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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}