Effects of pro-opiomelanocortin (POMC) on food intake and body weight: mechanisms and therapeutic potential?

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ABSTRACT

POMC (pro-opiomelanocortin) is a complex polypeptide precursor which is cleaved into smaller biologically active peptides such as the melanocortins, α-, β- and γ-melanocyte-stimulating hormone. Data from human genetic and murine studies convincingly show that an intact central melanocortin signalling pathway is critical for normal energy homoeostasis. Not only does a loss of normal melanocortin signalling lead to obesity, but there are also data implicating increased melanocortin activity in the pathogenesis of cachexia. The study of POMC biology has lead to some fundamental insights into the mechanisms controlling food intake and body weight. This increased understanding of the physiological roles of the melanocortin system has opened up the potential for the design and development of rational therapies to treat perturbations in energy homoeostasis.

INTRODUCTION

The biology of POMC (pro-opiomelanocortin) is complex and diverse. Smaller peptide fragments derived from the inert POMC precursor play a crucial role in integrating vital physiological functions, with POMC extensively processed in a highly tissue-specific manner to yield a range of peptides involved in a whole range of processes. Historically, the most well-known roles for these peptides have been in skin pigmentation, adrenal steroid synthesis and inflammation. However, over the last decade, a wealth of data have clearly shown that POMC-derived peptides, in particular those synthesized in neurons of the hypothalamus, play a critical role in controlling food intake and body weight.

Building upon these fundamental insights into the regulation of body weight, there is now the potential for the development of rational, mechanistic-based therapies to treat perturbations in energy homoeostasis. In this review, I will highlight some of the key findings from extensive murine and human genetic studies that have led to the central melanocortin system being the best-characterized neuronal pathway involved in the regulation of mammalian energy homoeostasis. Furthermore, I will outline current developments in potential therapies to manipulate this pathway and discuss their potential clinical uses in treating both obesity and cachexia.

POMC

In humans and mice the POMC gene consists of three exons. Exon 1 is untranslated, exon 2 codes for a signal peptide and the N-terminal region, with exon 3 coding for most of the translated mRNA [1]. Although POMC mRNA can be detected in a number of tissues, the gene is...
expressed at physiologically significant levels in a limited range of tissues. These include the corticotrophs of the anterior pituitary, neurons originating in the arcuate nucleus of the hypothalamus and the brainstem, plus cells in the dermis and the lymphoid system [1]. The transcribed POMC pro-peptide itself is functionally inert but, during translocation of the nascent protein through the endoplasmic reticulum and Golgi apparatus, it is extensively post-translationally processed, undergoing a series of proteolytic cleavages and chemical transformations to generate a series of smaller biologically active peptides [2].

Human POMC is made up of 241 amino acid residues [3]. It contains eight pairs and one quadruplet of basic amino acids which are cleavage sites for processing enzymes (Figure 1) [1]. The key cleavage enzymes which act at these sites are a family of endoproteases, the PCs (prohormone convertases), with the repertoire of POMC products seen in a particular tissue largely dependent on the range of processing enzymes expressed in that tissue. Thus pituitary corticotrophs express PC1 (prohormone convertase 1), but not PC2, resulting in the production of the N-terminal peptide, joining peptide, ACTH (adrenocorticotropic hormone) and β-lipotropin. In contrast, the expression of both PC1 and PC2 within the hypothalamus leads to the production of smaller peptide fragments such as α-, β- and γ-MSH (melanocyte-stimulating hormone). These three peptides, together with ACTH, are collectively known as the melanocortins.

**Figure 1** POMC processing in humans

POMC is a large precursor peptide processed into smaller biologically active fragments by cleavage at dibasic cleavage sites (solid lines). ACTH, together with α-, β- and γ-MSH are together known as the melanocortins (pale blue). NT, N-terminal fragment; JP, joining peptide; β-LPH, β-lipotropin; β-END, β-endorphin.

*MELANOCORTIN RECEPTORS*

The actions of the melanocortin peptides are mediated through a family of five melanocortin receptors (termed MC1R to MC5R; Table 1) [4]. These receptors show considerable homology, all being seven transmembrane domain G-protein-coupled receptors. MC1Rs are expressed within a range of cell types in the skin, including keratinocytes, melanocytes and endothelial cells. Signalling at MC1R is a key control point in melanogenesis, causing a switch in production from the red/yellow pigment phaeomelanin to the brown/black pigment eumelanin. MC2R is the classical adrenocortical ACTH receptor, expressed in the cortex of the adrenal gland [4]. MC3Rs are expressed in the brain, chiefly in the hypothalamus, cortex, thalamus and hippocampus [5,6]. Of note, MC3Rs can be found on some POMC hypothalamic neurons, where they may act as an autoinhibitory receptor.

MC4Rs are widely expressed within the central nervous system being present in the hypothalamus, thalamus, hippocampus, limbic system, brainstem and spinal cord [7]. Compelling human genetic and murine data have established that MC4R is a crucial molecular component of the homoeostatic circuit that regulates energy balance. Finally, MC5Rs are expressed at low levels in numerous tissues, including sebaceous, lachrymal and pheromone-producing exocrine glands [8,9]. Targeted disruption of MC5R in mice results in animals that have problems with thermoregulation and repelling water from their coat because of decreased production of sebaceous lipids [8].

**Table 1** Distribution and function of melanocortin receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Tissue</th>
<th>Ligand</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td>MC1R</td>
<td>Skin</td>
<td>α-MSH &gt; β-MSH &gt; γ-MSH &gt; ACTH</td>
<td>Pigmentation and immune function</td>
</tr>
<tr>
<td>MC2R</td>
<td>Adrenal cortex</td>
<td>ACTH</td>
<td>Steroidogenesis</td>
</tr>
<tr>
<td>MC3R</td>
<td>Hypothalamus, thalamus, kidney and gut</td>
<td>γ-MSH = α-MSH &gt; β-MSH</td>
<td>Cardiovascular control and sodium and energy homoeostasis</td>
</tr>
<tr>
<td>MC4R</td>
<td>Brain and spinal cord</td>
<td>β-MSH &gt; α-MSH &gt; γ-MSH</td>
<td>Food intake and energy expenditure</td>
</tr>
<tr>
<td>MC5R</td>
<td>Exocrine glands, lungs, spleen and pancreas</td>
<td>α-MSH = ACTH &gt; β-MSH &gt; γ-MSH</td>
<td>Sebaceous gland secretion</td>
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**MELANOCORTINS**

Melanocortins derive their name from the ability of the peptides to stimulate melanogenesis in the melanocyte and/or steroidogenesis in adrenal cortical cells [10]. This family of peptides possess structural similarity to a characteristic invariant tetrapeptide sequence (His–Phe–Arg–Trp) at their core being an absolute requirement for binding and activity at melanocortin receptors [11].

α-MSH is a 13-amino-acid-residue melanocortin agonist. α-MSH has a well-defined role in the skin where, acting via MC1R, it can influence pigmentation [12]. It also has anti-inflammatory and immunomodulatory
properties [13]. More recently, α-MSH has been proposed to be the melanocortin with the most important role in energy balance, acting upon MC4R (and to a lesser extent MC3R) in regions of the brain known to be involved in feeding behaviour. However, there remains contention regarding the physiological hierarchy of the melanocortin peptides and, in particular, whether α-, β- and γ-MSH have unique, overlapping or redundant roles in the processes that control energy balance.

β-MSH is a 22-amino-acid peptide which in vitro has a high affinity at MC4Rs [14,15]. Although historically the physiological role of β-MSH has been poorly defined, there are now data from human studies which report that β-MSH may play a critical role in the hypothalamic control of body weight in humans (reviewed below) [16,17].

The role of γ-MSH in energy homoeostasis is less well-defined, although it can bring about a reduction in food intake when given to a mouse lacking all endogenously derived melanocortin peptides [18]. More data suggest that the primary role of γ-MSH may be in the regulation of the cardiovascular system [19].

ACTH is a 39-amino-acid peptide that is able to activate all melanocortin receptors. However, ACTH is the only melanocortin that can act on MC2R [20] to cause the secretion of glucocorticoids and, to a lesser extent, androgenic steroids and mineralocorticoids.

AGOUTI AND AGRP (AGOUTI-RELATED PROTEIN) MELANOCORTIN ANTAGONISTS

Melanocortin receptors are unique among seven-transmembrane-domain-receptor families in that, in addition to a range of agonist ligands, there also exist two endogenously produced ligands which act as antagonists. This provides a mechanism to tightly regulate melanocortin receptor activity and to integrate opposing signals at the receptor level.

Agouti is a protein involved in regulating pigmentation [21]. It is secreted within the hair follicles to act in a paracrine fashion, antagonizing the action of α-MSH at MC1R expressed on the surface of melanocytes. Agouti induces a switch in pigment production from eumelanin (black/brown) to phaeomelanin (red/yellow). A number of dominant agouti alleles, such as A⁺ and A⁹, result in widespread ectopic expression of the agouti protein, giving rise to a phenotype of obesity, hyperphagia and yellow coat colour [21]. The link between hair colour and an obesity syndrome was made clearer when agouti was found to antagonize MC4R as well as MC1R. Additional insights came with the identification in the hypothalamus of a peptide named AgRP (due to its significant homology to agouti) [22]. AgRP acts as an antagonist at hypothalamic MC4Rs, with transgenic mice that ubiquitously express human AGRP developing hyperphagia and an obesity phenotype indistinguishable from that of the agouti mouse without an effect on pigmentation [23,24].

In a similar manner to POMC, AgRP undergoes processing by PC1 to produce a smaller, more biologically active peptide. The C-terminal fragment of AgRP, AgRP132, is 6-fold more potent at MC4Rs than full-length AgRP [25]. However, there are still some areas of uncertainty regarding the functions of AgRP and the roles of the N-terminal regions of AgRP have yet to be fully determined.

There are also some interesting in vitro data from two independent groups proposing that AgRP may be able to act not simply as an antagonist, but also as an inverse agonist at MC4R [26,27]. This agrees well with another model proposed by Srinivasan and co-workers [28] in which the N-terminal domain of MC4R functions as a tethered intra-molecular ligand, maintaining constitutive activity of the receptor and thereby bringing about tonic inhibition of food intake [28]. Such a model is intriguing not only because of the unique bi-directionality it would confer upon signalling at MC4Rs, but also because, if true, molecules based upon the N-terminal domain of the receptor may lead to novel therapeutic agents targeting this receptor.

MELANOCORTIN SIGNALLING IN THE HYPOTHALAMUS

An overriding theme which has come to prominence over the last decade is the critical role the central nervous system plays in co-ordinating metabolic functions in peripheral tissue. In particular, the hypothalamus is recognized to receive and integrate neural, metabolic and humoral signals from the periphery [29]. Within the hypothalamus, the arcuate nucleus, situated between the third ventricle and the median eminence, is considered to act as a primary sensor of alterations in energy stores to control appetite and body weight. Key to this role are two distinct subsets of arcuate neurons (Figure 2). The first population of neurons express POMC, with the majority of these cells also co-expressing the anorectic peptide CART (cocaine and amphetamine-related transcript). The mouse arcuate nucleus contains approximately 3000 of such POMC-positive cells. The second subset expresses the potent orexigenic peptides NPY (neuropeptide Y) and AgRP. Both sets of neurons send out dense projections to other nuclei within the hypothalamus, in particular to regions such as the paraventricular nucleus and lateral hypothalamus which express MC3R and MC4R. Additionally arcuate POMC neurons have widespread extra-hypothalamic descending projections to the brainstem, medulla and spinal cord [30]. The hypothalamic melanocortin system can therefore be defined as the neural circuits that include these two separate populations of neurons within the arcuate...
Neurons in the arcuate nucleus express leptin receptors and integrate peripheral signals to maintain energy homeostasis. Both NPY/AgRP and POMC neurons respond to leptin but do so in an opposite manner; NPY/AgRP neurons are inhibited but POMC neurons are activated. These first-order neurons signal through downstream second-order neurons to bring about changes in feeding behaviour and energy expenditure. 3v, third ventricle; PVN, paraventricular nucleus; LH, lateral hypothalamus.

These two populations of arcuate neurons express the long form of the leptin receptor (termed ObRb) [31] and are considered to be one of the key first-order neurons through which leptin exerts its effects. Leptin regulates these two neuronal populations in a reciprocal manner inhibiting NPY/AgRP neurons while stimulating POMC/CART neurons.

On the basis of these anatomical and functional data, a prevalent model of energy homeostasis has gained widespread acceptance (Figure 2). In situations of excess energy, high levels of leptin activate POMC neurons and trigger the release of melanocortins from POMC axon terminals. This in turn goes on to activate MC4R thereby leading to suppressed food intake and increased energy expenditure. Simultaneously, leptin suppresses the activity of arcuate AgRP/NPY, which would otherwise antagonize the effects of α-MSH on MC4R through the release of AgRP. In contrast, in times of energy depletion when leptin levels are low, there is reduced anorexigenic POMC neuron activity but increased orexigenic NPY/AgRP neuron activity. The NPY/AgRP system also robustly and directly inhibits POMC neurons through both NPY and the inhibitory neurotransmitter GABA (γ-aminobutyric acid) with this unidirectional interaction providing a tonic inhibition of POMC neurons whenever NPY/AgRP cells are active.

However, our knowledge of these pathways continues to rapidly evolve and, although the importance of the hypothalamic leptin-melanocortin signalling pathway in the control of energy homeostasis is still clear, the model outlined above is undoubtedly an oversimplified
paradigm. For example, POMC neurons and MC4Rs are also found in the brainstem [32], with melanocortin signalling in this region probably playing a role in integrating satiety signals from the gut [33]. Furthermore, the influence of leptin outside of the hypothalamus has been clearly shown by recent data indicating that it can act upon dopaminergic neurons in the ventral tegmental area (a region of the brain involved in reward and motivation) to influence food consumption and ingestive behaviour [34,35].

Finally, in addition to responding to peripheral leptin levels, arcuate neurons also integrate signals from a wide range of other nutrients and hormones (reviewed in [36]). For example, the effects of insulin on energy balance are likely to be integrated at the level of POMC neurons [37].

**MELANOCORTIN SIGNALLING AND OBESITY**

Genetically modified mouse models can be highly useful in gaining understanding of complex physiological systems. This is particularly true in the case of POMC biology and energy homoeostasis, where a powerful synergistic relationship between human genetics and murine modelling has been particularly fruitful in determining how the melanocortin system brings about changes in food intake and energy expenditure.

**Human POMC deficiency**

The first description of humans congenitally lacking the POMC gene products appeared in 1998. Krude et al. [38] reported two patients, one a compound heterozygote for two nonsense mutations in exon 3, and a second homozygous for a mutation that introduced an additional out-of-frame start site and interfered with POMC translational initiation [38]. As a consequence of ACTH deficiency, both subjects presented with the metabolic consequences of hypocortisolaemia in early childhood. Both went on to develop severe early-onset obesity associated with hyperphagia (due to reduced hypothalamic melanocortinergic signalling) and both subjects had pale skin and red hair (the result of reduced signalling through MC1R on melanocytes in skin and hair follicles). Since the first description of loss-of-function mutations in the human POMC gene, Gruter and co-workers have gone on to report three additional children with the same phenotype [39]. Co-workers here in Cambridge have also reported a patient from a Turkish family who is homozygous for a novel frameshift mutation which causes a stop codon to appear at the N-terminal end of POMC and therefore loss of all POMC-derived peptides [40]. Although affected by obesity and adrenal insufficiency, this child was the first reported patient with POMC deficiency who did not have red hair. This contrasts with the earlier other reported cases who were all white European Caucasians. This indicates that different ethnic groups may display a variable dependence on POMC peptides for eumelanin synthesis. Furthermore, this patient demonstrates that the absence of red hair in a non-Caucasian patient with obesity and adrenal insufficiency does not exclude POMC deficiency as a potential underlying diagnosis.

Humans heterozygous for mutations in POMC have also been studied. The heterozygous parents of the probands reported in the initial report of Krude et al. [38] were all found to have high, normal or mildly elevated body weight, suggesting a dosage effect of POMC gene products on human weight regulation. This was borne out when a number of extended family members of the Turkish patient were also studied [40]. Eleven out of 12 subjects heterozygous for the null mutation in POMC were either overweight or obese compared with only one out of seven wild-type family members. The finding that even haploinsufficiency of this gene appears to confer a substantial obesity risk emphasizes the critical role of the central POMC system in the control of human energy balance.

**Pomc-null mice**

There are now two independent mouse models with disruption of both alleles of the *Pomc* gene [41,42]. The phenotypes seen in both closely match the clinical picture reported in patients congenitally deficient in POMC peptides and indicate that the melanocortin pathways which regulate energy homoeostasis and adrenal function are very similar in humans and mice (Figure 3). Using such a model lacking all endogenously derived POMC peptides (*Pomc<sup>−/−</sup>*) our group has demonstrated that POMC deficiency results in an increase of both fat and lean tissue mass [41]. *Pomc<sup>−/−</sup>* mice also have a significant increase in body length compared with wild-type littermates. Additionally, *Pomc<sup>−/−</sup>* mice have a reduction in basal oxygen consumption and plasma thyroxine concentrations, indicating that the obesity phenotype may be the consequence of a reduced metabolic rate as well as increased food intake. We have also studied mice heterozygous for a null mutation in the *Pomc* allele (*Pomc<sup>+/−</sup>*) and, in keeping with the human data outlined above, have found they too have disordered energy homoeostasis. Although on standard chow, food intake and body weight in *Pomc<sup>+/−</sup>* mice are indistinguishable from wild-type, when challenged with a high-fat diet, although wild-type mice maintain the same body weight, *Pomc<sup>+/−</sup>* mice become significantly hyperphagic and develop obesity. This is in contrast with wild-type littermates which maintain the same energy intake and body weight [41].

**Disruption of activity at melanocortin receptors**

By screening the coding region of *POMC* in 262 Caucasian subjects with a history of severe obesity from childhood, Challis et al. [43] identified two children who were heterozygous for a missense mutation (Arg236Gly).
that disrupts the dibasic amino acid cleavage site between $\beta$-MSH and $\beta$-endorphin [43].

The Arg236Gly mutation completely prevented the normal processing of these two peptides, resulting in an aberrant $\beta$-MSH/$\beta$-endorphin fusion peptide. This fusion peptide had an affinity to MC4R comparable with that of $\beta$-MSH, but the ability to activate the receptor once bound was much reduced, thereby giving the mutant protein the capacity to interfere with melanocortin signalling. Indeed, mutations disrupting the $\beta$-MSH/$\beta$-endorphin cleavage site were found in 0.9 % of children with severe-onset obesity compared with only 0.2 % of normal weight controls, suggesting that mutations at this site may make an appreciable contribution to the genetic predisposition to severe childhood obesity.

Two more recent studies have directly addressed the uncertainty regarding the relative importance of particular POMC-derived melanocortin ligands in control of energy balance. Lee and co-workers [16] screened the coding regions of the POMC gene for mutations in 538 U.K. Caucasian subjects with severe early-onset obesity. They identified five subjects who were heterozygous for a missense variant in the region encoding $\beta$-MSH (Tyr221Cys). The obese children carrying the Tyr221Cys variant were hyperphagic and had increased linear growth, in a similar manner to subjects with MC4R deficiency. This variant was found at a significantly higher frequency in the obese study population than in unselected U.K. Caucasian controls (five out of 538 compared with four out of 5152 respectively) and Tyr221Cys co-segregated with obesity in affected family members. Finally, in vitro studies clearly demonstrated that, compared with wild-type $\beta$-MSH, the variant peptide had reduced binding and activity at MC4Rs. Interestingly, the same study also identified a single proband which was heterozygous for a missense mutation in $\alpha$-MSH, in which
the highly conserved histidine residue within the classical His–Phe–Arg–Trp receptor-binding motif was replaced by a glutamine residue. In keeping with canonical melanocortin biology, in vitro studies with a synthetic mutant α-MSH peptide showed this to have a deleterious effect on its function and, although the proband was obese, remarkably the same variant was found in one lean family member and one lean unrelated control. Thus this study favoured β-MSH rather than α-MSH as having a role in the control of energy balance.

Biebermann et al. [17] also reported a missense mutation within the coding region of β-MSH which was associated with early-onset human obesity. In addition, this study reported data from postmortem human brain studies to lend further support to the hypothesis that β-MSH plays a role in the hypothalamic control of human body weight. These findings have direct relevance to the development of novel anorexigenic compounds targeting the melanocortin system as they now provide a rationale to specifically base pharmacological analogues upon β-MSH.

**Loss of AgRP action**

Central administration of the potent orexin AgRP has a profound long-lasting effect upon food intake [44]. The first report of an Agrp-null mouse was therefore somewhat surprising because, in contrast with the hypoglycic lean animal one might expect with loss of antagonism at MC4R, the food intake and body composition of Agrp−/− mice was indistinguishable from wild-type littermates [45]. Interestingly, a more recent report has shown that Agrp−/− mice do actually display a modest lean phenotype late on in life, although this is the result of an increase in energy expenditure rather than hypophagia [46]. However, when AgRP neurons are ablated in the postnatal period, the results are very different [47]. In contrast with the very modest impact of Agrp deletion, loss of AgRP neurons in adult life leads to profound life-threatening hypophagia, thereby appearing to confirm that AgRP has a critical role in the regulation of food intake. However, deletion of a neuronal population is a more profound insult than removing a single peptide from the same neuronal population. AgRP neurons also express NPY and GABA and both have been shown to play a major role in the regulation of feeding. The loss of these or other, as yet uncharacterized, neurotransmitters from AgRP neurons may play a part in the dramatic result observed as much as the loss of the AgRP peptide.

At present, there is no compelling genetic evidence that abnormal elevation in AGRP expression or AgRP activity can cause an obesity phenotype in humans. However, there are reports of SNPs (single nucleotide polymorphisms) in both the promoter and the coding region that are associated with BMI (body mass index) and fat mass [48–50].

**Murine MC4R deficiency**

Mc4r-deficient mice develop a marked obesity syndrome associated with hyperphagia, hyperinsulinemia, hyperglycaemia and an increase in linear growth compared with wild-type [51]. Furthermore, heterozygous mice (Mc4r+/−) have an intermediate phenotype between wild-type and homozygous null mice, indicating a clear gene dosage effect. In the intervening decade since Huszar et al. [51] first reported the phenotype of Mc4r deficiency, Mc4r−/− mice have been extensively studied. Measurements of metabolic rate and data from pair-feeding studies have shown that, like Pomc-null mice, the obesity in Mc4r-null mice is as a result of defective regulation of energy expenditure as well as hyperphagia [52]. High-fat feeding also causes Mc4r−/− mice to develop obesity at an accelerated rate due to sustained hyperphagia, a much reduced level of diet-induced thermogenesis and a lack of increase in motor activity. Together with the data from Pomc null mice, this is highly suggestive that an intact melanocortin system is necessary to effect appropriate changes in homoeostatic mechanisms in response to changes in the calorific content of the diet [53].

**Human MC4R deficiency**

In 1998, two groups reported heterozygous mutations in humans in MC4R that were associated with dominantly inherited obesity [54,55]. Since then, mutations in MC4R have been reported in obese humans from various ethnic groups and are responsible for up to 5% of cases of severe childhood obesity and between 0.5 and 2.5% of adult obesity [56,57]. Furthermore, the finding that close to 1 in 1000 of the general population may have an MC4R mutation makes MC4R deficiency one of the most common single gene disorders [58].

As well as the increase in fat mass, MC4R mutant subjects also have an increase in lean mass that is not seen in other monogenic obesity syndromes such as leptin deficiency [59]. Affected children have increased linear growth with a height S.D. score of +2 compared with population standards. MC4R-deficient subjects also have higher levels of fasting insulin than age, sex and BMI SD score-matched children [59]. The accelerated linear growth and the disproportionate early hyperinsulinemia are consistent with observations in the Mc4r−/− mouse.

Affected subjects are objectively hyperphagic although this appears not to be as severe as that seen with leptin deficiency [59]. However, it is noteworthy that the severity of receptor dysfunction seen in in vitro assays can predict the amount of food ingested during a test meal by the subject harbouring that particular mutation [59].

**MC3R deficiency**

Homozygous-null Mc3r (Mc3r−/−) mice have an unusual phenotype, in that, although not significantly heavier than wild-type mice, they have an increased fat mass with a
reduction in lean mass [60,61]. *Mc3r*−/− mice also have a reduction in the body length. Our current understanding of the role of MC3R is that it influences feeding efficiency and the partitioning of fuel stores into fat. The non-redundant role of MC3R and MC4R is clearly illustrated by the finding that mice lacking both central melanocortin receptors become heavier than mice lacking MC4R alone [60].

Direct sequencing of the *MC3R* gene-coding sequence in populations with Type 2 diabetes mellitus and obesity has identified a number of sequence variants. However, these have all been detected in unaffected controls with similar frequencies or have been absent in family members who were also obese, and as yet there is no convincing evidence for a major role of *MC3R* mutations in causing a severe metabolic phenotype in humans [62,63].

**MELANOCORTIN PATHWAYS AND CACHEXIA**

As outlined above, disruption of central melanocortin pathways clearly leads to obesity in humans and rodents. It is perhaps not surprising then that overactivity of this important system is also being increasingly implicated in pathological states characterized by energy wasting.

Cachexia is a state of malnutrition characterized by a decrease in appetite and food intake combined with an inappropriate increase in metabolic rate, resulting in a loss of both fat and lean mass [64]. It is not the same as a voluntary fast when the sensation of hunger is not dulled and mechanisms entrained by reduced energy intake aim to restore energy supplies by increasing intake and reducing energy expenditure. Cachexia is commonly a feature of malignant diseases but is also associated with cardiac and renal failure, connective tissue disorders and chronic suppurative conditions. It impacts heavily upon quality of life and tolerance to therapy and has an impact on overall morbidity and mortality.

All of these disease have in common the production of systemic pro-inflammatory cytokines with the characteristic deleterious changes in appetite and metabolism ascribed to the direct action of cytokines altering the release and function of a number of neurotransmitters [65]. In particular, an increasing number of studies of melanocortin signalling have demonstrated that POMC neurons may be key transducers of stimuli which drive cachexia. For example, not only are the phenotypic features of MC4R deficiency the opposite of those seen in cachexia, but also activation of central MC4R leads to reduced food intake, increased energy expenditure and loss of body weight, replicating the response seen with cytokine administration. Further evidence that blockade of the central melanocortin signalling pathway can ameliorate the effects of cytokine administration comes from the findings that *Mc4r*−/− mice are resistant to LPS (lipopolysaccharide)-induced anorexia [66], whereas melanocortin antagonists given to wild-type animals can significantly ameliorate the reduction in food intake seen following LPS administration [66].

Perturbations in central melanocortin signalling can also result in differential responses to the induction of cachexia by tumours [67]. The hypophagia and weight loss induced by sarcoma growth can be reversed and prevented by administration of AgRP. Tumour-bearing *Mc4r*−/− mice are able to continue accumulating both lean and fat mass in the face of tumour growth, whereas wild-type control animals lose both lean and fat mass under identical conditions [67].

*Mc4r*−/− mice are also resistant to cachexia associated with renal disease. A subtotal nephrectomy in wild-type animals causes loss of lean and fat mass with an increase in resting metabolic rate, whereas *Mc4r*−/− mice undergoing this procedure continue to increase their lean and fat mass [68].

Intriguingly, *Mc3r*−/− mice suffer enhanced cachexia, both in response to a challenge with LPS and with tumour growth, when they lose more weight than wild-type mice during tumour-induced cachexia [67]. These data lend support to the hypothesis that MC3R may function, in part, as inhibitory autoreceptors on POMC neurons. In the absence of this putative braking function, a cachecic stimulus upon POMC neurons can proceed unabated leading to an overall increase in melanocortin tone and a more marked impact on energy balance [67].

**THERAPEUTIC MANIPULATION OF THE MELANOCORTIN PATHWAY**

The majority of the evidence presented above is based on a strategy of studying loss of gene function, be it naturally occurring or genetically engineered. However, this does not conclusively prove that therapeutic strategies directed to increase gene expression levels or activity of the gene product will ameliorate the phenotype seen with gene loss. A number of studies have addressed this issue and investigated the effect of increased melanocortin signalling brought about either by overexpressing *Pomc* or by administering potent melanocortin analogues.

**Genetic manipulation**

Li et al. [69] transgenically overexpressed neuronal POMC in genetically obese Zucker rats. There was a sustained reduction in food intake, a significant attenuation of weight gain and a 24% decrease in visceral adiposity. There are also data demonstrating that targeted *Pomc* gene therapy in the hypothalamus can reduce body weight and visceral adiposity in aged obese rats [70].

Savontaus et al. [71] generated transgenic mice overexpressing α- and γ-MSH under the control of the CMV (cytomegalovirus) promoter [71]. This resulted in a 2-fold increase in both α- and γ-MSH within the
hypothalamus and reduced weight gain and adiposity in lean wild-type male mice. Transgenic homozygous mice were also crossed with two obese strains of mice with disrupted melanocortin signalling, leptin-receptor-deficient db3J/db3J and yellow agouti A+/a mice and again obesity was attenuated. The same group used a similar strategy of transgenic overexpression to determine whether long-term melanocortinergic activation could attenuate the metabolic effects of a high-fat diet [72]. Their data showed that long-term melanocortin activation reduced body weight, adiposity and hepatic fat accumulation in the setting of diet-induced obesity.

Although such genetic manipulations are not in the realm of current available therapy, they nevertheless suggest that long-term melanocortinergic activation could serve as a potential strategy for the treatment of obesity.

**Pharmacological manipulation**

The fact that central melanocortin receptors are membrane-bound G-protein-coupled receptors with known peptide ligands, together with the wealth of evidence from the studies outlined above, makes the melanocortin system a very attractive target for rationally based therapies to treat disorders of energy homeostasis.

However, the endogenous melanocortins are linear peptides which are rapidly degraded by proteolytic enzymes and therefore hold limited promise as effective pharmacological agents. Thus many structural and functional studies over the last two decades have concentrated in trying to manipulate the basic melanocortin peptide sequence into compounds with improved potency and resistance to enzymatic degradation [73]. Several such compounds now exist (Figure 4). For example, if L-phenylalanine at position 7 of α-MSH is replaced by D-phenylalanine, the resultant peptide [NDP-α-MSH, also known as MTI (melanotan I)] is a more potent melanocortin agonist. Another potent melanocortin agonist is MTII (melanotan II), being a cyclic lactam ring derivative of the seven amino acids which make up α-MSH between positions 4–10. However, if the phenylalanine at position 7 of MTI is replaced by 2-naphthylalanine, this results in a high-affinity antagonist of both MC3R and MC4R called SHU9119.

Both MTII and SHU9119 have been used extensively in pharmacological studies of the melanocortin system [74]. In 1997, Fan et al. [75] demonstrated that central administration of MTII significantly decreased food intake in four different mouse models, including mice with a disrupted leptin–melanocortin system (ob/ob and A+/a mice) [75]. Furthermore, not only did SHU9119 bring about a significant increase in food intake, but co-administration of SHU9119 with MTII significantly abrogated the anorectic effect of the latter. More recently peripheral administration of MTII had been shown to suppress food intake and cause progressive weight loss in a dose-dependent manner in both lean and diet-induced obese mice [76,77].

In addition to manipulating the structure of melanocortin ligands, the concept of ‘privileged structure’ has been used to develop compounds active at melanocortin receptors. This approach is based upon the idea that a number of molecular structural scaffolds are able to bind with high affinity to multiple receptor families. Such organic cores, e.g. piperazine and cyclohexane, are often found in a number of commercially available drugs [73]. Using this approach, researchers at Merck have developed an active, small molecule peptide mimetic MC4R agonist named THIQ. Studies in mice demonstrate it is able to inhibit feeding in both acute and chronic study paradigms with no effect being seen in MC4R knockout mice [78].

A number of small molecule MC4R antagonists have also been successfully used in animal models of cachexia. NBI-12i is a potent MC4R antagonist that has nanomolar affinity and high specificity for MC4R [79]. As one might predict, it has no effect in MC4R−/− mice, but can significantly increase food intake and decrease the metabolic rate in wild-type mice when given peripherally. Importantly, when used in a murine cancer model (subcutaneous implantation of lung adenocarcinoma), NBI-12i can significantly attenuate the resultant cachexia with the ability to preserve lean body. Another compound, ML00253764, was discovered by high-throughput screening of non-peptide benzamidine compounds with potential activity at MC4Rs. This molecule was able to penetrate into the central nervous system after peripheral administration and has been shown to be able to effectively reduce tumour-induced weight loss in a xenograft mouse model [80].

**The melanocortin pathway: therapeutic promise and therapeutic problems**

There are drugs which specifically target melanocortin receptors that have undergone trials in humans [81]. Melanotan, as its name suggests, was developed to mimic the action of α-MSH on MC1R and induce melanogenesis in the hope that the resulting skin tan would protect against UV-related skin cancers. The related analogue MTII can also act upon skin melanocytes to
increase pigmentation but in early clinical trials had the unexpected side effect of producing spontaneous penile erections which were intermittently experienced for 1–5 h after MTII dosing.

Further studies have shown that MTII also appears to increase sexual desire but interestingly can also induce nausea, stretching and yawning [82], reminiscent in part of the ‘stretching–yawning syndrome’ seen many decades ago when POMC peptides were first centrally administered to dogs [83]. More recently, small trials of a nasally administered cyclic heptapeptide melanocortin analogue, PT-141, have also reported beneficial effects in men with erectile dysfunction [84].

Inevitably, a drug that can induce a bronzed tan, improve libido and sexual function and which may also have the potential to suppress appetite and cause weight loss has given rise to many titilating media commentaries with MTII attracting the unfortunate moniker of ‘The Barbie Drug’ [85].

As yet, the published data on melanocortin ligands for the treatment of obesity or cachexia are not as advanced. A small study looked at the effect of 6 weeks of intranasal ACTH4-10 on healthy normal-weight volunteers [86]. Compared with placebo, ACTH4-10 did have a significant effect, reducing body fat by 1.68 kg and body weight by 0.79 kg. Gruter and co-workers have reported a 3 month trial of treatment with intranasal ACTH4-10 in the two initial probands reported with total POMC deficiency but sadly there was no reduction in body weight or a change in eating behaviour in either [39]. One explanation may be that, compared with α-MSH, ACTH4-10 has a 1000-fold lower affinity at MC4R.

However, there are on-going ‘proof-of-concept’ trials involving MC4R agonists designed specifically for the treatment of obesity and the results from these are eagerly awaited.

CONCLUSIONS

Knowledge of the physiological roles of the melanocortin system has hugely increased in recent years, with POMC-derived peptides firmly placed at centre stage when it comes to our understanding of the molecular mechanisms controlling energy homeostasis. Much evidence from human and murine studies gives creedence to the possibility of successfully pharmacologically manipulating these pathways. The early evidence from clinical trials using MTII gives encouragement that administration of melanocortin analogues is, in the short term at least, safe. However, the unusual side effects observed with these agents add a note of caution. Although serendipitously leading to a new treatment for erectile dysfunction, they should provoke investigators to move away from a ‘hypothalamo-centric’ view of the melanocortin system and be wary of other hitherto unexpected effects when manipulating such a crucial central-nervous-system signalling pathway. Of course, unexpected does not always mean unwelcome and as melanocortin agents move over into the clinical arena we can anticipate interesting times ahead.

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