The role of the central melanocortin system in the regulation of food intake and energy homeostasis: lessons from mouse models

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A little more than a decade ago, the molecular basis of the lipostat was largely unknown. At that time, many laboratories were at work attempting to clone the genes encoding the *obesity*, *diabetes*, *fatty*, *tubby* and *agouti* loci, with the hope that identification of these obesity genes would help shed light on the process of energy homeostasis, appetite and energy expenditure. Characterization of *obesity* and *diabetes* elucidated the nature of the adipostatic hormone leptin and its receptor, respectively, while cloning of the *agouti* gene eventually led to the identification and characterization of one of the key neural systems upon which leptin acts to regulate intake and expenditure. In this review, we describe the neural circuitry known as the central melanocortin system and discuss the current understanding of its role in feeding and other processes involved in energy homeostasis.

Keywords: melanocortin; leptin; MC4-R; agouti

1. INTRODUCTION

(a) *The melanocortin system*

The melanocortin system plays a key role in a number of endocrine and homeostatic processes. Melanocortin agonists are all derived from the *proopiomelanocortin* (POMC) *prohormone* gene. POMC is cleaved by prohormone convertases PC1 and PC2 to produce a number of peptides including members of both the opioid and melanocortin families. The melanocortin peptides derived from POMC include adrenocorticotrophic hormone (ACTH) and α , β and γ -melanocyte-stimulating hormones (MSH), and all contain the same signature core amino acid sequence, His-Phe-Arg-Trp, required for binding to a family of five G-protein coupled melanocortin receptors. The term ‘melanocortin’ derives from the fact that all of these peptides exhibit melanotropic and/or adrenocorticotrophic activity. In addition to the melanocortin peptides, POMC cleavage also leads to the production of an opioid peptide, β -endorphin, an uncleaved product containing β -MSH and β -endorphin called β -lipotropin, and corticotrophin-like intermediate lobe peptide. In the rodent, POMC is principally expressed in the intermediate lobe of the pituitary and in the brain, in the arcuate nucleus of the hypothalamus (ARC) and the nucleus of the solitary tract (NTS) of the brainstem (Joseph *et al.* 1983). However, POMC is also expressed in the skin, gut, placenta and pancreas (Smith & Funder 1988). Additionally, the melanocortin system is unique among neuropeptide systems in that it is also regulated by two endogenous small protein antagonists, agouti (Bultman *et al.* 1992; Woychik 1992) and agouti-related protein (AgRP; Ollmann *et al.*

1997; Shutter *et al.* 1997). Agouti is expressed in hair follicular epithelial cells, from which it is secreted to regulate melanin expression by adjacent follicular melanocytes. AgRP is expressed in the adrenal cortex and in the neuropeptide Y (NPY) neurons of the ARC (Ollmann *et al.* 1997; Shutter *et al.* 1997).

(b) *The melanocortin receptors*

There are five known melanocortin receptors, MC1–5R corresponding to the order in which they were cloned, that are expressed in a number of sites and have varying affinities for the different melanocortin peptides. Table 1 shows a summary of the principal ligands and functions of each receptor. The melanocortin receptors are members of the G-protein coupled receptor family, which couple through Gs to stimulate an increase in intracellular cAMP. Little is known about the specific downstream signalling cascades resulting from activation of melanocortin receptors.

(i) *MC1-R*

The MC1-R is expressed largely in melanocytes in skin and hair where it is involved in stimulating eumelanin pigmentation in a variety of species including humans (for review see Rees (2003)). The action of the endogenous agonist α -MSH at the MC1-R increases the production of cAMP, which causes an increase in the eumelanin (black/brown): pheomelanin (red/yellow) ratio promoting darker pigmentation (Cone *et al.* 1996). The action of the endogenous antagonist agouti at the MC1-R acts to block the effect of α -MSH at this receptor, promoting pheomelanin expression, leading to a predominance of the red/yellow pigmentation. A classic example of this is the *agouti yellow* (A^y) mouse (for review see Wolff *et al.* (1999)), which ectopically overexpresses agouti in all somatic cells leading to the development of a yellow coat colour and

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Table 1. A summary of the primary function, ligand and site of expression of the melanocortin receptors. (* Indicates primary ligand.)

receptor	potency of ligand activation	principal site of expression	principal function
MC1-R	α -MSH = ACTH > β -MSH > γ -MSH	melanocytes	pigmentation
MC2-R	ACTH	adrenal gland	steroidogenesis
MC3-R	α -MSH = ACTH = β -MSH = γ -MSH*	brain	energy homeostasis, natriuresis
MC4-R	α -MSH = ACTH > β -MSH > γ -MSH	brain	energy homeostasis
MC5-R	α -MSH > ACTH* > β -MSH > γ -MSH	exocrine glands	exocrine gland secretion

obesity. The obesity is caused by the action of the ectopically expressed agouti at the centrally expressed MC4-R and this animal will be discussed in more detail later on in this review.

(ii) MC2-R

The MC2-R encodes the adrenocorticotropin (ACTH) receptor (Mountjoy *et al.* 1992), which is expressed on the adrenal cortex and is critical in regulation of the hypothalamic–pituitary–adrenal axis. The principal role for ACTH in this axis is to control the regulation of corticosteroid synthesis and secretion by the adrenal gland; however, ACTH is also critical for the development of the adrenal cortex itself.

(iii) MC3-R

The MC3-R is principally expressed in the central nervous system (Roselli-Rehfuß *et al.* 1993) and has been implicated in a number of physiological processes including natriuresis, cardiovascular regulation and energy homeostasis. In the rodent brain, MC3-R mRNA is largely expressed in the hypothalamus, but is also found at lower levels in limbic areas (Roselli-Rehfuß *et al.* 1993). The principal ligands for MC3-R are α - and γ -MSH and the MC3-R is believed to mediate the natriuretic effects of these peptides (Ni *et al.* 1998, 2003). Mice with a specific deletion of the MC3-R exhibit a complex obesity phenotype (Butler *et al.* 2000; Chen *et al.* 2000) implicating the MC3-R in the regulation of energy homeostasis. Briefly, MC3-R null mice show an increase in fat mass and a reduction in lean mass in the absence of any significant increase in body weight or in any measurable increase in food intake. In addition to being expressed in the brain, the MC3-R is also expressed at a number of peripheral sites including adipose tissue, heart, skeletal muscle, kidney (Chhajlani 1996), stomach, duodenum, placenta and pancreas (Gantz *et al.* 1993). MC3-R knockout mice have also been demonstrated to exhibit an increased sensitivity to salt-sensitive hypertension (Ni *et al.* 2003).

In the ARC, MC3-R mRNA is expressed in POMC neurons (Jegou *et al.* 2000) leading to the suggestion that the melanocortin system may use the MC3-R as part of a regulatory feedback mechanism, thus it has been proposed that the MC3-R acts as an inhibitory autoreceptor regulating melanocortinergic tone. Indeed, it has been demonstrated that an MC3-R specific agonist, D-Trp⁸- γ -MSH inhibits the spontaneous firing rate of POMC neurons (Cowley *et al.* 2001), and administration of the compound stimulates food intake (Marks *et al.* 2005). Thus, this melanocortin receptor has multiple functions, and its role in energy homeostasis appears complex, acting to inhibit

anorexigenic POMC neurons, yet appearing to have a collective anorexigenic effect since deletion of the gene causes obesity in the mouse.

(iv) MC4-R

MC4-R is also expressed principally in the central nervous system (CNS), but has a much wider distribution than the MC3-R. Studies using *in situ* hybridization (Mountjoy *et al.* 1994; Kishi *et al.* 2003) and transgenic mice expressing green-fluorescent protein under the control of the MC4-R promoter (Liu *et al.* 2003), have localized MC4-R to a variety of sites in the rodent brain including the cortex, cerebellum, striatum, hippocampus, hypothalamus, midbrain, amygdala, thalamus and brainstem. Indeed, MC4-R has been localized to all of the areas known to show α -MSH binding (Tatro 1990). The wide distribution of expression of MC4-R suggests that it may be involved in mediating a number of neuroendocrine and autonomic processes (Mountjoy *et al.* 1994b). This hypothesis has been extended using the MC4-R-GFP mouse. In the hindbrain of the MC4-R-GFP mouse, GFP-immunoreactivity is found in cells containing choline-acetyltransferase a marker of autonomic preganglionic neurons (Liu *et al.* 2003). Additionally, in the same mouse model, GFP-immunoreactivity in the paraventricular nucleus of the hypothalamus was found within thyrotropin releasing hormone and oxytocin neurons (Liu *et al.* 2003) implicating the MC4-R in modulating neuroendocrine processes. One of the most well-characterized functions of the MC4-R is the regulation of energy homeostasis (Fan *et al.* 1997a; Huszar *et al.* 1997). The MC4-R null mouse is hyperphagic, hyperinsulinemic, obese and shows increased linear growth, and as such mirrors the agouti obesity phenotype. The MC4-R null mouse will be discussed at much greater detail later in the review. Not surprisingly, given its widespread distribution, other functions appear to be modulated by the MC4-R as well, including cardiovascular regulation (Li *et al.* 1996) and erectile function (Poggioli *et al.* 1995; Wessells *et al.* 2003).

(v) MC5-R

The MC5-R is expressed largely in exocrine glands such as the adrenal, lacrimal and sebaceous glands, where it appears to regulate the synthesis and secretion of a wide variety of exocrine gland products (Chen *et al.* 1997). MC5-R null mice show defects in the production of a number of products secreted from exocrine glands, including porphyrin, sebaceous lipids and pheromones (Chen *et al.* 1997). As a result of presumed alterations in pheromone secretion, MC5-R null mice also show changes in aggression and defensive

behaviour (Caldwell & Lepri 2002; Morgan *et al.* 2004a,b).

This review will focus on the role of the central melanocortin system in the regulation of energy homeostasis and subsequent changes in body composition. The central melanocortin system is defined as the neurons expressing POMC and its cleavage products, the neurons expressing AgRP, and the CNS target neurons expressing receptors MC3-R and MC4-R. We will also briefly address the potential function in energy homeostasis of the MC3-R and MC4-R receptors expressed at peripheral sites.

2. THE CENTRAL MELANOCORTIN SYSTEM AND THE REGULATION OF ENERGY HOMEOSTASIS

(a) *The discovery of a role for the central melanocortin system in the regulation of energy homeostasis*

One of the oldest known genetic models of obesity, discovered early in the twentieth century, is the agouti mouse. There are two strains of agouti mouse, the lethal yellow, A^y (Wolff *et al.* 1999), and the viable yellow A^{vy} (Wolff 1990). The A^y mouse has ectopic expression of the endogenous melanocortin antagonist agouti caused by deletion of the *agouti* gene promoter and the coding region of the adjacent *Raly* gene. This deletion consequently causes the expression of the *agouti* gene to be driven by the promoter of the *Raly* gene, which is constitutively expressed, leading to the expression of agouti in all tissues (Bultman *et al.* 1992; Michaud *et al.* 1993). The A^y mutation is embryonically lethal in the homozygous state, however the heterozygous A^y/a mouse has been extensively studied. In the A^{vy} mouse, ectopic overexpression of agouti is caused by the insertion of an intracisternal A particle into the non-coding exon 1A (Yen *et al.* 1994). This mouse differs from the A^y in that it has a eumelanic mottling leading to the classic black/yellow agouti colouring as opposed to the bright yellow phenotype of the A^y mouse.

The agouti mouse has a distinct phenotype characterized by hyperphagia, hyperinsulinemia, hypometabolism and increased linear growth (for a comprehensive review of the agouti phenotype see (Wolff *et al.* 1999)). The cause of the obesity in the agouti mouse was not clear until the cloning of the melanocortin receptors in the early 1990s when it was shown that the agouti protein acts as an antagonist at the MC1-R and MC4-R (Lu *et al.* 1994). Indeed, when the MC4-R was cloned and found to be expressed throughout the hypothalamus, a key site in the brain for the regulation of energy homeostasis, it was postulated that the obesity in the agouti mouse may result via antagonism of this receptor (Lu *et al.* 1994; Mountjoy *et al.* 1994). This hypothesis was verified by the development and analysis of the MC4-R null mouse (Huszar *et al.* 1997) as well as by the development and use of the first melanocortin antagonist (Hruby *et al.* 1995; Fan *et al.* 1997b). Central administration of the MC3/4-R agonist melanotan II (MTII) reduced food intake in a wild-type, agouti and leptin deficient *ob/ob* mice, an effect that could be blocked by the MC3/4-R antagonist SHU9119 (Fan *et al.* 1997b). Additionally,

administration of SHU9119 alone caused an increase in nocturnal and fast-induced food intake, indicates that the melanocortin system exerts a tonic inhibitory effect on food intake via the centrally expressed MC3-R and MC4-R.

(b) *The interaction between leptin and the central melanocortin system*

The adipostatic hormone leptin, principally produced by adipocytes, is released into the circulation and exerts its effect on energy homeostasis predominantly via leptin receptors expressed in the brain. There is extensive evidence to demonstrate that the central melanocortin system is important in mediating the effects of leptin. First, leptin receptors are expressed on the majority of POMC neurons in the ARC (Cheung *et al.* 1997) and the anorectic effects of exogenously administered leptin in rodents are partially reversed by treatment with SHU9119 (Seeley *et al.* 1997), indicating that the central melanocortin system is downstream of leptin receptor signalling and plays a key role in mediating the effects of this important adipostatic hormone. Additionally, following peripheral leptin administration, the expression of proteins involved in the leptin receptor signalling cascade such as pSTAT-3 (phosphorylated signal transducer and activator of transcription 3) and SOCS-3 (suppressor of cytokine signalling 3) are upregulated in POMC neurons of the ARC (Elias *et al.* 1999; Munzberg *et al.* 2003). Moreover, POMC and AgRP mRNA levels are regulated by leptin and states of altered energy balance, such as fasting and lactation, (Smith 1993; Schwartz *et al.* 1997; Chen *et al.* 1999; Mizuno *et al.* 1999). Finally, leptin is able to alter the firing rate of ARC POMC and AgRP/NPY neurons in an *ex vivo* electrophysiological slice preparation (Cowley *et al.* 2001; Takahashi & Cone 2005).

However, the effects of the central melanocortin system on energy homeostasis are not restricted to mediating the effects of leptin. When the A^y/a mouse is crossed onto the leptin-deficient *ob/ob* background the resulting A^y/a *ob/ob* mice are even heavier than animals with the *ob/ob* or A^y/a mutations alone, suggesting that the effects of the central melanocortin system are independent and additive of those of the leptin pathway (Boston *et al.* 1997). This finding has recently been repeated in a study using double homozygous MC4-R null *ob/ob* mice (Trevaskis & Butler 2005). Additionally, pre-obese MC4-R null mice are able to reduce their food intake in response to peripheral leptin suggesting that while the melanocortin system may be important in mediating a component of the anorectic effects of leptin it is not essential (Marsh *et al.* 1999). However, in obese MC4-R null mice the anorectic effects of peripherally administered leptin are absent, suggesting that leptin resistance secondary to obesity also contributes to the lack of leptin responsiveness in these animals.

(c) *The involvement of the central melanocortin system in mediating hunger and satiety cues*

In addition to mediating the effects of factors that are implicated in controlling energy homeostasis in the long-term, such as leptin, there is also evidence to suggest that the central melanocortin system plays a

role in mediating hunger and satiety. POMC-immunoreactivity and MC4-R mRNA are found in the dorsal vagal complex (DVC) of the brainstem (Joseph *et al.* 1983; Palkovits *et al.* 1987; Mountjoy *et al.* 1994a). The DVC, consisting of the area postrema, the NTS and the dorsal motor nucleus, is the site of termination of vagal afferent nerve fibres and as such is critical in the rapid gut-brain communication necessary for the transmission of information regarding meal-termination and the onset of satiety. Indeed, central administration of MTII in rodents causes a reduction in meal-size but not frequency (Azzara *et al.* 2002; Zheng *et al.* 2005) indicative of the induction of satiety, while the antagonist SHU9119 causes the opposite effect, increasing meal size in the absence of changes in meal-frequency (Sutton *et al.* 2005). Additionally, NTS POMC neurons are activated, as assayed by the expression of immunoreactivity for the immediate early gene and marker of neuronal activation *c-fos*, by the well-characterized satiety factor cholecystokinin (CCK) and by feeding-induced satiety (Fan *et al.* 2004). CCK and MTII both increase the phosphorylation of extracellular-signal regulated kinase (pERK) in the NTS (Sutton *et al.* 2005). The increase in pERK induced by CCK can be reversed with prior administration of SHU9119, suggesting that the CCK-induced increase in pERK is dependent on intact MC3/4-R signalling, providing further evidence for the interaction between CCK and the central melanocortin system.

In addition to mediating signals associated with meal-termination i.e. satiety cues, the central melanocortin system has been implicated in mediating meal-initiation or hunger cues. Ghrelin, the only known endogenous ligand for the growth hormone (GH) secretagogue receptor (GHS-R), stimulates food intake and GH release following systemic administration in humans and both systemic and central administration in rodents. In humans, circulating ghrelin levels are known to increase pre-prandially and decrease post-prandially suggesting a role in meal-initiation (Cummings *et al.* 2001; Callahan *et al.* 2004), with the post-prandial reduction proportional to the amount of calories ingested.

In rodents the ghrelin system interacts with the central melanocortin system to modulate energy homeostasis. Although ghrelin is principally expressed in the stomach, there are also reports of ghrelin immunoreactivity in the hypothalamus where ghrelin-containing fibres are believed to make axo-somatic and axo-dendritic contact with both POMC and NPY/AgRP neurons, suggesting a direct link between brain-derived ghrelin and the central melanocortin system (Cowley *et al.* 2003). This link is further reinforced by electrophysiological data demonstrating that ghrelin increases the firing of ARC NPY/AgRP neurons known to express the GHS-R (Willesen *et al.* 1999) which, through previously characterized synaptic contacts (Cowley *et al.* 2001), are postulated to increase GABAergic inhibitory post-synaptic currents in POMC neurons. There is also evidence to suggest a direct inhibition of firing of POMC cells by ghrelin (Cowley *et al.* 2003; Riediger *et al.* 2003). Thus, with respect to food intake, ghrelin appears to have an opposing action

to leptin on the ARC POMC-NPY/AgRP neuronal network. Acute and chronic peripheral administration of ghrelin in rodents causes an increase in both AgRP and NPY mRNA (Asakawa *et al.* 2001; Kamegai *et al.* 2001), further suggesting an interaction between ghrelin and both the AgRP and NPY systems.

(d) Changes in energy homeostasis in models of altered central melanocortin signalling; the MC4-R null mouse

The generation of the MC4-R null mouse in 1997 (Huszar *et al.* 1997) reinforced the important role for the central melanocortin system in the regulation of energy homeostasis. Genetic deletion of receptor function, achieved following insertion of a neomycin cassette into the MC4-R gene, replacing the entire coding region, resulted in a mouse that, as predicted by the pharmacological antagonism studies of Fan and colleagues discussed above, recapitulated the phenotype of the agouti mouse and produced what has become known as the melanocortin obesity syndrome. The latter consists of a group of co-morbidities including hyperphagia, hypometabolism, hyperinsulinemia and increased linear growth, associated with alterations in central melanocortin signalling. The cause of the increase in linear growth in these animals has not been studied in detail. Animals that are heterozygous for the MC4-R deficiency (MC4-R^{+/-}) show an intermediate phenotype for all aspects of the syndrome, suggesting that two copies of the gene are required for normal energy homeostasis. Indeed, in humans haploinsufficiency at the MC4-R is believed to be responsible for up to 5% of cases of severe obesity in children (Vaisse *et al.* 1998; Yeo *et al.* 1998).

Obesity in the MC4-R null mouse is caused by a combination of hyperphagia and hypometabolism, but which of these symptoms occurs first and is the principal cause of the obesity seen is the source of some debate. However, prior to the onset of hyperphagia or an increase in body weight, the MC4-R null mouse displays hyperinsulinemia suggesting that the MC4-R may have effects on insulin secretion and or sensitivity independent of changes in body composition (Fan *et al.* 2000). Additionally, MTII administration is able to dose-dependently reduce serum insulin levels by a mechanism that can be inhibited by administration of the α -adrenoreceptor antagonist, phenotolamine, suggesting that the melanocortin system modifies sympathetic outflow to the pancreas.

One of the most intriguing phenotypes of the MC4-R null mouse is that it has a profound defect in normal homeostatic responses to changes in fat in the diet (Butler *et al.* 2001). When placed on a moderate fat diet, wild-type mice initially increase their caloric intake but then reduce their food intake until they become isocaloric. MC4-R null mice switched to high-fat food do not sense the change and remain hyperphagic and hypometabolic. Additionally, they respond with reduced fatty acid oxidation (Albarado *et al.* 2004). Taken together, these multiple homeostatic defects make the MC4-R null hypersensitive to the obesogenic effects of a high fat diet. This is in contrast to the leptin deficient *ob/ob* mice, which are equally hyperphagic on both diets (Butler *et al.* 2001).

One caveat of this study is that it did not address whether the defect in MC4-R null results from a defective homeostatic response to increased fats or increased caloric intake in general.

In addition to becoming hyperphagic on a moderate fat diet, the MC4-R null mice fail to increase their oxygen consumption or wheel running activity to modulate their energy expenditure, thus contributing to even greater weight gain (Butler *et al.* 2001). However, this failure to modify food intake and energy expenditure does not extend to other environmental factors. For example, MC4-R null mice show a normal re-feeding response after a fast and increase their energy intake in response to a reduction in ambient temperature to 4 °C (Butler *et al.* 2001). Indeed, in contrast to *ob/ob*, mice MC4-R nulls are able to thermoregulate normally and maintain their core body temperature when placed at 4 °C (Ste Marie *et al.* 2000). In the same study it was shown that following leptin administration, unlike wild-type animals, MC4-R null mice do not increase the levels of mitochondrial uncoupling protein 1 (UCP-1) mRNA in brown adipose tissue (BAT) which is a major thermogenic organ in rodents. In BAT, UCP-1 increases energy expenditure by increasing uncoupling of the respiratory transport chain leading to an increase in energy loss as heat. Thus, MC4-R null mice appear to be unable to reduce their energy expenditure in response to leptin, at least with respect to UCP-1 mRNA in BAT. Cold-exposure, fasting and high-fat diets are also known to cause changes in BAT UCP-1 mRNA, but it is not known whether these responses are intact in the MC4-R null mouse.

The obesity and hyperinsulinemia seen in the MC4-R null mice resemble aspects of the metabolic syndrome seen in humans. Metabolic syndrome is a group of co-morbidities including visceral obesity, hyperinsulinemia, dyslipidemia and increased circulating prothrombotic and proinflammatory factors associated with an increased risk of cardiovascular disease. In this context, it is interesting that the central melanocortin system has effects on the cardiovascular system. Pharmacological studies in rodents indicate that central administration of MTII causes an increase in mean arterial blood pressure (MAP) in addition reducing food intake (Kuo *et al.* 2003). Additionally, antagonism of the MC3/4-R by SHU9119 causes an increase in food intake and heart rate but has no effect on MAP despite an increase in body weight. These studies suggest that the central melanocortin system may be another potential link between increases in body weight and changes in cardiovascular function. This hypothesis is further reinforced by studies in MC4-R null mice, which show that despite obesity and elevated serum insulin, leptin and glucose, these animals show no significant difference in either basal blood pressure or the development of salt-sensitive hypertension (Tallam *et al.* 2005). Indeed, the MC4-R null mice have significantly reduced heart rate compared to wild-type, but not MC4-R heterozygous mice. These data suggest that intact MC4-R signalling may be an important link between the development of obesity and risk factors for cardiovascular disease such as increased heart rate and hypertension.

3. THE CENTRAL MELANOCORTIN SYSTEM AND ADIPOSITY

(a) *Increased adiposity in models of defective melanocortin signalling; hyperphagia versus hypometabolism*

Several mouse models with defective melanocortin signalling show the unique melanocortin obesity syndrome, but it remains controversial as to whether it is hyperphagia or hypometabolism that is responsible for the observed obesity, particularly in the MC4-R null mouse. Pair-feeding studies suggest that in the absence of hyperphagia, when the available food is restricted to the same level as wild-type mice, both MC4-R null males and females show an increase in fat pad mass (Ste Marie *et al.* 2000). Intriguingly, in this study the females became significantly heavier than the wild-type controls even though they were consuming the same number of calories, while the male mice were not significantly heavier than controls despite their increase in fat pad mass. This suggests that the female animals have a greater metabolic defect than the males, implicating an effect of sex steroids on the phenotype. Additionally, the finding that the male mice showed greater adiposity in the absence of an increase in body weight suggests that melanocortin signalling may be directly involved in regulating the propensity to store fat. Restricting the food intake of the MC4-R null animals of both sexes normalized the serum insulin levels to that of wild-type (Ste Marie *et al.* 2000). This study demonstrated that MC4-R null mice were hypometabolic but the authors did not address whether they thought the increased adiposity seen in the paired animals might be due to the hypometabolism alone or whether there may be direct regulation of adiposity by MC4-R. In a separate study examining the relative contribution of hyperphagia and hypometabolism to the obesity in the MC4-R null, Weide and colleagues proposed that hyperphagia driven by increases in ARC NPY mRNA (Weide *et al.* 2003) and not hypometabolism was solely responsible for the obesity seen in the MC4R-null animals. However, in this study the authors did not confirm their findings by pair-feeding the animals and did not take into account the potential for a direct effect of melanocortin signalling on adiposity.

There is evidence to suggest that the melanocortin system may directly affect adiposity via altering fat storage. In a study examining the development of hepatic steatosis in the MC4-R null mouse, Albarado and colleagues demonstrated that obese MC4-R null mice showed an increase in mRNA for fatty acid synthase (FAS), an important lipogenic liver enzyme (Albarado *et al.* 2004), suggesting that defective MC4-R signalling had a direct effect on lipid storage in this organ. However, the authors went on to propose that the increase in liver FAS mRNA and the subsequent development of hepatic steatosis was highly dependent on the level of obesity and the genetic background of the animals. In this study the levels of FAS mRNA were not measured in any organ other than the liver. It would be interesting to examine whether an increased FAS levels in adipose tissue may be partially responsible for the development of adiposity in these animals.

In contrast to the MC4-R null mouse, the MC3-R null has an increase in adipose mass combined with a decrease in lean mass leading to little overall change in body weight compared to wild-type control animals (Butler *et al.* 2000; Chen *et al.* 2000). The increase in adiposity in these animals has been attributed to reduced activity and increased feed efficiency i.e. the ratio of food intake to weight gain, as these animals are not hyperphagic on standard or high-fat chow (Butler *et al.* 2000; Chen *et al.* 2000). The expression of MC3-R on adipocytes may reflect an ability of this receptor to effect fat mass directly. However, the level of expression of genes known to regulate adipocyte function and fat storage such as FAS or peroxisome proliferator-activated receptor γ (PPAR- γ) has not been examined in these animals.

POMC-deficient animals are obese, hypometabolic and hyperphagic (on regular and high-fat chow) despite an absence of circulating corticosterone (Yaswen *et al.* 1999; Challis *et al.* 2004). In common with the MC4-R null, the increase in body-weight in these animals is caused by an increase in both lean and fat mass (Challis *et al.* 2004).

(b) The central melanocortin system and the sympathetic innervation of adipose tissue

One proposed mechanism by which the central melanocortin system affects body composition is via altering the activity of sympathetic nerves that innervate adipose tissue. Central administration of the agonists α -MSH or MTII and the antagonist AgRP increase and decrease, respectively, sympathetic activity in BAT (Haynes *et al.* 1999; Yasuda *et al.* 2004). Additionally, in mice both third and fourth ventricle administration of MTII causes an up-regulation of UCP-1 mRNA in BAT (Williams *et al.* 2003) thus, melanocortin signalling may regulate energy homeostasis by promoting energy expenditure via thermogenic mechanisms. The adipostatic factor leptin also increases the sympathetic nerve activity to BAT, but some studies suggest that unlike the effects of leptin in food intake, the increase in BAT sympathetic nerve activity cannot be blocked following the administration of the MC3/4-R antagonist SHU9119 (Haynes *et al.* 1999) suggesting that the effect of leptin on food intake and energy expenditure may partially be mediated by divergent pathways. However, this is controversial as another group has found that the MC3/4-R antagonist SHU9119 is able to inhibit the leptin induced increase UCP-1 mRNA levels in BAT (Satoh *et al.* 1998).

POMC neurons of the lateral ARC have been shown using retrograde tracing to project transynaptically to BAT (Oldfield *et al.* 2002) providing anatomical evidence for a link between the central melanocortin system and this critical effector site. However, MC4-R null mice are able to thermoregulate normally at 4 °C and have no significant increase in UCP-1 mRNA levels in BAT under basal conditions, indicating that the central melanocortin system does not seem particularly important in the regulation of BAT by ambient temperature (Butler *et al.* 2001).

4. THE CENTRAL MELANOCORTIN SYSTEM AND THE REGULATION OF LEAN MASS

(a) Linear growth

In childhood, obesity is often associated with an increase in linear growth (Vignolo *et al.* 1988), with obese children being taller than their non-obese counterparts. This difference is reduced through puberty, as obese children do not tend to show a notable growth spurt, resulting in no significant difference in height as adults between the obese and non-obese children. Importantly, however, in addition to their profound obesity, children with defects in the MC4-R gene are also tall (Yeo *et al.* 1998; Farooqi *et al.* 2000). Surprisingly, obese children show a reduction in serum GH levels due to decreased secretion and increased plasma clearance (Veldhuis *et al.* 1991; Vanderschueren-Lodeweyckx 1993). A number of hypotheses have been proposed to explain the increase in growth seen in these children despite the low circulating GH levels, including hyperinsulinemia (Bucher *et al.* 1983), hyperprolactinemia (Bucher *et al.* 1983), increased free insulin-like growth factor 1 (IGF-1; Attia *et al.* 1998), and increased insulin-like growth factor binding protein 3 (IGF-BP3) levels (Park *et al.* 1999). One of the most distinct characteristics of animals and humans with alterations in their MC4-R is an increase in linear growth in addition to obesity. This increase in linear growth in MC4-R null mice is combined with an increase in muscle mass leading to an overall increase in lean mass.

The cause of the increase in linear growth in animals and humans with defects in the MC4-R is not known. The agouti mouse, perhaps one of the best characterized of the mouse models of altered melanocortin signalling, shows an increase in linear growth despite low circulating GH levels, which show little of the normal diurnal variation. However, these mice do show a greater increase in the peak levels of serum IGF-1 during the surge at 2–4 weeks of age compared with wild-type mice; this could affect growth at a crucial stage in development and may account for some of the observed phenotype (Wolff *et al.* 1999). There are a number of possible sites of interaction between the melanocortin system and the regulation of growth.

Very little is known about potential interactions between the melanocortin system and the GH axis. Chronic central infusion of an MC3/4R antagonist into rats has no effect on the levels of circulating GH, IGF-1 or GHRH mRNA levels (Raposinho *et al.* 2000) suggesting that in adult animals antagonism of the central melanocortin system has no effect on the somatotrophic axis. Additionally, in an acute study, central administration of melanocortin receptor antagonists failed to alter the levels of spontaneous GH secretion in rats, however, there did appear to be an alteration in the pattern of GH secretion, with melanocortin antagonists advancing the morning plasma GH peak (Watanobe & Yoneda 2003).

There is anatomical evidence to suggest that the central melanocortin system may act to regulate GH secretion at the level of the hypothalamus via interaction with somatostatin expressing neurons. In the ARC, 35% of POMC neurons have been shown to express binding sites for somatostatin, while α -MSH

immunoreactive fibres are also believed to make contact with somatostatin-immunoreactive perikarya in the periventricular nucleus (Fodor *et al.* 1998) suggesting the potential for regulation of GH secretion from within the hypothalamus. Growth hormone releasing hormone (GHRH), POMC, AgRP and somatostatin receptors (sst1 and sst2) are all expressed highly in the ARC, however, a detailed study of their relative expression has not been performed. Clearly, much remains to be learned concerning the mechanisms underlying the increased linear growth that results from blockade of melanocortin signalling.

(b) Regulation of bone metabolism

There is increasing evidence to suggest that the melanocortin system may have direct effects on bone metabolism. In addition to increased linear growth, increased bone mineral density is also a feature of mice and humans with altered MC4-R signalling, suggesting that altered melanocortin signalling has a direct or indirect effect on bone (Farooqi *et al.* 2003). It has been proposed that the increase in bone mineral density occurs due to reduced osteoclast number in MC4-R null mice, which in turn may lead to reduced bone resorption (Eleftheriou *et al.* 2005).

Messenger RNAs for all the melanocortin receptors i.e. MC1-5R, have been detected to varying degrees in osteoblast-like, and osteoblast cell lines (Zhong *et al.* 2005), and POMC mRNA has also been detected in osteoclastic cells. Moreover, melanocortin agonists α -MSH and ACTH stimulate cell proliferation in osteoblast and or chondrocyte cultures (Cornish *et al.* 2003; Evans *et al.* 2004; Zhong *et al.* 2005). Repeated subcutaneous administration of α -MSH in mice causes a reduction in trabecular bone volume in the proximal tibiae and reduced trabecular number, indicating that a melanocortin ligand has effects on bone metabolism *in vivo* (Cornish *et al.* 2003).

(c) Cachexia

Cachexia is a state of malnutrition caused by chronic disease or infection. Characterized by a decrease in appetite/food intake combined with an increase in metabolism of both fat and lean mass, the causes of cachexia are unknown, thus the treatments of this debilitating condition are currently limited. Animals with defects in central melanocortin signalling show differential responses to the induction of cachexia by infection, tumours or kidney disease (Marks *et al.* 2001; Marks *et al.* 2003b; Cheung *et al.* 2005). MC4-R null mice or wild-type mice treated with AgRP, maintain their food intake and body weight following lipopolysaccharide (LPS) treatment, tumour implantation or uremia-associated cachexia, which cause significant reductions in food intake and body weight, in control animals (Marks *et al.* 2001; Marks *et al.* 2003b; Cheung *et al.* 2005). This finding was also confirmed in rats, where tumour-induced cachexia was prevented by treatment with the MC3/4-R antagonist SHU9119 (Wisse *et al.* 2001). In contrast to MC4-R null mice, the MC3-R null mice are more sensitive to cachexia, whether LPS or tumour-induced, leading to a greater reduction in body weight compared with wild-type mice. Of particular interest is the finding that the MC3-R

null mice lose significantly more lean mass than wild-type animals during tumour induced cachexia (Marks *et al.* 2003a). This finding suggests that the MC3-R may be critical for the regulation of lean mass during physiological challenge, perhaps through its ability to regulate melanocortinergic tone.

5. SUMMARY AND CONCLUSIONS

The data described above illustrate that the central melanocortin system is broadly involved in the complex physiological functions underlying energy homeostasis. The system alters feeding in response not only to acute hunger (ghrelin) and satiety (CCK) signals, as well as signals relating to composition of the diet, but also provides input from the adipostatic hormone leptin, as well. Furthermore, the melanocortin system not only regulates food intake, but plays a role in regulation of energy expenditure, via effects on autonomic outflow to a variety of tissues, as well as effects on endocrine axes. A detailed analysis of the neuroanatomy of the melanocortin system demonstrates that this system provides an excellent substrate for the integration of a wide variety of signals involved in the control of energy homeostasis (Cone 2005). The data that has been collected thus far on the physiological role(s) of the central melanocortin system suggests a number of possible therapeutic applications to disorders of weight regulation, including obesity and cachexia, and possibly diabetes, as well. Assuming potent MC3/MC4-R specific compounds can be derived that access the central nervous system, the actions of melanocortins on heart, kidney, and sexual function may also provide the challenge of potential unwanted side effects.

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