substrate-1 (IRS-1) in rat brain: colocalization with phosphotyrosine. *Regul. Pept.* 48:257–66
5. Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, et al. 2003. Inhibition of food intake in obese subjects by peptide YY3-36. *N. Engl. J. Med.* 349: 941–48
6. Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, et al. 2002. Gut hormone PYY(3–36) physiologically inhibits food intake. *Nature* 418:650–54
7. Benoit SC, Air EL, Coolen LM, Strauss R, Jackman A, et al. 2002. The catabolic action of insulin in the brain is mediated by melanocortins. *J. Neurosci.* 22:9048–52
8. Benoit SC, Schwartz MW, Lachey JL, Hagan MM, Rushing PA, et al. 2000. A novel selective melanocortin-4 receptor agonist reduces food intake in rats and mice without producing aversive consequences. *J. Neurosci.* 20:3442–48
9. Bertolini A, Poggioli R, Vergoni AV. 1988. Cross-species comparison of the ACTH-induced behavioral syndrome. *Ann. NY Acad. Sci.* 525:114–29
10. Brüning JC, Gautam D, Burks DJ, Gillette J, Schubert M, et al. 2000. Role of brain insulin receptor in control of body weight and reproduction. *Science* 289:2122–25

11. Caro JF, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, et al. 1996. Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* 348:159–61
12. Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, et al. 1998. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 392:398–401
13. Cohen P, Zhao C, Cai X, Montez JM, Rohani SC, et al. 2001. Selective deletion of leptin receptor in neurons leads to obesity. *J. Clin. Invest.* 108:1113–21
14. Comuzzie AG, Hixson JE, Almasy L, Mitchell BD, Mahaney MC, et al. 1997. A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. *Nat. Genet.* 15:273–76
15. Cowley MA, Cone RD, Enriori P, Louiselle I, Williams SM, Evans AE. 2003. Electrophysiological actions of peripheral hormones on melanocortin neurons. *Ann. NY Acad. Sci.* 994:175–86
16. Cowley MA, Smart JL, Rubinstein M, Cerdan MG, Diano S, et al. 2001. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature* 411:480–84
17. Cragnolini A, Scimonelli T, Celis ME, Schioth HB. 2000. The role of melanocortin receptors in sexual behavior in female rats. *Neuropeptides* 34:211–15
18. de Wildt DJ, Kasbergen CM, Versteeg DH. 1995. Effect of gamma 2-melanocyte-stimulating hormone on cerebral blood flow in rats. *J. Cardiovasc. Pharmacol.* 25:898–905
19. de Wildt DJ, Krugers H, Kasbergen CM, De Lang H, Versteeg DH. 1993. The hemodynamic effects of gamma 2-melanocyte-stimulating hormone and related melanotropins depend on the arousal potential of the rat. *Eur. J. Pharmacol.* 233: 157–64
20. Fan W, Boston B, Kesterson R, Hruby V, Cone R. 1997. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* 385:165–68
21. Fan W, Dinulescu DM, Butler AA, Zhou J, Marks DL, Cone RD. 2000. The central melanocortin system can directly regulate serum insulin levels. *Endocrinology* 141:3072–79
22. Farooqi IS, O'Rahilly S. 2000. Recent advances in the genetics of severe childhood obesity. *Arch. Dis. Child.* 83:31–34
23. Farooqi IS, Yeo GS, O'Rahilly S. 2003. Binge eating as a phenotype of melanocortin 4 receptor gene mutations. *N. Engl. J. Med.* 349:606–9
24. Fong T, Mao C, MacNeil T, Kalyani R, Smith T, et al. 1997. ART (protein product of agouti-related transcript) as an antagonist of MC-3 and MC-4 receptors. *Biochem. Biophys. Res. Commun.* 237: 629–31
25. Friedman JM. 2002. The function of leptin in nutrition, weight, and physiology. *Nutr. Rev.* 60:S1–14; discussion S68–84, 5–7
26. Gantz I, Fong TM. 2003. The melanocortin system. *Am. J. Physiol. Endocrinol. Metab.* 284:E468–74
27. Grill HJ, Ginsberg AB, Seeley RJ, Kaplan JM. 1998. Brainstem application of melanocortin receptor ligands produces long-lasting effects on feeding and body weight. *J. Neurosci.* 18:10128–35
28. Grill HJ, Kaplan JM. 2002. The neuroanatomical axis for control of energy balance. *Front. Neuroendocrinol.* 23:2–40
29. Grill HJ, Schwartz MW, Kaplan JM, Foxhall JS, Breininger J, Baskin DG. 2002. Evidence that the caudal brainstem is a target for the inhibitory effect of leptin on food intake. *Endocrinology* 143:239–46
30. Hagan M, Rushing P, Schwartz M, Yagaloff K, Burn P, et al. 1999. Role of the CNS melanocortin system in the response to overfeeding. *J. Neurosci.* 19:2362–67
31. Hagan MM, Benoit SC, Rushing PA, Pritchard LM, Woods SC, Seeley RJ. 2001. Immediate and prolonged patterns

of agouti-related peptide-(83–132)-induced c-Fos activation in hypothalamic and extrahypothalamic sites. *Endocrinology* 142:1050–56
32. Hagan MM, Rushing PA, Benoit SC, Woods SC, Seeley RJ. 2001. Opioid receptor involvement in the effect of AgRP-(83–132) on food intake and food selection. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 280:R814–21
33. Hagan MM, Rushing PA, Pritchard LM, Schwartz MW, Strack AM, et al. 2000. Long-term orexigenic effects of AgRP-(83–132) involve mechanisms other than melanocortin receptor blockade. *Am. J. Physiol.* 279:R47–52
34. Hahn TM, Breininger JF, Baskin DG, Schwartz MW. 1998. Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nat. Neurosci.* 1:271–72
35. Havel PJ. 2002. Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. *Curr. Opin. Lipidol.* 13:51–59
36. Heisler LK, Cowley MA, Tecott LH, Fan W, Low MJ, et al. 2002. Activation of central melanocortin pathways by fenfluramine. *Science* 297:609–11
37. Hinney A, Schmidt A, Nottebom K, Heibult O, Becker I, et al. 1999. Several mutations in the melanocortin-4 receptor gene including a nonsense and a frameshift mutation associated with dominantly inherited obesity in humans. *J. Clin. Endocrinol. Metab.* 84:1483–86
38. Hochgeschwender U, Costa JL, Reed P, Bui S, Brennan MB. (Sept. 11, 2003.) Altered glucose homeostasis in POMC null mouse mutants lacking central and peripheral melanocortin. *Endocrinology* 10.1210/en.2003-1008
39. Horvath TL, Diano S, Sotonyi P, Heiman M, Tschop M. 2001. Minireview: ghrelin and the regulation of energy balance—a hypothalamic perspective. *Endocrinology* 142:4163–69
40. Deleted in proof
41. Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, et al. 1997. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 88:131–41
42. Inui A. 2001. Ghrelin: an orexigenic and somatotrophic signal from the stomach. *Nat. Rev. Neurosci.* 2:551–60
43. Jackson I. 1997. Homologous pigmentation mutations in human, mouse and other model organisms. *Hum. Mol. Genet.* 6:1613–24
44. Khorram O, Bedran de Castro JC, McCann SM. 1984. Physiological role of alpha-melanocyte-stimulating hormone in modulating the secretion of prolactin and luteinizing hormone in the female rat. *Proc. Natl. Acad. Sci. USA* 81:8004–8
45. Kishi T, Aschkenasi CJ, Lee CE, Mountjoy KG, Saper CB, Elmquist JK. 2003. Expression of melanocortin 4 receptor mRNA in the central nervous system of the rat. *J. Comp. Neurol.* 457:213–35
46. Knudtzon J. 1984. Alpha-melanocyte stimulating hormone increases plasma levels of glucagon and insulin in rabbits. *Life Sci.* 34:547–54
47. Knudtzon J. 1986. Effects of pro-opiomelanocortin-derived peptides on plasma levels of glucagon, insulin and glucose. *Horm. Metab. Res.* 18:579–83
48. Kowalski TJ, Liu SM, Leibel RL, Chua SC Jr. 2001. Transgenic complementation of leptin-receptor deficiency. I. Rescue of the obesity/diabetes phenotype of LEPR-null mice expressing a LEPR-B transgene. *Diabetes* 50:425–35
49. Deleted in proof
50. Krude H, Biebermann H, Luck W, Horn R, Brabant G, Gruters A. 1998. Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. *Nat. Genet.* 19:155–57
51. Limone P, Calvelli P, Altare F, Ajmone-Catt P, Lima T, Molinatti GM. 1997. Evidence for an interaction between alpha-MSH and opioids in the regulation of gonadotropin secretion in man. *J. Endocrinol. Invest.* 20:207–10

52. Lipton JM, Macaluso A, Hiltz ME, Catania A. 1991. Central administration of the peptide alpha-MSH inhibits inflammation in the skin. *Peptides* 12:795–98
53. Lipton JM, Zhao H, Ichiyama T, Barsh GS, Catania A. 1999. Mechanisms of antiinflammatory action of alpha-MSH peptides. In vivo and in vitro evidence. *Ann. NY Acad. Sci.* 885:173–82
54. Liu H, Kishi T, Roseberry AG, Cai X, Lee CE, et al. 2003. Transgenic mice expressing green fluorescent protein under the control of the melanocortin-4 receptor promoter. *J. Neurosci.* 23:7143–54
55. Macaluso A, McCoy D, Ceriani G, Watanabe T, Biltz J, et al. 1994. Antiinflammatory influences of alpha-MSH molecules: central neurogenic and peripheral actions. *J. Neurosci.* 14:2377–82
56. Marsh D, Hollopeter G, Huszar D, Laufer R, Yagaloff K, et al. 1999. Response of melanocortin-4 receptor-deficient mice to anorectic and orexigenic peptides. *Nat. Genet.* 21:119–22
57. McGowan MK, Andrews KM, Grossman SP. 1992. Chronic intrahypothalamic infusions of insulin or insulin antibodies alter body weight and food intake in the rat. *Physiol. Behav.* 51:753–66
58. Meigs JB. 2003. Epidemiology of the insulin resistance syndrome. *Curr. Diab. Rep.* 3:73–79
59. Miller MW, Duhl DM, Vrieling H, Cordes SP, Ollmann MM, et al. 1993. Cloning of the mouse agouti gene predicts a secreted protein ubiquitously expressed in mice carrying the lethal yellow mutation. *Genes Dev.* 7:454–67
60. Mizuno T, Kleopoulos S, Bergen H, Roberts J, Priest C, Mobbs C. 1998. Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting and in ob/ob and db/db mice, but is stimulated by leptin. *Diabetes* 47:294–97
61. Mizuno TM, Makimura H, Silverstein J, Roberts JL, Lopingco T, Mobbs CV. 1999. Fasting regulates hypothalamic neuropeptide Y, agouti-related peptide, and proopiomelanocortin in diabetic mice independent of changes in leptin or insulin. *Endocrinology* 140:4551–57
62. Mizuno TM, Mobbs CV. 1999. Hypothalamic agouti-related protein messenger ribonucleic acid is inhibited by leptin and stimulated by fasting. *Endocrinology* 140:814–17
63. Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, et al. 1997. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 387:903–8
64. Moussa NM, Claycombe KJ. 1999. The yellow mouse obesity syndrome and mechanisms of agouti-induced obesity. *Obes. Res.* 7:506–14
65. Nijenhuis WA, Oosterom J, Adan RA. 2001. AgRP(83–132) acts as an inverse agonist on the human-melanocortin-4 receptor. *Mol. Endocrinol.* 15:164–71
66. Obici S, Feng Z, Tan J, Liu L, Karkanias G, Rossetti L. 2001. Central melanocortin receptors regulate insulin action. *J. Clin. Invest.* 108:1079–85
67. Ollmann M, Wilson B, Yang Y, Kerns J, Chen Y, et al. 1997. Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science* 278:135–38
68. Polonsky KS, Given BD, Hirsch L, Shapiro ET, Tillil H, et al. 1988. Quantitative study of insulin secretion and clearance in normal and obese subjects. *J. Clin. Invest.* 81:435–41
69. Polonsky KS, Given E, Carter V. 1988. Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. *J. Clin. Invest.* 81:442–48
70. Qian S, Chen H, Weingarth D, Trumbauer ME, Novi DE, et al. 2002. Neither agouti-related protein nor neuropeptide Y is critically required for the regulation of energy homeostasis in mice. *Mol. Cell Biol.* 22:5027–35
71. Reizes O, Lincecum J, Wang Z, Goldberger O, Huang L, et al. 2001. Transgenic expression of syndecan-1 uncovers a

physiological control of feeding behavior by syndecan-3. *Cell* 106:105–16

72. Rossi M, Kim M, Morgan D, Small C, Edwards C, et al. 1998. A C-terminal fragment of Agouti-related protein increases feeding and antagonizes the effect of alpha-melanocyte stimulating hormone in vivo. *Endocrinology* 139:4428–31
73. Rotimi CN, Cooper RS, Okosun IS, Olatunbosun ST, Bella AF, et al. 1999. Prevalence of diabetes and impaired glucose tolerance in Nigerians, Jamaicans and US blacks. *Ethn. Dis.* 9:190–200
74. Schioth HB, Watanobe H. 2002. Melanocortins and reproduction. *Brain Res. Brain Res. Rev.* 38:340–50
75. Schwartz MW, Figlewicz DP, Baskin DG, Woods SC, Porte D Jr. 1992. Insulin in the brain: a hormonal regulator of energy balance. *Endocr. Rev.* 13:387–414
76. Schwartz MW, Peskind E, Raskind M, Nicolson M, Moore J, et al. 1996. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nat. Med.* 2:589–93
77. Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG. 1996. Identification of hypothalamic targets of leptin action. *J. Clin. Invest.* 98:1101–6
78. Schwartz MW, Seeley RJ, Weigle DS, Burn P, Campfield LA, Baskin DG. 1997. Leptin increases hypothalamic proopiomelanocortin (POMC) mRNA expression in the rostral arcuate nucleus. *Diabetes* 46:2119–23
79. Scimonelli T, Celis ME. 1990. A central action of alpha-melanocyte-stimulating hormone on serum levels of LH and prolactin in rats. *J. Endocrinol.* 124:127–32
80. Seeley RJ, Yagaloff K, Fisher S, Burn P, Thiele T, et al. 1997. Melanocortin receptors in leptin effects. *Nature* 390:349
81. Seeley RJ, van Dijk G, Campfield LA, Smith FJ, Nelligan JA, et al. 1996. The effect of intraventricular administration of leptin on food intake and body weight in the rat. *Horm. Metab. Res.* 28:664–68
82. Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. 1998. A leptin missense mutation associated with hypogonadism and morbid obesity. *Nat. Genet.* 18:213–15
83. Thiele T, van Dijk G, Yagaloff K, Fisher S, Schwartz M, et al. 1998. Central infusion of melanocortin agonist MTII in rats: assessment of c-Fos expression and taste aversion. *Am. J. Physiol.* 274:R248–54
84. Trivedi P, Jiang M, Tamvakopoulos CC, Shen X, Yu H, et al. 2003. Exploring the site of anorectic action of peripherally administered synthetic melanocortin peptide MT-II in rats. *Brain Res.* 977:221–30
85. Tsujii S, Bray GA. 1989. Acetylation alters the feeding response to MSH and beta-endorphin. *Brain Res. Bull.* 23:165–69
86. Van der Ploeg LH, Martin WJ, Howard AD, Nargund RP, Austin CP, et al. 2002. A role for the melanocortin 4 receptor in sexual function. *Proc. Natl. Acad. Sci. USA* 99:11381–86
87. Vergoni AV, Bertolini A, Mutulis F, Wikberg JE, Schioth HB. 1998. Differential influence of a selective melanocortin MC4 receptor antagonist (HS014) on melanocortin-induced behavioral effects in rats. *Eur. J. Pharmacol.* 362:95–101
88. Versteeg DH, Van Bergen P, Adan RA, De Wildt DJ. 1998. Melanocortins and cardiovascular regulation. *Eur. J. Pharmacol.* 360:1–14
89. Wessells H, Gralnek D, Dorr R, Hruby VJ, Hadley ME, Levine N. 2000. Effect of an alpha-melanocyte stimulating hormone analog on penile erection and sexual desire in men with organic erectile dysfunction. *Urology* 56:641–46
90. Wilson BD, Ollmann MM, Kang L, Stoffel M, Bell GI, Barsh GS. 1995. Structure and function of ASP, the human homolog of the mouse agouti gene. *Hum. Mol. Genet.* 4:223–30
91. Deleted in proof
92. Woods SC, Chavez M, Park CR, Riedy C, Kaiyala K, et al. 1996. The evaluation of insulin as a metabolic signal controlling behavior via the brain. *Neurosci. Biobehav. Rev.* 20:139–44

93. Woods SC, Lotter EC, McKay LD, Porte D Jr. 1979. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature* 282:503–5
94. Woods SC, Seeley RJ. 2000. Adiposity signals and the control of energy homeostasis. *Nutrition* 16:894–902
95. Woods SC, Seeley RJ. 2001. Insulin as an adiposity signal. *Int. J. Obes. Relat. Metab. Disord.* 25:S35–38
96. Yaswen L, Diehl N, Brennan MB, Hochgeschwender U. 1999. Obesity in the mouse model of pro-opiomelanocortin deficiency responds to peripheral melanocortin. *Nat. Med.* 5:1066–70
97. Yeo G, Farooqi I, Aminian S, Halsall D, Stanhope RG, O'Rahilly S. 1998. A frameshift mutation in MC4R associated with dominantly inherited human obesity. *Nat. Genet.* 20:111–12

Annual Review of Nutrition
Volume 24, 2004

CONTENTS