



Review

Structure, function and regulation of the melanocortin receptors

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ABSTRACT

Melanocortin receptors belong to the seven-transmembrane (TM) domain proteins that are coupled to G-proteins and signaled through intracellular cyclic adenosine monophosphate. Many structural features conserved in other G-protein coupled receptors (GPCRs) are found in the melanocortin receptors. There are five melanocortin receptor subtypes and each of the melanocortin receptor subtypes has a different pattern of tissue expression and has its own profile regarding the relative potency of different melanocortin peptides. α -, β -, and γ -MSH and ACTH are known endogenous agonist ligands for the melanocortin receptors. Agouti and AgRP are the only known naturally occurring antagonists of the melanocortin receptors. We have examined the molecular basis of all five human melanocortin receptors for different ligand binding affinities and potencies using chimeric and mutated receptors. Our studies indicate that human melanocortin MC₁ receptor, human melanocortin MC₃ receptor, human melanocortin MC₄ receptor and human melanocortin MC₅ receptor utilize orthosteric sites for non selective agonists, α -MSH and NDP- α -MSH, high affinity binding and utilize allosteric sites for selective agonist or antagonist binding. Furthermore, our results indicate that molecular determinants of human melanocortin MC₂ receptor for ACTH binding and signaling are different from that of other melanocortin receptors. Many studies also indicate that agonists can induce different conformation changes of melanocortin receptors, which then lead to the activation of different signaling pathways, even when the expression level of receptor and the strength of stimulus–response coupling are the same. This finding may provide new information for the design of drugs for targeting melanocortin receptors.

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1. Introduction

The melanocortin system consists of 1) the melanocortin peptides α -, β -, and γ -melanocyte-stimulating hormone (α -, β -, γ -MSH) and adrenocorticotrophic hormone (ACTH), 2) a family of five seven-transmembrane G protein-coupled melanocortin receptors, and 3) the endogenous melanocortin antagonists agouti and agouti-related

protein (AgRP) (Cone et al., 1996; Gantz and Fong, 2003; Holder and Haskell-Luevano, 2004; Yang and Harmon, 2003). The melanocortins are involved in a diverse number of physiological functions, including pigmentation, steroidogenesis, energy homeostasis, exocrine secretion, sexual function, analgesia and inflammation. Melanocortin MC₁ receptor is the melanocyte α -MSH receptor, expressed on cutaneous melanocytes, where it has a key role in determining skin and hair pigmentation. Melanocortin MC₁ receptor is also expressed at leukocytes, where it may mediate the anti-inflammatory property. Melanocortin MC₂ receptor is the adrenocortical ACTH receptor, expressed in the adrenal cortex zona reticularis and zona fasciculata, where it mediates the effects of ACTH on steroid secretion.

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Melanocortin MC₃ receptor is identified in many areas of the central nervous system and peripheral tissues and involved in energy homeostasis. Melanocortin MC₄ receptor is expressed predominantly in the central nervous system and regulates both food intake and sexual function. Melanocortin MC₅ receptor is expressed in numerous human peripheral tissues and is mainly involved in exocrine function, particularly sebaceous gland secretion discovered by targeted deletion of that receptor (Adan et al., 1999; Cone, 1999, 2005; Gantz and Fong, 2003). The physiological significance of the melanocortin receptor family has promoted considerable research activity over the past decade (Adan et al., 1997; Adan and Vink, 2001; Chen et al., 2000a,b, 2007a; Huszar et al., 1997; Hwa et al., 2001). The ultimate goal for the development of any new therapeutic agent for melanocortin receptors is to identify a drug that produces the desired effect with minimal side effects. To this end, the concept of directed signaling and functional selectivity has generated significant interest as a means to develop compounds that can selectively activate or block receptor-signaling pathways that lead only to the desired therapeutic effect. This is of particular importance for the melanocortin receptors as a potential target for the treatment of skin cancer, food intake, and exocrine disorders because many existing melanocortin receptor agonists for melanocortin receptors are not receptor subtype specific and have unwanted side effects. Several approaches have been undertaken to develop melanocortin receptor agonists and antagonists. In this review, current understanding of the molecular basis of human melanocortin receptors responsible for ligand binding and signaling will be discussed.

2. Structural features of the melanocortin receptors

Melanocortin receptors belong to the seven-transmembrane (TM) domain receptor proteins that are coupled to proteins G [G-protein-coupled receptors (GPCRs)] and signaled mainly through intracellular cyclic adenosine monophosphate. The cloning of the human melanocortin receptor genes has led to a tremendous progress in understanding the biological effects of the melanocortin peptides and the melanocortin receptors. So far, five human melanocortin receptor genes, including human melanocortin MC₁ receptor, human melanocortin MC₂ receptor, human melanocortin MC₃ receptor, human melanocortin MC₄ receptor, and human melanocortin MC₅ receptor, have been cloned (Gantz et al., 1993, 1994a,b, 1996, 1997; Mountjoy et al., 1992). Melanocortin receptors consist of a single polypeptide featuring seven α -helical TM domains, an extracellular N-terminus, and an intracellular C-terminus. Melanocortin receptors share many structural features conserved in other GPCRs: the consensus N-linked glycosylation sites near the amino terminus, a palmitoylation site in the COOH-terminal tail, and sites for phosphorylation in the first and third intracellular domains and in the COOH-terminal tail. All human melanocortin receptors contain the conserved amino acids, aspartic acid–arginine–tyrosine (DRY) motif at the junction of the TM3 domain and contain cysteine at C terminus. However, melanocortin receptors have several features which are different from other GPCR. Melanocortin receptors are the smallest G protein-coupled receptors known, with short amino- and carboxyl-terminal ends and a very small second extracellular loop. Human melanocortin receptors also lack several features found in most GPCRs; one, or two, cysteine residues in the first and second extracellular loops and prolines found in the fourth and fifth TM domains. The melanocortin receptors contain conserved cysteine residues at different regions of the receptor, including cysteine residues within extracellular loops (ELs) and cysteine residues within TMs, and cysteine residue in C terminus. Functional studies indicate that cysteines in EL3 may form a disulfide bond which is crucial for receptor function while cysteine residues in TMs of the human melanocortin receptors may have different roles in receptor expression, ligand binding and receptor activation (Yang et al., 2007).

3. The orthosteric binding pocket in the melanocortin receptors

The orthosteric site is defined as the receptor area where the endogenous agonist binds. Melanocortin receptors share same endogenous ligands and therefore should have orthosteric binding sites for endogenous melanocortins. Sequence comparison of human melanocortin receptors shows high sequence homologies, ranging from 60% identity between human melanocortin MC₄ receptor and melanocortin MC₅ receptor, 45% identity between human melanocortin MC₃ receptor and melanocortin MC₁ receptor and between human melanocortin MC₃ receptor and melanocortin MC₂ receptor to 38% identity between human melanocortin MC₂ receptor and melanocortin MC₄ receptor (Gantz et al., 1993; Yang and Harmon, 2003). The alignment of the human melanocortin receptor subtype primary sequences also highlights the highly conserved residues among these receptors (Fig. 1). To determine the molecular basis of human melanocortin receptors for the endogenous ligand binding, site-directed mutagenesis is used to examine the role of the conserved amino acid residues in human melanocortin receptors for ligand orthosteric binding and chimeric receptors are used to examine the role of non-conserved amino acid residues for ligand allosteric binding. We have examined all five human melanocortin receptor binding sites by using the above two approaches. Based on the assumption that receptor residues conserved among the human melanocortin receptors might participate in ligand orthosteric binding, conserved basic, negative TM residues and aromatic, hydroxyl and sulfhydryl containing residues of human melanocortin receptors have been mutated and tested. Glutamine acids 2.4 and aspartic acid 3.2 and 3.6 in TM3 of the melanocortin receptors are conserved among all five melanocortin receptor subtypes. Mutation of this glutamic acid or aspartic acid to alanine resulted in significantly decrease in endogenous agonist binding and receptor signaling, suggesting that these residues are crucial for the melanocortin orthosteric binding. Phenylalanine F6.51 and histidine 6.54 are also conserved among all five melanocortin receptors. Mutation of these residues to alanine resulted in a significant decrease in endogenous agonist binding and receptor signaling, suggesting that these residues are also involved in melanocortin orthosteric binding (Fleck et al., 2005; Haskell-Luevano et al., 2001; Nickolls et al., 2003; Pogozheva et al., 2005; Yang et al., 2000). Table 1 enumerates the human melanocortin receptor residues involved in ligand orthosteric binding. For human melanocortin MC₁ receptor, glutamic acid 94 in TM2, aspartic acid 117 and 121 in TM3, tryptophan 254, phenylalanine 257 and histidine 260 in TM6 were identified to be involved in MSH orthosteric binding (Yang et al., 1997). For human melanocortin MC₃ receptor, glutamic acid 131 in TM2, aspartic acid 154 and 158 in TM3, tryptophan 292, phenylalanine 295 and histidine 298 in TM6 are identified to be involved in MSH orthosteric binding (Chen et al., 2006). For human melanocortin MC₄ receptor, glutamic acid 100 in TM2, aspartic acid 122 and 126 in TM3, tryptophan 258, phenylalanine 261 and histidine 264 in TM6 are important for MSH orthosteric binding (Chen et al., 2007c; Yang et al., 2000). For human melanocortin MC₅ receptor, glutamic acid 92 in TM2, aspartic acid 115 and 119 in TM3, tryptophan 251, phenylalanine 254 and histidine 257 in TM6 were identified to be involved in MSH orthosteric binding (unpublished data).

Melanocortin MC₂ receptor is unique among melanocortin receptors. Although melanocortin MC₂ receptor shares some conserved amino acid residues in TM region with other melanocortin receptors, it shows low sequence homology with other melanocortin receptors, only 38% identity between melanocortin MC₂ receptor and melanocortin MC₄ receptor. Pharmacological characterization also indicates that melanocortin MC₂ receptor has different pharmacological profiles compared with other melanocortin receptors. First, ACTH is the only endogenous ligand for melanocortin MC₂ receptor. α -MSH, β -MSH and γ -MSH are unable to bind to melanocortin MC₂ receptor

| | | TM1 | | | loop 1 | |
|-------|-----|---------|----------|---------|-------------|----------|
| hMC1R | 39 | SISDGL | FLSL | GLVSLV | ENAL | VVATIA |
| hMC2R | 25 | VLPEEII | FTTI | SIVGVLE | NLI | VLLAVF |
| hMC3R | 76 | FIKPEII | FLSL | GIVSLLE | NIL | VILAVV |
| hMC4R | 45 | FVSPEV | FTVL | GVISLLE | NIL | VIVAIA |
| hMC5R | 37 | GIAVEV | FTTL | GVISLLE | NIL | VIGAIV |
| | | | | | | |
| | | TM2 | | | loop 2 (1e) | |
| hMC1R | 71 | SPMYC | FIICCL | ALS | LLVSGT | NVLE |
| hMC2R | 57 | APMYF | FIICSL | AIS | MLGSLY | KILE |
| hMC3R | 108 | SPMYF | FIICSL | AVAD | MLVSVS | NAL |
| hMC4R | 77 | SPMYF | FIICSL | AVAD | MLVSVS | NGS |
| hMC5R | 69 | SPMYF | FIICSL | AVAD | MLVSM | SAW |
| | | | | | | |
| | | TM3 | | | loop 3 (2i) | |
| hMC1R | 117 | DNVID | IVITCS | SMLS | SLCFLG | AIADV |
| hMC2R | 103 | NDI | ISLFLV | SLLGS | IFLS | VIAADV |
| hMC3R | 154 | DNIF | DSMICI | SLVAS | ICNLL | AIADV |
| hMC4R | 122 | DNVID | SVICS | SLLAS | ICSL | SIADV |
| hMC5R | 115 | DNVF | DSMICI | SVVASM | CSLL | AIADV |
| | | | | | | |
| | | TM5 | | | loop 5 (3i) | |
| hMC1R | 189 | LLCLV | VFLA | MLV | MAVLYV | FML |
| hMC2R | 175 | VITFT | SLFPL | MLV | FILCLYV | FMF |
| hMC3R | 226 | IVCL | ITMFA | MML | MGTLVY | FMF |
| hMC4R | 194 | IICLI | TMFA | MLA | MALVLYV | FMF |
| hMC5R | 187 | ILCL | ISMFA | MLF | LVLSYI | FMF |
| | | | | | | |
| | | TM6 | | | loop 6 (3e) | |
| hMC1R | 240 | AVTL | TILLGI | FFLC | GGPFL | ELTLIVL |
| hMC2R | 218 | AITL | TILLGV | FIFC | NAPFL | ELVLLMTF |
| hMC3R | 278 | AVT | TILLGV | FIFC | NAPFL | ELVLLMTF |
| hMC4R | 244 | AITL | TILLGV | FVVC | NAPFL | ELIFYIS |
| hMC5R | 237 | AVTV | TMLLGV | ETVC | NAPFL | ELTLMLS |
| | | | | | | |
| | | TM7 | | | C-terminal | |
| hMC1R | 279 | N | NLFLALII | CNAI | IDPLIY | AFHSQ |
| hMC2R | 257 | L | QVNGMLIM | CNAI | DPFIY | AFRSP |
| hMC3R | 317 | H | NTYLVLIM | CNSV | IDPLIY | AFRSL |
| hMC4R | 283 | H | NLYLILIM | CNSI | IDPLIY | ALRSQ |
| hMC5R | 276 | H | NMYLILIM | CNSV | IDPLIY | AFRSQ |

e: extracellular loop
i: intracellular loop
TM: Transmembrane domain

Fig. 1. Primary sequences of human melanocortin receptors. The conserved TM residues in these receptors are denoted as italic. The residues involved in ligand orthosteric binding were highlighted by bold.

and activate receptor. Second, substitution of Phe7 with DPhe at ACTH decreases ligand binding affinity and potency at melanocortin MC₂ receptor whereas substitution of phenylalanine (Phe)7 at MSH with DPhe results in increased ligand binding affinity and potency at melanocortin MC₁ receptor, melanocortin MC₃ receptor, melanocortin MC₄ receptor and melanocortin MC₅ receptor. Furthermore, Phe7-ACTH1-17 is a potent melanocortin MC₂ receptor agonist but DPhe7-ACTH1-17 loses its binding ability at melanocortin MC₂ receptor. Thirdly, DNal (2')-ACTH1-17 is a potent agonist at melanocortin MC₂ receptor but substitution of Phe7 at MSH with D-naphthylalanine (2') [DNal (2')] switches ligand from agonist to antagonist at melanocortin MC₃ and MC₄ receptors. All these results suggest that melanocortin MC₂ receptor has a different ligand binding pocket compared with other melanocortin receptors. Mutagenesis study indicates that human melanocortin MC₂ receptor utilizes some conserved amino acid residues for ACTH binding. However, the study also indicates that melanocortin MC₂ receptor has a broad binding pocket in which both conserved and unique amino acid residues in TM2, TM3, TM4 and TM6 are involved. Mutations of the conserved amino acid residues,

glutamic acid 80 in TM2, aspartic acid 107 in TM3, phenylalanine 178 in TM4, tryptophan 232, phenylalanine 235 and histidine 238 in TM6 and phenylalanine 258 in TM7, significantly reduced ACTH binding affinity and signaling that is similar to other melanocortin receptors. Mutations of unique amino acids aspartic acid 104, phenylalanine 108 in TM3, phenylalanine 168 in TM4 and phenylalanine 178 in TM4 significantly decreased ACTH binding and signaling, implying that melanocortin MC₂ receptor orthosteric binding sites are much broader than that of other melanocortin receptors (Chen et al., 2007b).

4. An allosteric binding pocket in the melanocortin receptors

The allosteric site is defined as the receptor area that is distinct from the binding site of the endogenous ligands. Allosteric ligands alter receptor conformation through binding at sites distinct from the orthosteric ligand binding site. Melanocortin receptor family is a member of G-protein-coupled receptor families that share the same endogenous ligand. While ligand selectivity for melanocortin receptor family is usually achievable, it is often difficult to obtain selectivity among subtypes of the family. Thus selective orthosteric ligands, which presumably interact with the same amino acid sequences, would tend to be non-selective within a family. Allosteric ligands may imprint specific conformations on the receptor protein that are not induced by orthosteric agonists. Therefore, targeting allosteric domains allows to achieving binding site selectivity to an extent that is not achieved by traditional orthosteric agonists. Furthermore, selectivity at the allosteric site of the melanocortin receptors allowed for the pharmacological separation of receptors that previously could not be distinguished using orthosteric ligands. Increasing evidence demonstrates that melanocortin receptors allow for ligand interactions outside the domain where the endogenous ligand binds. Agouti

Table 1
Receptor mutation on NDP- α -MSH binding affinity and potency.

| Residue mutation | Receptor binding affinity and potency | | | | |
|------------------|---------------------------------------|--------|--------|--------|--------|
| | hMC1R | hMC2R | hMC3R | hMC4R | hMC5R |
| TM2 (2.6) | E94 ↓ | E80 ↓ | E131 ↓ | E100 ↓ | E92 ↓ |
| TM3 (3.25) | D117 ↓ | D103 ↓ | D154 ↓ | D122 ↓ | D115 ↓ |
| TM3 (3.29) | D121 ↓ | D107 ↓ | D158 ↓ | D126 ↓ | D119 ↓ |
| TM6 (6.48) | W254 ↓ | W232 ↓ | W292 ↓ | W258 ↓ | W251 ↓ |
| TM6 (6.51) | F257 ↓ | F235 ↓ | F295 ↓ | F261 ↓ | F254 ↓ |
| TM6 (6.54) | H260 ↓ | H238 ↓ | H298 ↓ | H264 ↓ | H257 ↓ |

↓: binding affinity and potency decrease.

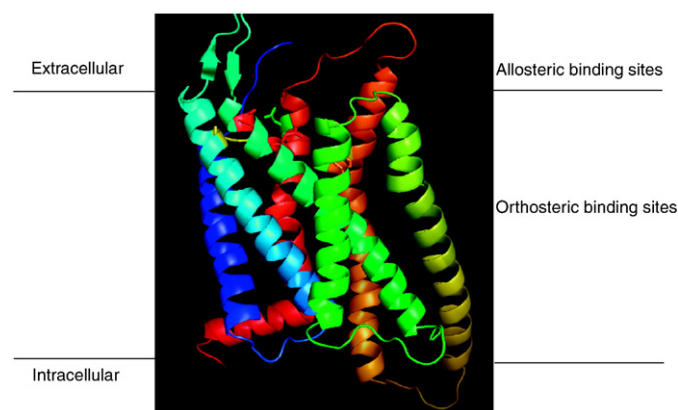


Fig. 2. Schematic representation of antagonist AgRP binding pocket at melanocortin receptor. AgRP utilizes both orthosteric and allosteric sites for selective binding at melanocortin receptor.

signaling protein (ASIP) and AgRP are endogenous melanocortin receptor antagonists. ASIP is selective for melanocortin MC₁ receptor and melanocortin MC₄ receptor but AgRP is selective antagonist for melanocortin MC₃ receptor and melanocortin MC₄ receptor. Study indicates that AgRP selectively binds to melanocortin MC₃ receptor and melanocortin MC₄ receptor using orthosteric and allosteric binding (Yang et al., 1999). The allosteric site is located extracellular loop and the orthosteric site is located at transmembrane region (Oosterom et al., 2001; Yang et al., 1999, 2003) (Fig. 2). This selective allosteric binding is achieved from less well conserved amino acids among the extracellular regions of the melanocortin receptors while orthosteric binding is shared by MSH and AgRP.

Many small synthetic melanocortin MC₄ receptor selective agonists have been developed and characterized. These synthetic compounds have different chemical structures from that of MSH but they are selective for different melanocortin receptor subtypes. For example, synthetic nonpeptide compound N-(3R)-1 4-tetrahydroisoquinolinium-3-ylcarbonyl-(1R)-1-(4-chlorobenzyl)-2-4-cyclohexyl-4-(1 H-1,2,4-triazol-1-ylmethyl) piperidin-1-yl-2-oxoethylamine (THIQ) is a synthetic small compound which is a selective melanocortin MC₄ receptor agonist. Many studies indicate that both conserved amino acid residues and non-conserved residues are involved in THIQ binding (Pogozheva et al., 2005; Yang et al., 2009). Mutations of the conserved residues, aspartic acids 122 and 126 in the TM3 of the melanocortin MC₄ receptor, reduce the binding affinity and potency of both NDP-MSH and THIQ. However, mutation of non-conserved residues, isoleucine 129 and leucine 125 in the TM3 of the melanocortin MC₄ receptor, significantly reduces the potency of THIQ but not on binding affinity or potency of the classic orthosteric agonists, suggesting that these residues are involved in allosteric binding (Haskell-Luevano et al., 2001; Pogozheva et al., 2005; Yang et al., 2000).

5. Agonist-selective signaling of melanocortin receptors: Mechanism and implication

Melanocortin agonists bind to melanocortin receptors with high affinity and shift the receptor to its active conformation and induce a physiological effect. Recent studies indicate that agonists possess different efficacies on different signaling pathways of particular melanocortin receptors (1, 2). A new theory may accommodate these phenomena that melanocortin agonist can induce different conformation changes of one particular melanocortin receptor, which then lead to the activation of different signaling pathways. Activation of different signaling pathways may lead to the different cellular responses *in vitro* or physiological responses *in vivo* which were shown in a schematic diagram (Fig. 3). Agonist I and agonist II activate

the receptor, which transduce the signal to two pathways, pathway A and pathway B. Agonist I is a full agonist on pathway A and pathway B. However, agonist II is a full agonist on pathway B but has no effect on pathway A.

Many studies indicate that melanocortin MC₄ receptor agonist can induce different conformation changes of melanocortin MC₄ receptor, which then leads to the activation of different signaling pathways, even when the expression level of receptor and the strength of stimulus–response coupling are the same. For example, the classical signaling pathway for the melanocortin MC₄ receptor is to couple to the heterotrimeric stimulatory G protein (Gs) and receptor activation leads to increased cAMP production, and consequently protein kinase A (PKA) activation. Recently, many studies indicate melanocortin MC₄ receptor can couple to all three major classes of G proteins, Gs, Gi/o, and Gq, changing second messengers such as cAMP and calcium and activating MAPK including ERK1/2 and JNK (Chai et al., 2006a,b, 2009). Functional studies indicate that synthetic melanocortin MC₄ receptor agonists differ in their ability to couple the same receptor to different G proteins. The peptide agonists exhibited high intrinsic activity in cAMP, calcium and receptor internalization, whereas nonpeptide agonists only exhibited high intrinsic activity in the cAMP signal pathway and impaired ability to mobilize calcium or internalize the receptor, suggesting that these agonists induce different receptor conformational states. NDP-MSH activates receptor and induces receptor internalization while nonpeptide THIQ activates melanocortin receptor but failed to induce receptor internalization. Further analysis indicates that different regions of the melanocortin MC₄ receptor are involved in receptor activation and internalization. Deletion of the partial C terminal of the melanocortin MC₄ receptor completely abolished agonist induced receptor internalization but still maintained agonist mediated receptor activation (Yang et al., 2005).

Molecular analysis of the melanocortin receptors has indicated that different amino acid residues at melanocortin receptors are crucial for agonist or antagonist mediated receptor activation or inhibition. SHU 9119 is a cyclic melanocortin analogue that contains D-naphthylalanine instead of D-phenylalanine in the core melanocortin sequence which loses agonist activity at human melanocortin MC₃ receptor and human melanocortin MC₄ receptor (Yang et al., 2002). Molecular analysis of the melanocortin MC₃ receptor and MC₄ receptor indicate that leucine 165 at melanocortin MC₃ receptor and leucine 133 at melanocortin MC₄ receptor are crucial for SHU9119 antagonist activity. Replacement of leucine 165 to methionine 165 and leucine 133 to methionine 133 switch SHU9119 from antagonist to agonist at melanocortin MC₃ receptor and melanocortin MC₄ receptor (Yang et al., 2002). Evidence indicates that different amino acid residues of the melanocortin receptors are involved in different G protein binding. Mutation of aspartic acid 90 to asparagine 90 at melanocortin MC₄ receptor abolishes Gs pathway but remains Gq pathway.

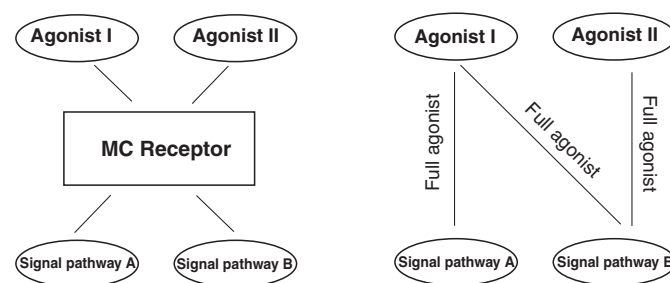


Fig. 3. Schematic representation of melanocortin receptor agonist-selective signaling. Agonist I and II exhibit different efficacies in pathways A and B. Agonist I is a full agonist for pathways A and B. Agonist II is a full agonist for only pathway B.

6. Melanocortin receptor modeling using computational methods

To obtain accurate information on the three-dimensional surrounding the binding pocket of melanocortin receptors is a big challenge and the knowledge of the melanocortin receptor's three-dimensional structure is critical to understanding how melanocortin receptors carry out their functions. For GPCR, the three dimensional structure of a receptor is usually obtained from X-ray crystallography studies, but for most GPCRs crystallization is still an unresolved problem. An alternative approach to building a molecular model of a protein is the homology modeling procedure where the target protein is built starting from the experimental known 3d-structure of a related protein. Four GPCR crystal structures have been identified. Their structures reveal two important features. One is structural convergence (the similarities in structure). Another is structural divergence (the difference in structure). It is evident from superimposition of the TM domains that these are very similar. The docked ligands are also similar in occupying much the same space. Thus GPCRs tend to accommodate these small molecules with similar spatial arrangement, but interactions with amino acid chains of ligands are quite different. Many groups have developed 3-dimensional models for human melanocortin MC₁ receptor and human melanocortin MC₄ receptor (Fleck et al., 2007; Haskell-Luevano et al., 1996; Hogan et al., 2006; Pogozheva et al., 2005; Prusis et al., 1997; Sun and Fry, 2007). We utilized automatic comparison of the melanocortin receptor with already known four GPCRs and developed 3 dimensional models for human melanocortin MC₂ receptor, human melanocortin MC₃ receptor and human melanocortin MC₅ receptor. Our results indicate that these melanocortin receptors are more similar to adenosine receptor than other three GPCR (rhodopsin, β 1-adrenergic and β 2-adrenergic receptors). We utilized adenosine receptor as template since crystal structure of this receptor has been identified. A three-dimensional molecular model of NDP-MSH interacting with the human melanocortin MC₃ receptor and human melanocortin MC₅ receptor suggests that NDP-MSH binding sites at human melanocortin MC₃ receptor and human melanocortin MC₅ receptor are similar to that of human melanocortin MC₁ receptor and human melanocortin MC₄ receptor while ACTH binding sites at human melanocortin MC₂ receptor is different from all other melanocortin receptors. Melanocortin MC₂ receptor binding sites include a series of amino acids in TM domains 2, 3, 5 and 7 which form a broad binding pocket for ACTH.

7. Conclusion

Melanocortin receptor family is of the most important class of GPCR in the genome because of its tremendous molecular diversity and potential targets for therapeutic application. The majority of current ligands affect melanocortin receptor activity by binding to orthosteric site as the endogenous cognate ligand for the receptor. Over the past one decade, novel opportunities for drug discovery have risen from a greater understanding of the complexity of melanocortin receptor signaling. A striking example of this is the appreciation that melanocortin receptors possess functional allosteric binding sites. Allosteric modulator ligands bind receptor domains topographically distinct from the orthosteric site, altering the biological activity of the orthosteric ligand by changing its binding affinity, functional efficacy, or both. This additional allosteric ligands offer the way for not only receptor-selective but also signaling pathway-selective therapies. While still a relatively new concept in melanocortin receptor pharmacology, allosteric ligands provide a remarkable precision in the targeting of drugs to closely related melanocortin receptor subtypes and engendering stimulus-bias in orthosteric ligand signaling, opening up new avenues for not only receptor-selective but also signaling-pathway-selective therapies.

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