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Review

Physiological roles of the melanocortin MC_3 receptor

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ABSTRACT

The melanocortin MC_3 receptor remains the most enigmatic of the melanocortin receptors with regard to its physiological functions. The receptor is expressed both in the CNS and in multiple tissues in the periphery. It appears to be an inhibitory autoreceptor on proopiomelanocortin neurons, yet global deletion of the receptor causes an obesity syndrome. Knockout of the receptor increases adipose mass without a readily measurable increase in food intake or decrease in energy expenditure. And finally, no melanocortin MC_3 receptor null humans have been identified and associations between variant alleles of the melanocortin MC_3 receptor and diseases remain controversial, so the physiological role of the receptor in humans remains to be determined.

Contents

1. Structure and function of the receptor

The melanocortin MC_3 receptor belongs to the G-protein coupled receptor family (Gantz et al., 1993; Roselli-Rehfuss et al., 1993). It is positively coupled to adenylyl cyclases through Gs and, upon activation, stimulates cAMP production. A few studies suggest that overexpressed melanocortin MC_3 receptor activation can also induce calcium release from intracellular stores (Kim et al., 2002b; Konda et al., 1994; Mountjoy et al., 2001). The mechanism of calcium release is unclear and the role of IP_3 generation is controversial (Kim et al., 2002a; Konda et al., 1994; Mountjoy et al., 2001). Based on the discrepancy observed in this signaling cascade when studied in different in-vitro models, it will be important to validate the activation of calcium signaling in melanocortin MC_3 receptor neurons in ex-vivo or in-vivo models. Another pathway activated downstream of melanocortin MC_3 receptor is the MAPK pathway. Indeed, Chai et al.

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(2007) showed that, in HEK293 cells transfected with the melanocortin MC_3 receptor, NDP-αMSH triggers a significant phosphorylation of ERK1/2. In addition, they established that melanocortin MC_3 receptor-mediated MAPK activation is PI3K dependant and pertussis toxin sensitive (Chai et al., 2007). Interestingly, as with the melanocortin MC_4 receptor (Nijenhuis et al., 2001), the melanocortin MC_3 receptor was reported to have a constitutive activity (Nijenhuis et al., 2001) but the physiological relevance of this finding is still unclear. Importantly, the melanocortin MC_3 receptor is one of the rare G protein-coupled receptors to have a natural inverse agonist, agouti-related protein (Nijenhuis et al., 2001; Ollmann et al., 1997), a protein homologous to agouti (Ollmann et al., 1997). Like most G protein-coupled receptors, following activation, the melanocortin MC_3 receptor recruits β-arrestin and internalizes (Breit et al., 2006; Nyan et al., 2008). However, a surprising feature of the melanocortin MC_3 receptor was uncovered when Breit et al. (2006) showed that both melanocortin MC_3 receptor agonists and its natural antagonist agouti-related protein can promote its internalization. This is very unusual as internalization is thought to be a G protein-coupled receptor signaling "turn off" mechanism, and antagonist-mediated blockade of receptor signaling usually causes a compensatory increase in surface receptor expression rather than receptor internalization. This observation could suggest the existence of a yet unidentified agouti-related protein-mediated signaling pathway.

The natural agonists for the melanocortin receptors are α, β, and γ-melanocyte stimulating hormone, and adrenocorticotropic (ACTH) hormone. They are all proteolytic products of the proopiomelanocortin preprohormone precursor, and all contain the tetrapeptide pharmacophore His-Phe-Arg-Trp. Melanotropins differ in their potency at the five members of the melanocortin receptor family. The melanocortin MC_2 receptor is the only melanocortin receptor to be specifically activated by only one of the melanotropins, namely, ACTH. Also, γ-MSH has over 100 fold higher affinity and 45 fold higher potency at the melanocortin MC_3 receptor than at the other melanocortin receptors. This selectivity is likely to be physiologically important since γ-MSH has been reported to be expressed in the brain (Kawai et al., 1984).

As with most G protein-coupled receptors, the mapping of the melanocortin MC_3 receptor's ligand binding pocket is incomplete. Site directed mutagenesis of amino acids putatively involved in melanocortin MC_3 receptor–ligand interaction, based on knowledge acquired from similar studies on melanocortin MC_1 receptor and melanocortin MC_4 receptor, was performed by Chen et al. (2006). They showed that alanine substitution of amino acids E131, D154, and D158 in TM2 and 3, predicted to form an ionic binding pocket for α-MSH, caused a significant decrease in agonist binding and receptor signaling. Mutagenesis of aromatic amino acids F295 and F296 as well as residue H298, all located in the TM6, also impaired agonist binding and were hypothesized to be part of a hydrophobic binding pocket (Chen et al., 2006). In the same study, the authors also established a requirement for residues D121 and D332 in order to achieve proper expression of the melanocortin MC_3 receptor at the plasma membrane; it is however unclear if the lack of receptor at the plasma membrane is due to deficient trafficking, or reduced receptor synthesis or stability. Another interesting finding is the conversion of SHU9119 from antagonist to agonist by mutating the leucine at position 165 in the melanocortin MC_3 receptor. This result mimics the previous identification of the same behavior for the corresponding L133 in the TM3 of melanocortin MC_4 receptor (Yang et al., 2002) suggesting a role for the described leucine residue in agonist vs. antagonist selectivity for both melanocortin MC_3 receptor and melanocortin MC_4 receptor.

The development of biologically active melanocortin MC_3 receptor specific ligands, both agonist and antagonists, will be instrumental to the elucidation of melanocortin MC_3 receptor roles in vivo. To this end, several approaches were used, such as a D-amino acid scan of γ-MSH (Grieco et al., 2000) leading to the discovery of D-Trp^8-γ-MSH, a compound reported to be the most selective melanocortin MC_3 receptor agonist known today with 250 fold and 300 fold higher potency at the melanocortin MC_3 receptor than at the melanocortin MC_5 receptor and at the melanocortin MC_4 receptor respectively. However, when the same compound was independently tested using a different cAMP assay (Promega P-Glo) the EC_{50} at the melanocortin MC_3 receptor was found to be 0.17 nM, corresponding to a 15 fold selectivity only for melanocortin MC_3 receptor compared to the melanocortin MC_4 receptor (Table 1). These divergent results demonstrate the importance of independent testing of the ligands developed using different methods to validate their potency and specificity. In a separate study, an α-MSH/γ-MSH hybrid (peptide 4) created by Cai et al. (2005) showed specific antagonist activity at the melanocortin MC_3 receptor with an IC_{50} of 6 nM, however, this hybrid is also a potent agonist of the melanocortin MC_1 receptor and the melanocortin MC_4 receptor and a partial agonist of the melanocortin MC_5 receptor. Balse-Srinivasan et al. (2003) synthesized a cyclic α-MSH/β-MSH analogue (peptide 9) with potent antagonist properties at the melanocortin MC_3 receptor (IC50 = 3 nM), however, this compound is not specific (melanocortin MC_5 receptor/melanocortin MC_3 receptor = 31). Kavarana et al. (2002) synthesized a series of cyclic analogues of α-MSH from which the peptide MK-9 is a potent melanocortin MC_3 receptor antagonist with a Ki of 5.9 nM, however this petide is also poorly selective (melanocortin MC_4 receptor/melanocortin MC_3 receptor = 37) and is a potent agonist at melanocortin MC_5 receptor (EC_{50} = 1.01 nM) (Kavarana et al., 2002). Other studies produced a variety of ligands with activity at the melanocortin MC_3 receptor with different affinity, potency and specificity but none of those compounds demonstrated satisfactory selectivity for the melanocortin MC_3 receptor over the other melanocortin receptors.

Manipulation of known peptidic ligands of the melanocortin receptors has provided us with a tremendous amount of information in the requirement for receptor binding affinity and selectivity, and additional work will be required to achieve compounds with 100–1000 fold selectivity for melanocortin MC_3 receptor. More extensive modification and testing of compounds already available as well as identification of small molecule ligands or allosteric modulators could prove successful at producing molecules highly specific for the melanocortin MC_3 receptor. Such ligands would allow targeted and specific manipulation of melanocortin MC_3 receptor signaling in vivo and, hopefully, will lead to a better understanding of the physiological roles of the melanocortin MC_3 receptor.

2. Expression of the receptor

2.1. Central expression

Both melanocortin MC_3 receptor and MC_4 receptor are expressed in hypothalamic, midbrain, and brainstem, nuclei, however the similarity in CNS expression ends there (Mountjoy et al., 1994;

Table 1
EC50 values for α-MSH and D-Trp-8-γ-MSH at the human melanocortin MC_3 receptor and melanocortin MC_4 receptor using the pGLO cAMP detection system. Human HEK293 cells were cotransfected with plasmids encoding the human melanocortin MC_4 receptor or melanocortin MC_3 receptor cDNAs (pCDNA3.1 vector) and with a plasmid encoding an engineered cAMP sensitive luciferase (pGLO sensor™-20FcAMP plasmid, Promega) and stable clones were selected for their ability to respond to α-MSH. Cells were seeded in a 384 well plate in 10 μL of culture medium without antibiotics and were incubated by adding 10 μL of the substrate containing media (GloSensor™ cAMP assay, Promega) diluted at 4% in CO_2-independent medium (Gibco). The luminescence was recorded before and after injection of a range of concentrations of α-MSH or D-Trp-8-γ-MSH for 15 min to obtain the maximal luminescent responses on a Spectramax M5 (Molecular Devices) plate reader (100 msec integration). Incubations were performed in triplicate and curves and EC_{50} values were determined using Prism (Graphpad).

Compound	hMC4-GLO (EC_{50})	hMC4R-GLO (EC_{50})
α-MSH	2.38×10^{-10}	4.5×10^{-10}
D-Trp-8-γ-MSH	1.7×10^{-10}	2.5×10^{-9}

Roselli-Rehfuss et al., 1993). Studies of the receptor show discrete regions of CNS expression for the melanocortin MC_3 receptor signal relative to other melanocortin receptor subtypes. Therefore, despite the relative redundancy of melanocortin receptor signaling and activation, there are clear functionally distinct roles for the melanocortin MC_3 receptor.

Expression studies of melanocortin MC_3 receptor mRNA have primarily been focused in the rodent brain. Northern blot hybridization experiments demonstrate the greatest expression of the melanocortin MC_3 receptor gene in the hypothalamus (Roselli-Rhefus et al., 1993). *In situ* hybridization demonstrate approximately 35 different nuclei expressing the receptor, with the highest expression in the arcuate nucleus, ventromedial hypothalamus, medial habenula, ventral tegmental area, and raphe (Roselli-Rhefus et al., 1993). Not surprisingly, melanocortin MC_3 receptor mRNA is found primarily in areas of the brain which receive direct innervation from proopiomelanocortin immunoreactive neurons. However, the arcuate nucleus, which contains all of the forebrain proopiomelanocortin expressing neurons, displays moderate levels of melanocortin MC_3 receptor mRNA, while the nucleus of the solitary tract (NTS) containing the other central proopiomelanocortin expressing neurons apparently does not express melanocortin MC_3 receptor mRNA (Roselli-Rhefus et al., 1993). The expression of melanocortin MC_3 receptor in the arcuate nucleus and ventromedial hypothalamus are particularly intriguing with regard to the observations that the receptor appears to inhibit anorexigenic proopiomelanocortin neurons and yet when deleted causes obesity, perhaps via as yet uncharacterized actions in the ventromedial hypothalamus or other nuclei. A mouse line containing GFP under the control of a melanocortin MC_3 receptor BAC clone is available, and is a helpful tool for better defining the properties of melanocortin MC_3 receptor neurons in the CNS (Fig. 1).

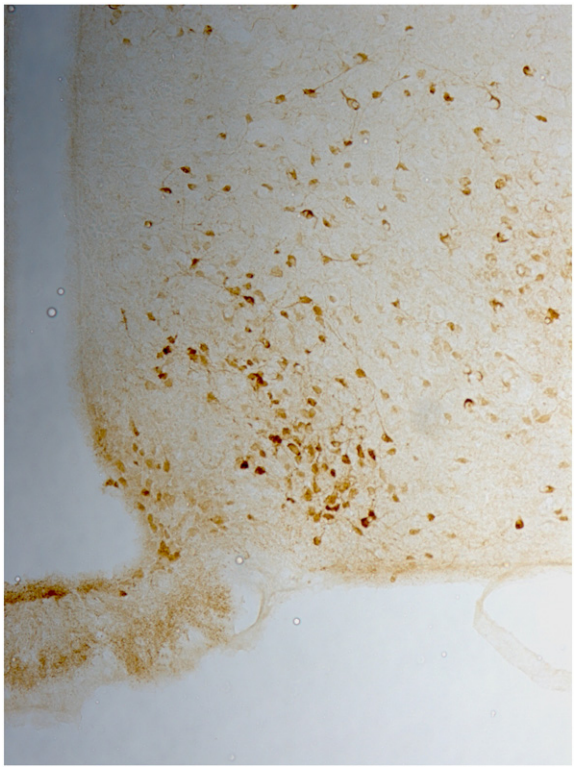

Fig. 1. MC3-GFP positive cell bodies in the arcuate nucleus and ventromedial hypothalamic nucleus. A transgenic mouse (MMRRC stock number 00264-UNC) containing the GFP protein under the control of a melanocortin MC_3 receptor BAC clone was used for the preparation of coronal brain slices. GFP was identified immunohistochemically.

2.2. Development

Melanocortin MC_4 receptor binding predominates centrally in various embryonic stages of development, however, a rapid increase in ventromedial and arcuate nuclei expression of melanocortin MC_3 receptor mRNA postnatally with higher CNS expression evident at postnatal day 27 is described by Kistler-Heer et al. (1998). Interestingly, a transition is shown in melanocortin receptor subtype in regions of the brain including the ventral tegmental area and the dorsoposterior hypothalamus from melanocortin MC_4 receptor to melanocortin MC_3 receptor-dominant throughout development. This change in receptor subtype suggests a differentiation in the regional melanocortin MC_3 receptor signaling in order to more acutely regulate specific neuronal populations. Further studies to support this idea show a 3–4 fold increase in melanocortin MC_3 receptor mRNA in the ventral tegmental area, habenula, and ventromedial hypothalamus from birth to adulthood in rats (Xia and Wikberg, 1997).

2.3. Peripheral expression

To add to the complexity of this receptor, expression outside the CNS has been documented, and physiological actions outside the CNS have been demonstrated as well. Northern analysis of poly(A) + RNA has established the presence of melanocortin MC_3 receptor transcripts of the appropriate size in human placenta, and in several human gut tissues including the stomach, duodenum, and pancreas using a combination of RT-PCR and southern blotting techniques (Gantz et al., 1993). In another study, PCR analysis of human tissues similarly detected melanocortin MC_3 receptor cDNA in the heart, while southern blotting of amplified cDNA detected expression in the testis, ovary, mammary gland, skeletal muscle, and kidney (Chhajlani, 1996). Further studies in rodents have confirmed melanocortin MC_3 receptor expression in the kidney and peritoneal macrophages (Getting et al., 2003; Ni et al., 2006b). Melanocortin MC_3 receptors in these regions may function in modulating natriuresis and immune function, which will be further elaborated later in this review.

3. Genetics

The importance of melanocortin MC_3 receptor in human obesity was first suggested by a study that showed a QTL for % body fat in the region of the melanocortin MC_3 receptor gene (Lembertas et al., 1997). Further analysis showed that peak LOD scores for body mass index (BMI), fat mass, and subcutaneous fat were localized near the melanocortin MC_3 receptor gene (Lembertas et al., 1997). Since then studies have focused on the identification of the common and rare allelic variants of the melanocortin MC_3 receptor that predispose to obesity (Fig. 2).

Common variants that may affect melanocortin MC_3 receptor expression or function have been found 5′, within, and 3′ of the open reading frame (ORF). 4 common polymorphisms (−201C>G, −239A>G, −762A>T, and −769T>C) have been identified 5′ of the ATG site for melanocortin MC3 receptor (Li et al., 2000). The −239A>G variant falls directly within a GATA binding site, and mutation at this site decreases binding affinity for GATA4 (Schalin-Jantti et al., 2003). In fact, a frequency of 4.5 to 21% has been reported for the −239A>G variant (Obregon et al., 2010; Schalin-Jantti et al., 2003). Despite a known function, this variant has not been shown to be present at a higher rate in obese than in lean controls (Li et al., 2000). In fact, none of the common 5′ variants are present more frequently in obese population than in lean controls.

Within the ORF, two common variants have received the majority of interest. Nucleotide substitutions 17C>A and 241G>A result in missense mutations T6K and V81I, respectively (Obregon et al., 2010; Rutanen et al., 2007; Wong et al., 2002. Minor allele frequency for each of these variants is reported to be 5.6–16% (Schalin-Jantti,

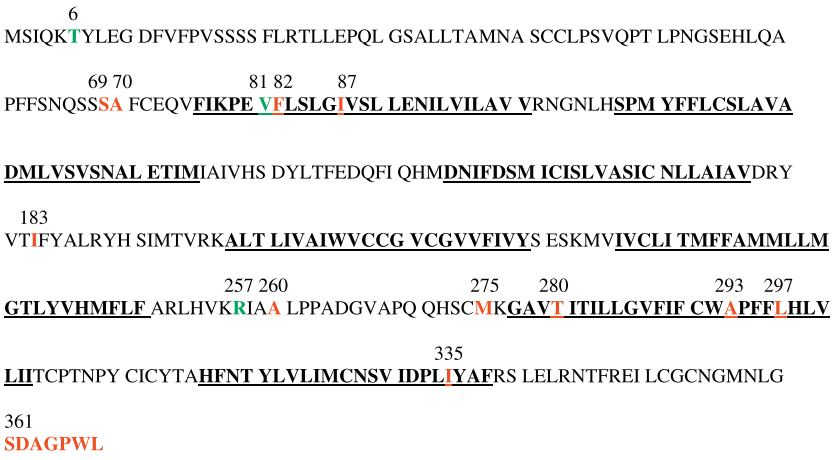

Fig. 2. N-terminus to C-terminus FASTA protein sequence of the human melanocortin MC_3 receptor with sites of common (green) and rare (red) variants highlighted. Underlined sequences are transmembrane domain regions.

2003 #16). Because they are in linkage disequilibrium, the effects of these variants have been studied in conjunction (Lee et al., 2007; Mencarelli et al., 2008; Rutanen et al., 2007). In a cell culture model, expression of the double mutant has been shown to decrease maximal binding to NDP-MSH by 50% and NDP-stimulated cAMP accumulation by approximately 30% (Feng et al., 2005). Feng et al. report homozygous presentation of both polymorphisms with a prevalence of 15.8 and 1.7% in African American and Caucasian populations, respectively. Together these results suggest that co-presentation of these common variants occurs at a high frequency and may result in a measurable phenotype.

The initial report of the Lys6 Ile81 variants suggested that neither correlated with obesity or glucose tolerance (Wong et al., 2002). However, subsequent studies with larger sample populations have shown that homozygous presentation of the Lys6 Ile81 variant can affect body weight, BMI, fat mass, % body fat, and energy intake (Feng et al., 2005; Lee et al., 2007; Savastano et al., 2009). Additionally, homozygous expression of the Lys6 or Ile81 variants results in decreased HOMA, insulin:glucose ratio, and fasting glucose (Lee et al., 2007). The decreased fasting glucose may result from increased glucose oxidation, previously shown for carriers of the Lys6 and Ile81 alleles (Rutanen et al., 2007). Circulating triglycerides and fasting free fatty acids are lower in homozygous carriers of the Lys6 and Ile81 minor alleles (Lee et al., 2007; Rutanen et al., 2007). Lipid oxidation was lower in carriers of the two minor alleles in both the basal and insulin stimulated state than in individuals that had the Thr6 Val81 genotype (Rutanen et al., 2007). Combined, these results suggest that the common variants T6K and V81I do affect melanocortin MC_3 receptor function resulting in measurable phenotypes.

Less well studied common variants include a recently reported ORF variant and a 3′ insertion variant (Boucher et al., 2002; Calton et al., 2009). Calton et al. report an ORF sequence variant R257S that was present in both obese and lean humans and occurred with a prevalence of 0.4%. However, no investigation into possible functions of this variant was reported. The 3′ insertion +2138CAGACC occurs with a minor allele frequency of 17.6% (Obregon et al., 2010). However, the effects of this common variant are not well established (Boucher et al., 2002).

In contrast to the melanocortin MC_4 receptor, however, the prevalence of rare melanocortin MC_3 receptor variants was not associated with obesity and was found to be 0.49% when sequencing 889 obese subjects and 932 lean controls (Calton et al., 2009). A total of 12 rare mutations have been reported in the literature (Calton et al., 2009; Lee et al., 2007; Mencarelli et al., 2008). The most studied of the rare mutations is the I183N mutation, which results in a complete lack of signaling in response to agonist stimulation (Lee et al., 2002; Rached et al., 2004; Tao and Segaloff, 2004). The muted signaling associated with the I183N mutation appears to result from decreased trafficking of the melanocortin MC_3 receptor to the cell membrane (Rached et al., 2004; Tao and Segaloff, 2004). Two additional mutations T280S and I335S, first identified in humans, have been found to nearly completely mute melanocortin MC_3 receptor activity (Calton et al., 2009; Mencarelli et al., 2008). Similar to the I183N mutation the I335S mutation is shown to eliminate cell surface expression. In fact, I335 of melanocortin MC_3 receptor correlates with I301 of the melanocortin MC_4 receptor, for which the I301T mutation was previously reported to be a loss-of-function mutation (Vaisse et al., 2000). Three additional melanocortin MC_3 receptor mutations, first identified in human samples, have been reported to affect receptor activity in a cell culture model. The two robust mutation effects occur with the S69C mutation, which decreases maximal receptor activity to 55% of WT levels, and the F82S mutation which increases the EC_{50} more than 100 fold and decreases maximal receptor activity more than 50% (Calton et al., 2009). Cells expressing the A70T mutant melanocortin MC_3 receptor display a slightly reduced maximal cAMP response to MSH (Lee et al., 2007). Rare mutations for which no obvious signaling defects exist include I87T, A260V, M275T, L297V, A293T, and X361S (Calton et al., 2009; Mencarelli et al., 2008). The X361S mutation abolishes the stop codon leading to the addition of seven extra amino acids to the intracellular C terminus of the receptor (Mencarelli et al., 2008).

Genetic studies have identified mutations in human melanocortin MC_1 receptor (Koppula et al., 1997; Valverde et al., 1995), MC2-R (Clark et al., 1993; Tsigos et al., 1993), melanocortin MC_4 receptor (Farooqi et al., 2000; Vaisse et al., 2000), and proopiomelanocortin (Krude et al., 1998) that lead to distinct syndromes. Yet, despite the significant obesity syndrome seen on deletion of the melanocortin MC_3 receptor in the mouse, an obesity syndrome associated with loss of melanocortin MC_3 receptor expression in humans has not yet been clearly demonstrated.

4. Melanocortin MC_3 receptor as an autoreceptor

Melanocortin MC_3 receptor is expressed widely within the CNS with abundant expression in the proopiomelanocortin and NPY neurons of the arcuate nucleus (Bagnol et al., 1999; Mounien et al., 2005). In fact, melanocortin MC_3 receptor is expressed in a rostral caudal gradient in both proopiomelanocortin (43%→13%) and AgRP/NPY (55→28%) neurons. An auto-inhibitory functional role of melanocortin MC_3 receptor on proopiomelanocortin neurons was first suggested when it was shown that bath application of the melanocortin MC_3 receptor agonist, D-trp^8-γ-MSH (7 nM), increased IPSC frequency on

proopiomelanocortin neurons (Cowley et al., 2001). This was in direct opposition to the inhibitory effect of NPY (100 nM) on IPSC frequency in proopiomelanocortin neurons (Cowley et al., 2001). Subsequently, in vivo effects of D-trp^8-γ-MSH have supported a role for melanocortin MC_3 receptor in the dampening of proopiomelanocortin neuronal activity. Peripheral administration of D-trp^8-γ-MSH has been shown to cause a dose responsive increase in food intake that peaked at 5 μg/animal and was absent in the melanocortin MC_3 receptor$^{-/-}$ mouse (Marks et al., 2006). Using the melanocortin MC_4 receptor$^{-/-}$ mouse, reductions in food intake at higher doses of D-trp^8-γ-MSH were shown to result from non-specific activity at the MC4R (Marks et al., 2006). Lending further credence to an auto-inhibitory role of melanocortin MC_3 receptor on proopiomelanocortin neurons, 66 h melanocortin MC_3 receptor stimulation by ICV infusion of D-trp^8-γ-MSH decreases proopiomelanocortin mRNA expression (Lee et al., 2008). While melanocortin MC_4 receptor$^{-/-}$ mice appear relatively insensitive to the food intake and body weight effect of illness induced cachexia, melanocortin MC_3 receptor$^{-/-}$ mice are hypersensitive to these same cachexia models (Marks et al., 2003). The opposite effects of melanocortin MC_3 receptor and melanocortin MC_4 receptor ablation in cachexia models provide further evidence for a role of melanocortin MC_3 receptor as a brake on proopiomelanocortin neuron activity and subsequently melanocortin MC_4 receptor stimulation.

5. Physiology of the melanocortin MC_3 receptor

Our understanding of the physiology of the melanocortin MC_3 receptor has lagged behind that of the other centrally expressed receptor, melanocortin MC_4 receptor. Much of what we do know about the physiological function of this receptor has come from studies using the melanocortin MC_3 receptor$^{-/-}$ mouse and the comparatively melanocortin MC_3 receptor specific agonist γ-MSH and its stable analogues.

5.1. Energy homeostasis

The melanocortin MC_3 receptor$^{-/-}$ mouse has a unique phenotype characterized by an increase in adiposity on a standard chow diet in the absence of a notable difference in body weight, total food intake or energy expenditure (Butler et al., 2000; Chen et al., 2000). The increase in adiposity in these animals is exacerbated by feeding a high-fat diet (Butler et al., 2000; Ellacott et al., 2007; Sutton et al., 2006; Trevaskis et al., 2007) suggesting an alteration in nutrient partitioning. Despite the increase in adiposity in the melanocortin MC_3 receptor$^{-/-}$ mouse these animals are relatively protected from the development of metabolic syndrome, compared with other mouse models with comparative levels of adiposity, due to a reduced inflammatory response to obesity (Ellacott et al., 2007; Trevaskis et al., 2007). The obesity phenotype in the melanocortin MC_3 receptor$^{-/-}$ mouse occurs by a mechanism distinct from the obesity phenotype in the melanocortin MC_4 receptor deficient animals as combined melanocortin MC_3 receptor/melanocortin MC_4 receptor deletions have an additive effect on adiposity (Chen et al., 2000). Recent studies have proposed that elements of the phenotype in the melanocortin MC_3 receptor$^{-/-}$ mouse may be caused by an alteration in circadian rhythm in this model (for review see (Begriche et al., 2009)), which will be discussed in a later section of this review.

Despite the increase in adiposity in the melanocortin MC_3 receptor$^{-/-}$ mouse, a striking phenotype in these animals is an increased susceptibility to weight loss in experimental models of cachexia (Marks et al., 2003; Marks et al., 2001). This is in sharp contrast to the melanocortin MC_4 receptor$^{-/-}$ mouse which is protected from weight loss in numerous cachexia paradigms relative to wild-type animals (Cheung et al., 2005; Marks et al., 2001, 2003; Scarlett et al., 2010). The differential response to cachexia in these two models of central melanocortin receptor deficiency, which both show increased adiposity, is likely to be connected at least in part to the differences in lean body mass phenotype. In melanocortin MC_4 receptor$^{-/-}$ animals obesity is associated with increased lean body mass (Huszar et al., 1997) while the increased adiposity in the melanocortin MC_3 receptor$^{-/-}$ mouse does not correlate with an increase in lean mass (Butler et al., 2000; Chen et al., 2000). Furthermore, the enhanced cachexia seen in the melanocortin MC_3 receptor$^{-/-}$ animals may support the hypothesis that the melanocortin MC_3 receptor functions, at least in part, as an autoinhitory receptor on hypothalamic proopiomelanocortin neurons.

There are a limited number of pharmacological studies examining the role of the melanocortin MC_3 receptor in the regulation of energy homeostasis. The melanocortin MC_3 receptor agonist D-Trp^8-γ-MSH, a stabilized γ-MSH analogue which has significant selectivity for the melanocortin MC_3 receptor over the melanocortin MC_4 receptor (Grieco et al., 2000), has been used to examine alterations in feeding behavior in response to pharmacological modulation of the melanocortin MC_3 receptor. Intracerebroventricular (i.c.v.) administration of D-trp^8-γ-MSH in rats (Lee et al., 2008) and peripheral administration of the same compound in mice (Marks et al., 2006) stimulate food intake. In these studies intake was measured in freely-feeding animals following chronic administration via an osmotic mini-pump (Lee et al., 2008) or following acute administration prior to the normal nocturnal intake period or in satiated animals (Marks et al., 2006). Some early studies using γ-MSH as opposed to D-trp^8-γ-MSH failed to see any effect of i.c.v. administration of this peptide on food intake of rodents in a fast-induced refeeding paradigm (Abbott et al., 2000; Kask et al., 2000). The differences in outcome between these studies are likely due to the increased stability of D-Trp^8-γ-MSH compared with γ-MSH and the different feeding paradigms used. Currently, due to a lack of pharmacological antagonists with the ability to cleanly differentiate between melanocortin MC_3 receptor and melanocortin MC_4 receptor much of what we know about the effect of the loss of melanocortin MC_3 receptor signaling in the regulation of energy homeostasis comes from studies in animals with genetic deficiency of these receptors, as described above.

5.2. Natriuresis

The cardiovascular and natriuretic effects of γ-MSH in rodents have been documented since 1985 (Callahan et al., 1985; Lymangrover et al., 1985). While there are some debates over whether the cardiovascular effects of exogenously administered γ-MSH are mediated via the melanocortin MC_3 receptor (Gruber et al., 2009; Mioni et al., 2003; Ni et al., 2006b), the importance of the melanocortin MC_3 receptor in mediating natriuresis is established. Gamma-MSH plays a critical role in reflex natriuresis after unilateral nephrectomy (Lin et al., 1987; Ni et al., 1998). In addition to circulating γ-MSH being elevated after unilateral nephrectomy (Lin et al., 1987; Ni et al., 1998), both circulating γ-MSH and kidney melanocortin MC_3 receptor mRNA levels are also increased in rodents following ingestion of a high-salt diet (Chandramohan et al., 2009; Mayan et al., 1996; Ni et al., 2006a) implicating melanocortin MC_3 receptor signaling in mediating the natriuretic response in two distinct paradigms. The results of these studies are reinforced by studies demonstrating that the genetic disruption of γ-MSH signaling in either the melanocortin MC_3 receptor deficient mouse or prohormone convertase 2 (PC2) deficient mouse (which is unable to process proopiomelanocortin to γ-MSH) results in salt-sensitive hypertension (Ni et al., 2003). In the case of the PC2 deficient animal, this salt-sensitive hypertension can be overcome by infusion of exogenous NDP-γ-MSH, a stable γ-MSH analogue (Ni et al., 2003). A detailed review of the cardiovascular and renal actions of γ-MSH can be found elsewhere (Humphreys, 2007).

5.3. Immune function

Peptides of the melanocortin family have been shown to exert anti-inflammatory effects in vivo and in vitro (for review see (Catania et al., 2004)). Many of the anti-inflammatory effects of melanocortin peptides are believed to be mediated via the melanocortin MC_1 receptor, which is expressed on cell types involved in mediating the inflammatory response including neutrophils, monocytes, dendritic cells and B-lymphocytes (for review see (Catania, 2007)). Melanocortin MC_3 receptor mRNA has also been detected in macrophages (Getting et al., 1999a,b). Melanocortin MC_3 receptors on macrophages have been proposed to mediate some of the anti-inflammatory effects of γ-MSH. In a model of gouty arthritis, D-Trp^8-γ-MSH dose dependently reduces interleukin-1 and chemokine CXCL1 release from primary peritoneal macrophages induced by monosodium urate crystals in a melanocortin MC_3 receptor dependent mechanism (Getting et al., 2006a; Getting and Perretti, 2001). The anti-inflammatory effects of D-Trp^8-γ-MSH in this paradigm are intact in macrophages obtained from the recessive yellow *e/e* mouse which has defective melanocortin MC_1 receptor signaling, further supporting the contribution of the melanocortin MC_3 receptor in mediating this effect (Getting et al., 2006b). The same group also reports melanocortin MC_3 receptor dependent anti-inflammatory efficacy of D-Trp^8-γ-MSH in models of vascular inflammation (Leoni et al., 2008), and lung inflammation (Getting et al., 2008).

5.4. Circadian rhythm

Rodents maintain a clear circadian rhythm of food intake, with the majority of energy intake taking place during the night. Animals with defective clock genes exhibit defects in this rhythm, with increased food intake during the day and increased susceptibility to diet-induced obesity (Turek et al., 2005). Behavioral and endocrine rhythms are entrained to photic cues by virtue of the retinal hypothalamic tract, however diurnal energy intake patterns also impact on circadian oscillators. For example, restricting food intake to limited time periods can uncouple peripheral clocks from the central circadian oscillator in the suprachiasmatic nucleus (Damiola et al., 2000). One assay of this coordinated effect of photic and metabolic cues is food anticipatory locomotor activity (FAA). Briefly, when animals are food restricted to approximately 50–60% of their normal intake during a 4 h window of time during the day, they will eventually exhibit increased locomotor activity 1–2 h prior to the presentation of food. Butler and colleagues have discovered that melanocortin MC_3 $receptor^{-/-}$ mice exhibit a striking reduction in FAA (Sutton et al., 2008). The pattern of expression of cellular clock genes in peripheral tissues, such as Bmal1 and Rev-erbα, can also be shifted with this restricted daytime food presentation. However again, the melanocortin MC_3 $receptor^{-/-}$ shows a defect in the entrainment of peripheral clock genes in this food restriction paradigm (Sutton et al., 2010). While perturbations in circadian rhythms are associated with obesity (Fonken et al., 2010), circadian rhythms of food intake and locomotor activity are largely intact in the melanocortin MC_3 $receptor^{-/-}$. Additional data will be required to determine if the defective entrainment to restricted food presentation can elucidate a mechanism for the obesity syndrome and metabolic defects in the melanocortin MC_3 $receptor^{-/-}$ mouse.

6. Conclusions

Many potential functions of the melanocortin MC_3 receptor have now been elucidated, primarily using the melanocortin MC_3 $receptor^{-/-}$ mouse model. These include effects on lean and adipose mass, natriuresis, immune function, susceptibility to aspects of metabolic function, and entrainment to restricted food presentation. In the case of the melanocortin MC_4 receptor, we understand the basics of many of the regulatory inputs, neural circuits and effector pathways that cause the obesity syndrome in mice and humans with defective melanocortin MC_4 receptor signaling. However, in the case of the melanocortin MC_3 receptor there remain more questions than answers. What are the effector pathways mediating these physiological responses to melanocortin MC_3 receptor blockade? For example, what are the endocrine or autonomic mechanisms by which melanocortin MC_3 receptor blockade causes obesity and reduced metabolic syndrome? What are the behavioral pathways mediating the defective entrainment to reduced food presentation? Are there specific inputs to melanocortin MC_3 receptor neurons in the CNS, such as γ-MSH? What is the source of the physiological ligand for peripheral melanocortin MC_3 receptor? And, what are the respective contributions of the central and peripheral melanocortin MC_3 receptor to the physiological functions identified thus far? Future research in the field is needed to address these problems.

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