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Review

Design of novel melanocortin receptor ligands: Multiple receptors, complex pharmacology, the challenge

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ABSTRACT

The major pharmacophore for the melanocortin 1, 3, 4 and 5 receptors is the sequence -His-Phe-Arg-Trp-. There is a need for potent, biologically stable, receptor selective ligands, both agonists and antagonists, for these receptors. In this report we briefly examine the structural and biophysical approaches we have taken to develop selective agonist and antagonist ligands that can cross (or not) the blood brain barrier. Remaining questions and unmet needs are also discussed.

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Contents

1. Introduction

Proopiomelanocortin, a primordial animal gene, its processed products, the melanotropin peptides, and their receptors have a long and storied history (e.g. Hadley, 1988; Vaudry and Eberle, 1993). Early work (until the 1990s) concentrated primarily on the functions of adrenal corticotropic hormone (ACTH) and the adrenal gland, α-melanocyte stimulating hormone (α-MSH) and pigmentation, and β-endorphin and pain. In the 1990s five melanocortin receptors were cloned (Cone, 2000). In addition to the melanocortin receptor 1 (melanocortin MC_1 receptor, pigmentary receptor) and the melanocortin receptor 2 (melanocortin MC_2 receptor, adrenal receptor), three other receptors were discovered, and their expression in the periphery and in the central nervous system suggested that numerous possible

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biological activities might be associated with the POMC ligands and these receptors including, in addition to pigmentary effects and the production of glucocorticoids in response to stress: effects associated with feeding behaviors (obesity, anorexia), sexual behavior (erectile dysfunction, sexual motivation), temperature control, pain, inflammatory response, immune response, and many others (e.g. Cone, 2000). A vexing problem is the observation that the primary native ligand for the melanocortin MC_1 receptor, melanocortin MC_3 receptor, melanocortin MC_4 receptor and melanocortin MC_5 receptor is α-melanocyte stimulating hormone (α-MSH) or possibly γ-melanocyte stimulating hormone (γ-MSH). These native ligands are rather non-selective for these 4 receptors, and the basic pharmacophore is the sequence -His-Phe-Arg-Trp- (Hruby et al., 1987) which is found in both α-MSH and γ-MSH (Fig. 1). α-MSH and other native melanotropin peptides are biologically unstable, being easily hydrolyzed by proteases, and in addition the Met residue in α-MSH is easily oxidized which leads to a modified ligand with decreased biological activity. Thus a primary need in this field is to obtain melanotropin analogues which are: 1) biologically stable to proteolysis and oxidation; 2) agonists or antagonists; 3) selective for one of the melanocortin receptors; 4) able to cross the blood-brain-barrier (or not); and 5) orthosteric or allosteric ligands. Development of such ligands has been critical not only to establish structure–activity relationships but equally important, to establish the biological functions of the various melanocortin receptors and melanocortin systems. In this paper, we will briefly discuss the fundamental approach we have developed toward these objectives which requires a highly interdisciplinary program involving asymmetric synthesis, peptide and peptidomimetic synthesis, computational chemistry, biophysics and conformational determination, molecular pharmacology, molecular biology and close collaboration with biologists (e.g. Hruby, 1982; Hruby et al., 1990; Hruby et al., 1997; Hruby, 2001; Hruby, 2002; Hruby, 2009).

2. Materials and methods

2.1. Asymmetric synthesis

It often is necessary to synthesize novel amino acids in order to examine the conformational or topographical properties of a peptide or peptidomimetic. This important topic will not be discussed here except to say we have concentrated on chi-constrained aromatic amino acids (for reviews see Hruby et al., 1990, 1997; Hruby and Qian, 1994) and constrained structures of proline (e.g. Hruby, 2009) primarily as a way to examine the importance of chi space in SAR studies.

2.2. Peptide and peptidomimetic synthesis

Most of the peptide and peptidomimetic syntheses have been done using either the N^{α}-Boc or the N^{α}-Fmoc strategies of peptide synthesis (Hruby et al., 1992; Hruby et al., 1998). Though the native melanotropin peptides are all linear compounds, we and others have made many cyclic analogues since our initial synthesis of c[Cys^4, Cys^{10}]-α-MSH (Sawyer et al., 1982). For each of these cases whether disulfides, sulfides, lactams, or others, appropriate synthetic conditions needed to be developed for the particular cyclic template. In some cases cyclization could be done on the solid support, whereas in others it needed to be done in solution, often assisted by microwave conditions. In general, orthogonal protection of the functional group(s) to be cyclized is required. Details are found in the individual publications which first established the use of various cyclic templates.

2.3. Peptide and peptidomimetic purification and analysis

Most purifications of our peptides and peptidomimetics are done by high performance liquid chromatography (HPLC) which, in its analytical format, also serves to evaluate purity. We usually do thin layer chromatography (TLC) with 2 or 3 different solvent systems. Also of great use are gel filtration for final purification, and ion exchange chromatography.

2.4. Conformational analysis, computational chemistry and modeling

Thus far, X-ray crystal structures of the melanocortin receptors have not been obtained, and in general it has been very difficult to obtain X-ray crystal structures of membrane proteins especially G-protein coupled receptors (GPCRs) such as the melanocortin receptors. Furthermore, none of the native melanotropin peptides have had their X-ray structures determined. Thus obtaining conformational and topographical information about the melanotropin ligands via computer assisted modeling and computational chemistry in conjunction with biophysical studies has played a crucial role in the development of three dimensional structure activity relationships (SARs) from the earliest days of our studies in this area in the early 1980s (e.g. Sawyer et al., 1980, 1982). This approach includes 2-dimensional NMR studies in conjunction with computational chemistry. Though details will not be discussed, they can be found in the papers which involve the development of our new, mostly cyclic templates.

2.5. Biological activity assays. Receptor binding assays

Competition binding assays and functional assays are carried out using HEK293 cells stably expressing the melanocortin MC_1 receptor, melanocortin MC_3 receptor, melanocortin MC_4 receptor and melanocortin MC_5 receptor as described previously (Cai et al., 2005a; Cai et al., 2005b; Cai et al., 2004a) using [^{125}I]-[Nle^4,D-Phe^7]-α-MSH (Perkin Elmer Life Science) as the radioligand and [Nle^4,D-Phe^7]α-MSH (NDP-α-MSH) and Ac-Nle^4-c[Asp^5,D-Phe^7,Lys^{10}]-α-MSH(4–10)-NH_2 (MT-II) as the standard control ligands.

2.6. Data analysis

IC_{50} and EC_{50} values generally represent the mean of 2 experiments run in triplicate. The values and their associated standard errors are determined by fitting the data using nonlinear least square analysis via GraphPad Prism 4 (GraphPad Software, San Diego, CA).

3. Results and discussion

In our early work the major goals were to obtain α-MSH analogues (agonists and antagonists) which would be more biostable than the native ligand α-MSH. Our initial modifications were to introduce the pseudoisosteric amino acid norleucine (Nle) into position 4 in place of methionine to prevent oxidation of Met^4 to its sulfoxide or sulfone, and to replace Phe^7 with D-Phe^7 to enhance stability against

α-MSH Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2

γ-MSH H-Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-Gly-OH

[Nle^4, D-Phe^7]α-MSH Ac-Ser-Tyr-Ser-Nle-Glu-His-D-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2

Fig. 1. Structure of some key melanotropin peptides and peptidomimetics.

proteolytic enzymes. The resulting peptide [Nle^4, D-Phe^7] α-MSH (Sawyer et al., 1980, NDP-α-MSH, MT-I) (Fig. 1) was found to be more potent than α-MSH and it was significantly more stable *in vivo* (Fig. 2). In addition, it was found to have highly prolonged biological activities in a variety of species. This molecule has been used by many investigators for studies of biological functions of melanocortin receptors since it has high potency at the melanocortin MC_1 receptor, melanocortin MC_3 receptor, melanocortin MC_4 receptor and melanocortin MC_5 receptor and good *in vivo* stability and biodistribution. Computational studies and molecular modeling suggested it had a β-turn structure centered at the D-Phe^7 residue, and to test this hypothesis we designed and synthesized the cyclic analogue c[Cys^4 Cys^{10}]-α-MSH (Ac-Ser-Tyr-Ser-c[Cys^4-Glu-His-Phe-Arg-Trp-Cys^{10}]-Lys-Pro-Val-NH_2 (Sawyer et al., 1982) which also was found to be a super potent analogue, though not with prolonged activity. Extensive SAR studies eventually led to the conclusion that the tetrapeptide sequence -His-Phe-Arg-Trp- was the principal pharmacophore for α-MSH bioactivity (Hruby et al., 1987) though smaller fragments especially with a D-Phe still could have potent bioactivity (e.g. Sawyer et al., 1993). Truncation studies, conformational studies, and molecular modeling and computational studies led to the design of the truncated cyclic lactam analogue of α-MSH, Ac-Nle-c[Asp^5, D-Phe^7, Lys^{10}]-α-MSH-(4-10)-NH_2 (Al-Obeidi et al., 1989a,b; MT-II). This analogue (Fig. 2) was highly potent in several assays and was found to be very stable *in vivo* and can cross the blood brain barrier (BBB). NMR based conformational analysis, in conjunction with computational chemistry provided evidence to support the β-turn conformation of this cyclic analogue of α-MSH (Ying et al., 2003). This compound also has been widely used for a very wide variety of biological activity studies by many investigators. We have used Ac-Nle^4-c[Asp^5,D-Phe^7,Lys^{10}]-α-MSH(4-10)-NH_2 (MT-II) and the linear analogue [Nle^4,D-Phe^7]-α-MSH (MT-I) (Table 2) for human clinical trials for pigmentation effects in humans (Hadley et al., 1993; Dorr et al., 1996) and for psychogenic erectile dysfunction in men (Wessells et al., 1998).

3.1. Recent orthosteric selective ligands

In our efforts to obtain selective ligands for the melanocortin receptors, we have examined a wide variety of scaffolds to test the basic hypothesis that selectivity could arise from two sources: 1) conformational and/or topographical (chi space) differences in the arrangements of pharmacophore elements; and/or 2) differences in receptor address moieties in the structure. In this paper we will illustrate examples of testing this strategy, but due to space limitation we will not discuss the biophysical studies, computational chemistry and computer assisted modeling that went into these considerations.

The first example H-Tyr-Val-Nle-Gly-His-D-Nal(2′)-Arg-Trp-Asp-Arg-Phe-Gly-NH_2, (Table 1, **1**) was made some time ago (Balse-Srinivasan et al., 2003a). This linear γ-melanocyte stimulating hormone analogue has, to our knowledge, a unique biological activity profile at the melanocortin MC_3 receptor, melanocortin MC_4 receptor and melanocortin MC_5 receptor. It is a potent agonist at the human melanocortin MC_4 receptor, but an antagonist at the human melanocortin MC_3 receptor and human melanocortin MC_5 receptor. It would be interesting to evaluate its *in vivo* biological activities in feeding behavior, erectile function and cardiovascular function. The cyclic analogue **2** (Table 1) has a unique biological activity profile being a potent and selective antagonist ligand at the human melanocortin MC_1 receptor with weak agonist activity at the human melanocortin MC_3 receptor and the human melanocortin MC_5 receptor, and no binding or functional activity at the human melanocortin MC_4 receptor. We currently are preparing a paper on its structure activity relationship. In the meantime, B. Kest and colleagues (Juni et al., 2010) have shown that this ligand can modulate hyperalgesia in a gender specific manner. An analogue of Ac-Nle^4-c[Asp^5,D-Phe^7,Lys^{10}]-α-MSH (4-10)-NH_2 in which the Nle^4 residue is replaced by a D-Phe and the His^6 residue by a Pro residue (**3**, Table 1) (Grieco et al., 2006) gave a ligand that was more selective as a human melanocortin MC_3 receptor agonist than [D-Trp^8] γ-melanocyte stimulating hormone (Grieco et al., 2000) since it had antagonist activities at both the human melanocortin MC_4 receptor and human melanocortin MC_5 receptor. A very interesting and unique conformationally and topographically constrained analogue **4** (Table 1) was examined for its biological activity profile. Aba is a cyclic aromatic amino acid mimetic 4-amino-1,2,4,5-tetrahydro-2-benzazepin-3-one. It has a unique conformation, particularly of the aromatic side chain groups relative to Ac-Nle^4-c[Asp^5,D-Phe^7,Lys^{10}]-α-MSH(4-10)-NH_2 (MT-II), which likely accounts for its unique biological activity profile with highly selective antagonist activity at the human melanocortin MC_3 receptor (Ballet et al., 2007). Another interesting human melanocortin MC_3 receptor antagonist is the ligand **5** (Table 1) which is a cyclic disulfide-containing ligand with constrained cystine amino acid penicillamine (β, β-dimethylcystine) in the 4 position and the C-terminal sequence of β-melanocyte stimulating hormone (β-MSH) (Balse-Srinivasan et al., 2003b). This compound has highly potent and selective melanocortin MC_3 receptor antagonist activity.

3.2. Alternative template designs

Based on the conformation properties of Ac-Nle^4-Ac-c[Asp^5,D-Phe^7, Lys^{10}]-α-MSH(4–10)-NH_2 (MT-II) and Ac-Nle^4-c[Asp^5,D-Nal(2′)^7, Lys^{10}]-α-MSH(4–10)-NH_2 (SHU9119) (Ying et al., 2003) we have utilized computer assisted modeling and computational chemistry to

α-MSH: Ac-Ser-Tyr-Ser-Met-Glu-*His-Phe-Arg-Trp*-Gly-Lys-Pro-Val-NH_2

MT-I: Ac-Ser-Tyr-Ser-Nle-Glu-*His-D-Phe-Arg-Trp*-Gly-Lys-Pro-Val-NH_2

(NDP-α-MSH)

MT-II: Ac-Nle-c[Asp-*His-D-Phe-Arg-Trp*-Lys]-NH_2

SHU-9119: Ac-Nle-c[Asp-*His-D-Nal(2')-Arg-Trp*-Lys]-NH_2

α-MSH – Biologically Unstable; $T_{½}$ - minutes
MT-I – Biologically More Stable; $T_{½}$ = 2 - 4 hours; Does Not Cross BBB. Non-Selective.
MT-II – Biologically Stable; $T_{½}$ = 1 - 2 days; Crosses BBB. Non-Selective.
SHU9119 – Antagonist – MC3R and MC4R; Agonist – MC1R and MC5R. Biologically Stable.

Fig. 2. α-MSH and its analogues developed in Hruby lab.

Table 1
Selective orthosteric ligands for melanocortin receptors.

Compound	Binding affinities IC_{50} (nM)[a]				Functional assays cAMP EC_{50} (nM)[b]			
	MC1R	MC3R	MC4R	MC5R	MC1R	MC3R	MC4R	MC5R
1.Tyr-Val-Nle-Gly-His-D-Nal(2′)-Arg-Trp-Asp-Arg-Phe-Gly-NH_2	–	0.84	2.5	1.0	–	>10,000	24	>10,000
2. c[$(CH_2)_3$CO-Gly-His-D-Phe-Arg-D-Trp-Cys(S)]-Asp-Arg-Phe-Gly-NH_2	17	3900	NB	1300	NA	100	NA	1300
3. D-Phe-c[Asp-Pro-D-Phe-Arg-Trp-Lys]-NH_2	-	3.7	700	1100	–	4.9	>10,000	>1000
4. Ac-Nle-c[Asp-Aba-D-Phe-Arg-Trp-Lys]-NH_2	NB	50	NB	2900	NA	NA	NA	NA
5. Ac-Nle-c[Pen-Glu-His-D-Nal(2′) –Arg-Trp-Cys]-Pro-Lys-Asp-NH_2	–	3.0	750	94	–	NA	NA	NA
10. Ac-Nle-c[Asp-N-MeHis-D-Phe-N-MeArg-N-MeTrp-N-MeLys]-NH_2	14	2200	NB	NB	13	NA	NA	NA

NB—no binding at 10^{-5}M ligand; NA—no activity at 10^{-5}M ligand.
[a] Competitive binding of ligands vs. [^{125}I]-NDP-α-MSH.
[b] Adenylate cyclase assay.

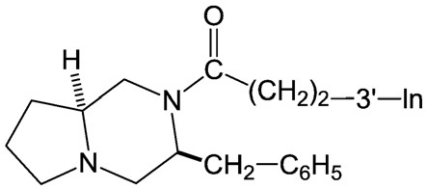
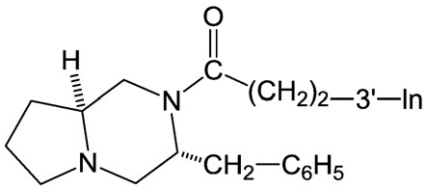
design a number of other novel scaffolds, and will discuss a few recent examples here. The first examples involve substitution of the Arg^8 residue in MT-II with a 3R-thiol-Arg residue or a Cys residue with N^{α}-guanidinylbutyl group at the terminal Trp-NH_2 residue (**6** and **7**, Table 2) (Ying et al., 2006). This novel design also gave novel biological activity results. As can be seen in Table 2, both **6** and **7** had no binding or functional activity up to 10^{-5}M at the human melanocortin MC_1 receptor, human melanocortin MC_3 receptor, or human melanocortin MC_5 receptor. At the melanocortin MC_4 receptor both **6** and **7** showed potent binding affinity, but no or minimal adenylate cyclase activity. Interestingly however, the ligands could only displace 50% of the radioactive ligand [^{125}I]-NDP-α-MSH in competitive binding experiments, indicating that they are potent allosteric antagonists of the human melanocortin MC_4 receptor. Furthermore, when the D-Phe^7residue was replaced with a D-Nal(2′) residue, the peptidomimetic ligand no longer binds to the human melanocortin MC_4 receptor (data not shown, Ying et al., 2006), but now binds only to the human melanocortin MC_5 receptor, though weakly (IC_{50} = 210 nM).

Based on computer assisted molecular modeling and computational chemistry, we have also designed a non-peptide, but amino acid based, scaffold to examine structure activity relationships (Cain et al., 2006). The bicyclic scaffold (**8** and **9**, Table 2) is derived from proline and its analogues and α- amino acids, and readily lends itself to asymmetric substitution at several positions in the bicyclic scaffold. The diastereoisomers **8** and **9** have quite different biological activity profiles, with the peptide mimetic **8** binding only to the human melanocortin MC_5 receptor as an allosteric antagonist. On the other hand, compound **9** binds in the nanomolar range as an allosteric antagonist at both the human melanocortin MC_1 receptor and human melanocortin MC_3 receptor. Further studies of related analogues are in progress.

3.3. N-methylated analogues of MT-II

Though backbone N-methylation has been used in structure activity relationship studies of backbone biologically active peptides for many years, a systematic examination of the effects of N-methyl modifications of peptides generally has not been examined due to the difficulties in the synthesis of multiple N-methylated peptides. Recently, however Kessler and co-workers have worked out a general synthetic approach for “in situ” N-methylation during solid phase peptide synthesis that greatly facilitates synthesis of multiple N-methylated peptides (e.g. Biron and Kessler, 2005). We have collaborated with Kessler and coworkers to systematically N-methylate the amide bonds in the cyclic structures MT-II and SHU-9119 (Fig. 2) (Doedens et al., 2010). The N-tetramethyl substituted analogue **10** (Table 1) is a potent and selective agonist for human melanocortin MC_1 receptors with very weak antagonist activities at

Table 2
Allosteric ligands for melanocortin receptors.

Compound	[a]Binding affinities a IC_{50} (nM)				[b]Functional assays cAMP EC_{50} (nM)			
	MC1R	MC3R	MC4R	MC5R	MC1R	MC3R	MC4R	MC5R
6. Ac-c[Cys-His-D-Phe-N^{α}-Gubu-Cys]-Trp-NH_2	NB	NB	1.8 (50)	NB	NA	Na	NA	NA
7. Ac-c[Cys-His-D-Phe-Cys]-N^{α}-Gubu-D-Trp-NH_2	NB	NB	0.7 (50)	NB	NA	NA	>1000	NA
8. 	NB	NB	NB	0.8 (50)	NA	NA	NA	NA
9. 	40 (50)	40 (50)	NB	NB	NA	NA	NA	NA

Gubu = guanidinylbutyl; NB = no binding at 10^{-5}M; NA = no cAMP up to 10^{-5}M ligand; In = indole.
[a] Competitive binding of ligands vs. [^{125}I]-NDP-α-MSH.
[b] Adenylate cyclase assay.

the human melanocortin MC_3 receptor and no binding activity at the human melanocortin MC_4 receptor and human melanocortin MC_5 receptor. NMR-based conformation analysis demonstrated that the more constrained conformation of **10** compared to Ac-Nle^4-c[Asp^5,D-Phe^7,Lys^{10}]-α-MSH(4-10)-NH_2 (MT-II) provides interesting insights into its selectivity for the human melanocortin MC_1 receptor. In this regard it is interesting to note that the corresponding trimethylated analogue's potency decreases 4 fold at the human melanocortin MC_1 receptor, but now the analogue does not bind to the human melanocortin MC_3 receptor (Doedens et al., 2010) (data not shown).

3.4. Melanocortin peptides vs. small molecule ligands for melanocortin receptors

It has become increasingly clear in the past few decades that G-protein coupled receptors such as the melanocortin receptor can choose more than one signaling pathway of intracellular communication depending upon the ligand. The vast majority of hormones and neurotransmitters are peptides (or proteins), but scientists often seek to develop non-peptide mimetics of the peptide or protein ligands. A question that arises is whether non-peptide ligands that are presumed to be mimetics, actually signal via the same mechanism. We have examined this question by comparing (Cai et al., 2004b) the signaling mechanism of Ac-Nle^4-c[Asp^5,D-Phe^7,Lys^{10}]-α-MSH(4–10)-NH_2 (MT-II), a potent human melanocortin MC_4 receptor agonist (vide supra) and N-[(3R)-1,2,3,4-tetrahydroisoquinolinium-3-ylcarbonyl]-(1R)-1-(4-chlorobenzyl)-2-[4-cyclohexyl-4-(1H-1,2,4-triazol-1ylmethyl)piperidin-1-yl]-2-oxoethylamine (THIQ) a non-peptide agonist selective for the human melanocortin MC_4 receptor. For this purpose we have examined phosphorylation in the human melanocortin MC_4 receptor following treatment with the two agonists. We have also examined C-terminal and third loop mutagenesis, and its effect on Ac-Nle^4-c[Asp^5,D-Phe^7,Lys^{10}]-α-MSH(4–10)-NH_2 (MT-II) and THIQ binding. We have used confocal and two photon laser scanning fluorescence microscopy to examine the ligand dynamics in live cells possessing the human melanocortin MC_4 receptor. In results to date, we have shown that third loop mutations do not affect Ac-Nle^4-c[Asp^5,D-Phe^7,Lys^{10}]-α-MSH(4–10)-NH_2 (MT-II) binding and only slightly affect piperidincyclic adenosine monophosphate (cAMP) formation, whereas for N-[(3R)-1,2,3,4-tetrahydroisoquinolinium-3-ylcarbonyl]-(1R)-1-(4-chlorobenzyl)-2-[4-cyclohexyl-4-(1H-1,2,4-triazol-1ylmethyl) piperidin-1-yl]-2-oxoethylamine (THIQ) there is some change in binding affinity and a more significant change in cAMP formation. In the C-terminal modifications, Ac-Nle^4-c[Asp^5,D-Phe^7,Lys^{10}]-α-MSH(4–10)-NH_2 (MT-II) treatment leads to green fluorescent protein-β-arrestin aggregation and internalization, whereas THIQ treatment causes no GFP-β-arrestin aggregation but nonetheless internalization occurs. Furthermore, Ac-Nle^4-c[Asp^5,D-Phe^7,Lys^{10}]-α-MSH(4-10)-NH_2 leads primarily to phosphorylation at the C-terminal of the human melanocortin MC_4 receptor, whereas THIQ treatment leads primarily to phosphorylation at the third and second intracellular loops. These results suggest that non-peptide and peptide agonist ligands for the human melanocortin MC_4 receptor have different signaling mechanisms. The significance of these differences for medical applications and for potential biological outcomes is unclear, but deserves further investigation.

3.5. Conclusions and unsolved questions

Using novel scaffolds and conformational constraints, it is slowly becoming possible to obtain potent (<10 nM IC_{50}s and EC_{50}s) and receptor selective (>200 fold) agonist and antagonist analogues for the human melanocortin MC_1 receptor, human melanocortin MC_3 receptor, human melanocortin MC_4 receptor, and human melanocortin MC_5 receptor. However, we still are not at the point where we know precisely the structural and conformation features that can reliably predict structure activity relationship differences for ligands for the melanocortin receptors. Clearly, we will have to continue with systematic, meticulous, 3-dimensional studies of carefully designed ligands with substantial differences in affinity and efficacy (>1000 fold). For these melanocortin receptors to have some certainty regarding the differential 3-dimensional structural pharmacophore for both agonists and antagonists (each will be different for each receptor) at the melanocortin MC_1 receptor, melanocortin MC_3 receptor, melanocortin MC_4 receptor, and melanocortin MC_5 receptor. In addition, there are a number of other outstanding unresolved issues to be addressed. In particular, when medical applications are sought, are the agonist, antagonist, cellular and animal screens we are using sufficiently discriminating? How are the central and peripheral actions of the melanocortin receptor different, and are there integrated feedback loops and so forth? How are orthosteric vs. allosteric ligands different in desired bioactivities and undesirable bioactivities? How can feeding behavior, sexual behavior, cardiovascular effects, and related functions be separated in a single ligand? Are modulation of pain, immune response, and other outstanding unresolved issues being adequately addressed? Are modulation of pain, immune response, and other 'bioactivities' of melanotropic peptides completely related to melanocortin receptors or are they also dependent on participation of other receptor systems? These and many other questions still remain. Our understanding of how the melanocortin receptors and their ligands are integrated into so many critical biological functions should keep us modest in what we know, but excited about the many opportunities we have to make a major impact in understanding human health and disease.

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