Melanocortin-4 Receptor (MC4R) Agonists for the Treatment of Obesity

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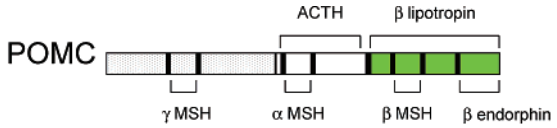
Introduction

Of the obesity targets currently being pursued in academia and in industry, rodent and human genetic data and pharmacological results from several rodent obesity models provide a high degree of target validation for the melanocortin-4 receptor (MC4R). MC4R is widely distributed throughout the brain.[1] This distribution pattern correlates with brain sites displaying high sensitivity to melanocortin-regulated feeding behavior. The ligands for MC4R are also expressed in the hypothalamus, within leptin-responsive neurons in the arcuate nucleus where their expression is regulated by alterations in energy balance. Therefore, it is hypothesized that MC4R agonists will cause sustained body weight loss by decreasing food intake without a compensatory reduction in energy expenditure. Hence, there has been an intense effort to identify selective agonists of the MC4 receptor as possible treatments for obesity.

Melanocortin Receptors and Ligands

The melanocortin receptors belong to the family of seven-transmembrane G-protein-coupled receptors. Five subtypes of melanocortin receptor have been identified, named MC1, MC2, MC3, MC4, and MC5.[2,3] They interact with their endogenous ligands (adrenocorticotropic hormone and melanocortins) to mediate a wide array of activities from the control of feeding and sexual behavior to skin pigmentation and neuroendocrine regulation. The MC1R subtype is primarily expressed in skin and contributes to pigmentation. The MC2R subtype is primarily expressed in adrenal gland and controls steroidogenesis. The MC3R and MC4R subtypes are primarily expressed in the brain and contribute to the control of energy balance. The MC5R subtype is primarily expressed in exocrine tissues. All endogenous agonists of melanocortin receptors are encoded by a single gene that generates a large precursor (proopiomelanocortin, POMC) that undergoes proteolytic processing to generate α-melanocyte stimulating hormone (α-MSH), adrenocorticotropic hormone (ACTH), *β*-MSH, *γ*-MSH, and *β*-endorphin in a tissue-specific manner (Figure 1).

The melanocortins have attracted the attention of peptide chemists for over 30 years. Among the first significant findings from research on α-MSH was the identification of NDP-α-MSH (see Figure 2). Incorporation of D-Phe in place of the Phe residue at position 7 led to a significant increase in binding and functional potency at the melanocortin receptors. Structure−activity relationship studies on ACTH and α-, *β*-, and *γ*-MSH melanocortin peptides established that the conserved tetrapeptide sequence His-Phe-Arg-Trp was the minimal core sequence required for biological activity. Cyclic peptide analogues were designed by Hruby and co-workers to improve the intrinsic potency and in vivo stability.[4] The design of Ac-Ile-c[Asp-His-D-Phe-Arg-Trp-Lys]-NH2 (**1**) and Ac-Ile-c[Asp-His-D-2-Nal-Arg-Trp -Lys]-NH_2 (**2**), which are among the most potent peptides synthesized, was based on the cyclization of the His-Phe-Arg-Trp "core" sequence of α-MSH.[4] Compound **1** is a nonselective MC1, MC3, MC4, and MC5 receptor agonist. Interestingly, replacement of the D-Phe in **1** by D-2-naphthylphenylalanine (D-2-Nal) led to **2**, which is an agonist for MC1 and MC5 receptors but a potent antagonist for MC3 and MC4 receptors. Compounds **1** and **2** have been very valuable tools for providing pharmacological proof-of-concept for the MC4 receptor as a target for the treatment of obesity. Additional research around **1** and the His-Phe-Arg-Trp "core" sequence generated various truncated peptides and cyclic analogues such as Ac-Ile-c[Asp-His-D-Phe-Arg-Trp-Lys]-OH (**3**), which are potent melanocortin agonists (Figure 1). Compound **3** is being evaluated in the clinic for the treatment of male and female dysfunction.[5,6] Peptidyl agonists and antagonists with improved selectivity toward MC4R have also been disclosed recently by Chu and co-workers[7a] and covered in a review paper by MacNeil and co-workers.[7b] Progress in the field was summarized in a recent publication by Haskell-Lauveno and co-workers.[8]



Receptor	Primary Ligands	Function
MC1R	α MSH, ACTH	Pigmentation
MC2R	ACTH	Steroidogenesis
MC3R	α MSH, γ MSH	Energy balance
MC4R	α MSH, ACTH	Energy balance, Erectile function
MC5R	α MSH, ACTH	Exocrine function

Figure 1. Melanocortin receptors, ligands, and function.

α-MSH	Ac-Ser-Tyr-Ser-Met-Glu- His- *L*-Phe-Arg-Trp -Gly-Lys-pro-$ValNH_2$
NDP-MSH	Ac-Ser-Tyr-Ser-Ile-Glu- His- *D*-Phe-Arg-Trp -Gly-Lys-pro-$ValNH_2$
1 (Agonist)	Ac-Ile-c[Asp- His-*D*- Phe- Arg- Trp -Lys]-NH_2
2 (Antagonist)	Ac-Ile-c[Asp- His-*D*-2-Nal-Arg-Trp -Lys]-NH_2
3 (Agonist)	Ac-Ile-c[Asp- His-*D*- Phe- Arg- Trp -Lys]-OH

Figure 2. Endogenous and synthetic peptidyl ligands with high affinity for the MC4 receptor.

Genetics and MC4R

The genetic validation for MC4R as an antiobesity target is particularly compelling. Loss-of-function variants in the MC4R are associated with hyperphagia, obesity, and metabolic defects with dominant heredity in humans as well as mice.[9,10] Both the MC4R KO ($mc4r^{-/-}$) mice and the yellow agouti mice, which overexpresses the endogenous MC3/MC4 antagonist agouti protein (AGRP), exhibit obesity, hyperinsulinemia, increased linear growth, normal basal corticosterone, and unchanged arcuate neuropeptide Y (NPY) level with elevated dorsal medial hypothalamus NPY level, further supporting the view that the agouti syndrome is due to antagonism of central MC4R.[9] Since

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a dominant negative effect has not been confirmed with human MC4R variants,[11,12] the dominant heredity is best explained by haploinsufficiency (i.e., gene dosage effect). Moreover, MC4R agonism is likely to be a titratable trait as suggested by the observation of haploinsufficiency phenotype for loss-of-function variants in both humans and mice. Therefore, activation of MC4R by exogenous agonists is expected to cause hypophagia and weight loss.

Numerous variants in the human MC4R gene have been identified with an overall frequency of 1−5% in the morbidly obese population.[10,13] The intrinsic function of these variants ranges from complete loss of function, to partial loss of function, to completely normal when evaluated in heterologous expression systems. For those variants with apparently normal function, the sequence variation can either reduce the expression level of MC4R or impair plasma membrane targeting.[14,15] For those partial loss of function variants, codominant and recessive inheritance has also been reported,[10,16] which is consistent with a gene dosage effect.

In light of the prominent association of MC4R variants with obesity, it is important to recognize that the melanocortin system (including AGRP) is remarkably conserved at the chromosomal organizational level in many species from zebrafish to mammals.[17−19] Hence, MC4 is a critical receptor from the phylogenetic point of view, and these data are consistent with its pivotal role in controlling feeding behavior and body weight.

Pharmacology of Melanocortin Peptides

A variety of nonselective peptide agonists and antagonists have been used as research tools to define the role of the MC4R in feeding and body weight regulation. Cyclic peptide **1** and [Nle(4),D-Phe(7)]-α-melanocyte stimulating hormone (NDP-α-MSH), which are potent agonists for the MC1, MC3, MC4, and MC5 receptors, have been used as agonist tools, and **2** (MC1 and MC5 receptor agonist; MC3 and MC4 receptor antagonist) and the endogenous melanocortin antagonist, agouti-related protein (MC1, MC3, MC4 receptor antagonist), have been used to study putative MC4R antagonist effects. In general, the pharmacological effects of these peptides are consistent with the role predicted by the phenotype of the $mc4r^{-/-}$ mouse.

Peptide agonist **1**, acutely administered either centrally or peripherally, decreases food intake and body weight in rats[20] and in mice.[21] Peptide antagonist **2**[22,23] and AGRP[24] given centrally have the opposite effects, increasing both food intake and body weight. When given at nonefficacious doses, both antagonists block evoked feeding suppression evoked by **1**. Compound **1** increases[25] and AGRP decreases[26] oxygen consumption, thus demonstrating an energy expenditure component in MC4R-mediated body weight regulation as well energy intake. The efficacy of NDP-α-MSH when given into a variety of hypothalamic sites[27] and into the brainstem region[23] and when administered peripherally lends credence to the idea that MC4Rs in multiple central nervous system (CNS) nuclei are a part of the central neural network regulating energy balance.

In keeping with the ability of melanocortin agonists to suppress food intake via multiple subpopulations of MC4Rs, there appear to be multiple ways melanocortin receptor activation can impact feeding pathways. Most often, hypothalamically regulated feeding[27] is associated with homeostatic need. Additionally, pharmacological studies have also implicated melanocortin agonism with reward-based feeding,[28] possibly via the MC4Rs localized in the nucleus accumbens.[29,30] In other studies, melanocortin agonists have been implicated in reduction of meal size.[31,32]

Sensitivity to melanocortin agonists appears increased in most obese rodent models: genetic, diet-induced, or lesion-induced. Multiple-day intraperitoneal administration of **1** decreases body weight to a greater extent in DIO mice than in chow-fed controls.[33] Similarly, studies in genetic[22] and diet-induced obese rats[34] and in hypothalamic lesioned models[35] have also shown increased sensitivity to **1** or α-MSH. With peripheral administration, the issue arises of whether the effect is that a greater dose is given when drug administration is normalized to total body weight instead of to lean (metabolically active) body mass. However, multiple-day central infusion of **1** in chow-fed and high-fat-fed Sprague-Dawley rats have resulted in similar suppression of food intake and body weight in the presence of a reduction of hypothalamic MC3 and MC4 receptor mRNA expression,[36] thus suggesting an increased sensitivity of the melanocortin system downstream to the receptor. Not all models behave similarly; the high-fat-fed Long-Evans rat appears to be an exception. It is less able to suppress food intake in response to acutely administered icv **1** than its counterparts on a low-fat diet.[37] In this instance, no changes were seen in mRNA expression of POMC, AGRP, or MC4R.

Melanocortin agonism may directly improve insulin sensitivity. Acute, intracerebral injection of **1** suppresses insulin secretion and increases plasma glucose levels in ob/ob mice.[38] Infusion of α-MSH or **2** shows opposite pharmacological effects to modulate glucose production during insulin clamp studies.[39] Long-term pair-feeding of $mc4r^{-/-}$ mice eliminates the significant hyperinsulinemia and hyperglycemia observed in ad libitum fed $mc4r^{-/-}$ mice. However, pair-fed $mc4r^{-/-}$ mice still exhibit trends toward elevated insulin levels relative to ad libitum fed wild-type controls.[40] In both of these studies, melanocortin-mediated food intake changes were controlled for with paired-feeding. Still, a partial role of altered adiposity in the modulation of insulin resistance cannot be eliminated.

Design Considerations for MC4R Agonists

Several academic and industry research groups have focused their attention on developing orally active MC4R agonists with suitable pharmacokinetic and pharmacodynamic properties for inducing weight loss in animals. Toward this end, considerable progress has been made in designing selective MC4R agonists following the publication of the results of Fan and co-workers[41] in 1997, wherein MC4R was shown to be an important obesity target. Thus far, however, there are no reports of MC4R agonists progressing into the clinic for the treatment of obesity. This in part may be due to the usual difficulties of designing agonists for peptide receptors and for the MC4R specifically, attaining high selectivity versus the MC1, MC3, and MC5 receptors. Furthermore, a significant challenge for the field is to identify MC4R agonists with adequate CNS penetration and distribution to attain occupancy at brain MC4 receptors.

Two approaches have been pursued to identify small-molecule MC4R agonists. First, peptidomimetics based on piperazine, cyclohexane, and pyrrolidine "cores" have been developed to present the key pharmacophoric groups from the His-Phe-Arg-Trp message sequence. Second, peptidyl privileged structures and non-peptide MC4R agonists have been identified by designing focused privileged structures libraries and/or modifying hits from high-throughput screening (HTS). Progress toward the development of peptidomimetics "core" designs has been reviewed recently in several excellent publications,[42,43] and therefore, this article will only cover some recent developments in the design of privileged structure-based and non-peptide MC4R agonists. In the literature, intrinsic binding IC_{50} values

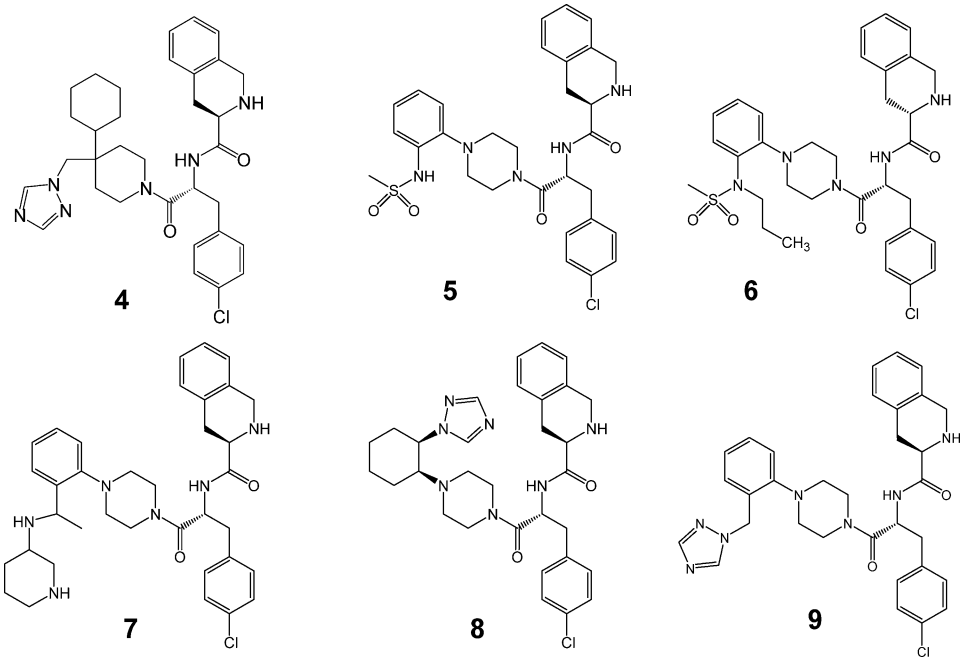

Figure 3. Agonists for the MC4 receptor based on the peptidyl privileged structure design. Privileged structure modifications are shown.

were determined in a radioligand ([^{125}I]-NDP-α-MSH) binding assay using cell membranes. Functional EC_{50} values were determined by measuring cAMP concentrations post incubation of compounds with intact cells expressing the melanocortin receptors. The percent activation, when disclosed, refers to levels of cAMP stimulation postincubation at 10 μM in comparison with α-MSH.

MC4R Agonist Ligand Design: Peptidyl Privileged Structures

Prior to the early 1990s, morphine and related opioids were identified as peptidomimetic agonists. Other non-peptide agonists included tifludom, a benzodiazepine-based opioid agonist, and erythromycin, a motilin receptor agonist. However, over the past several years, considerable progress has been made in the design of small-molecule agonists for peptide receptors. Non-peptide seven-transmembrane receptor agonists have been identified for the angiotensin AT1, CCK1, bradykinin B2 vasopressin V2, and NK1 receptors by empirical modifications of receptor antagonists.[44,45] In addition to the aforementioned examples, small-molecule receptor agonists have been developed by employing the peptidyl privileged structure design, where a privileged structure anchor is derivatized with a capped or uncapped dipeptide. Successful applications of this design concept include the identification of agonists for the ghrelin and C5a receptors, the somatostatin sst1, sst2, sst4, and sst5 receptors, and the MC4R receptor.[44] Among the first selective MC4R agonists to be reported in the literature was compound **4** (THIQ), which is a potent full agonist of MC4R (EC_{50} = 2.1 nM) with >100-fold functional selectivity vs the other melanocortin receptor subtypes.[46] In the design of **4**, Sebhat and co-workers[46] utilized a spiroindanylsulfonamide privileged structure to identify a partial agonist lead that was ultimately modified to **1** (Figure 3). Significant food intake reduction, which lasted for about 6 h, was observed in mice after intracerebroventricular (ICV) administration of 32 nmol of **4**.[47] This effect of **4** on nocturnal feeding was shown to be MC4R-mediated because no effect was found in MC4R knockout mice. Pharmacokinetics for **4** were modest in rats, characterized by an oral bioavailablity of 14%, a relatively short half-life ($T_{1/2}$ = 0.6 h), and high clearance (CL_p = 84 mL min^{-1} kg^{-1}). Hence, poor pharmacokinetics and an unacceptable off-target activity profile prevented further development of **4**. Significant tolerability for potency was found by modifying the N-terminal tetrahydroisoquinoline (Tic) side chain and the privileged structure. For example, moderate to excellent efficacy at MC4R has been reported by employing piperazine-based privileged structures, as exemplified in **5** (EC_{50} = 16 nM),[48] **6** (MC4R EC_{50} = 0.4 nM),[49] **7** (EC_{50} = 24nM),[50] **8** (binding K_i = 0.26 nM),[51] and **9** (EC_{50} = 24nM).[52] Of these, MC4R agonists **5** and **6** have been tested in animals for pharmacokinetic properties and/or efficacy. Compound **5** demonstrated 30% orally bioavailability in rats with a clearance of 43 mL min^{-1} kg^{-1}, a volume of distribution of 4.5 L kg^{-1}, and half-life of 1.7 h.[48] Fotsch and co-workers[49] reported that compound **6** showed modest bioavailability in mice (~20%), a relatively short terminal half-life ($T_{1/2}$ = 0.44 h), and moderate clearance (30 mL min^{-1} kg^{-1}).[49] Importantly, **6** was moderately brain-penetrant (C_{brain} = 130 ng g^{-1} at T_{max}) and reduced food intake over 6 h in fasted C57BL/6 mice. Consistent with the short half-life, cumulative food intake reduction 24 h after oral dosing at 50 mpk was similar to controls. Fostch and co-workers[49] did not report if the efficacy was mediated by MC4R because they did not test **6** in mc4r$^{-/-}$ and wild-type mice. In addition to the compounds cited above, in the past few years, several patent applications have appeared that contain MC4R agonists with either the piperidine or the piperazine core.[49]

A series of selective MC4R agonists with alternate N-terminal side chains have also been disclosed. For example, **10** (MB243; EC_{50} = 11 nM)[53] and **11** (EC_{50} = 1.2 nM)[53] are potent and selective MC4R agonists (Figure 4). Compound **10** shows modest oral bioavailability in rats, dogs, and rhesus monkeys (9−17%) and reduced body weight in diet-induced obese (DIO) rats at 20 mg kg^{-1} po, b.i.d. Significant covalent binding was observed when **10** was incubated with rat and human liver microsomes in vitro.[53] Mechanistic studies showed that covalent labeling occurred by metabolic activation of the N-terminal piperazine unit.[54] This information guided the identification of potent MC4R agonists with reduced covalent binding by incorporating small alkyl substituents on the piperazine.[54]

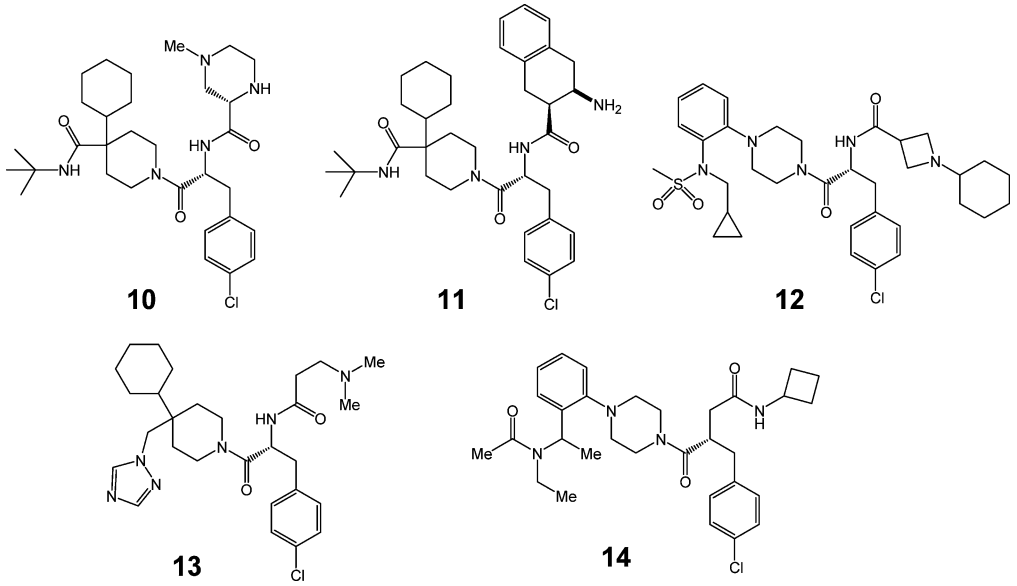

Figure 4. Agonists for the MC4 receptor based on the peptidyl privileged structure design. Modifications to the N-terminus are shown.

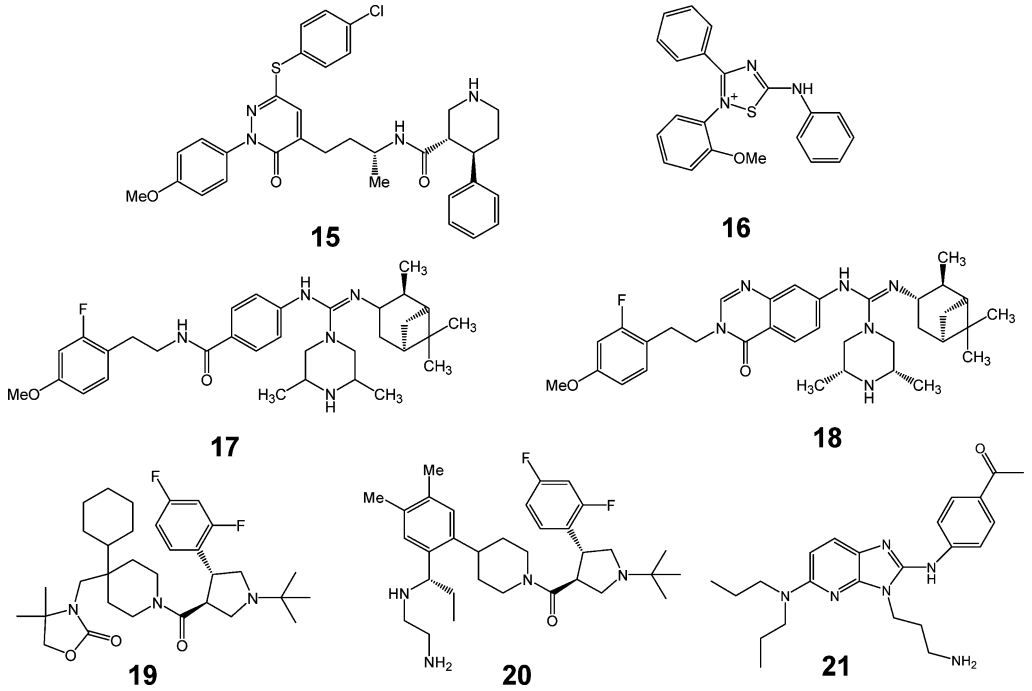

Figure 5. Non-peptide agonists for the MC4 receptor.

Additional compounds with good efficacy at MC4R include **12** (MC4R EC_{50} = 1.8 nM)[49], **13** (MC4R EC_{50} = 40 nM)[55] and **14** (MC4R EC_{50} = 15 nM (100%)).[56]

It is quite remarkable that potent MC4R agonists have been designed that are considerably smaller in size than the peptidyl ligands α-MSH and **1** that they mimic. Hruby and co-workers recently published their findings on conformational analysis of **1** and other agonist and antagonists peptides by employing NMR techniques.[57] They reported that the active pharmacophore is induced by a type II′ β turn structure around residues His-Phe-Arg-Trp of **1**. It is noteworthy that the MC4 agonist **1** was also modeled as a mimic of a turn structure in **1** peptide and that, consistent with SAR in the peptide series, use of *p*-Cl D-Phe increased potency toward MC4R.[46] Previously, agonists for the ghrelin and sst2 receptors were also suggested to mimic type II′ β turn structures in the ghrelin receptor agonist peptide GHRP-6 and the somatostatin analogue MK-0678, respectively.[44] Hence, the peptidyl privileged design may be broadly useful for designing peptidomimetics when the pharmacophore is induced by a β turn structure in peptide ligands. In addition, GPCR pahramcophores have also been utilized in designing enzyme inhibitors and ion channel modulators for which the binding motifs may be associated with α-helical structures.[44] Determining how privileged structures bind and activate GPCRs, including the MC4 receptor, may provide guidance in developing structurally distinct agonists with improved pharmacokinetics and brain penetration.

Non-Peptide MC4R Agonists

In addition to the MC4R agonists described above, significant progress has been made toward the design of non-peptide agonists (Figure 5). Non-peptide leads offer the best hope for crossing the blood−brain barrier and providing occupancy at the MC4 receptor at the relevant sites. Thus far, only a limited

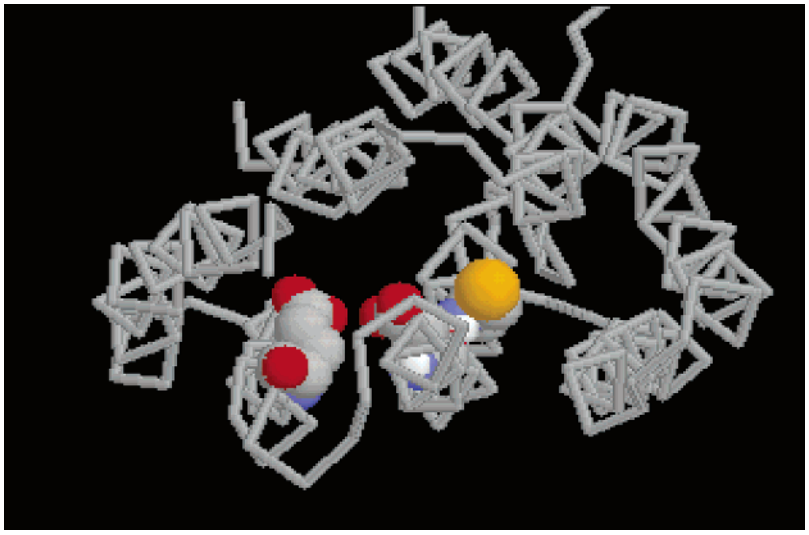

Figure 6. Homology model of MC4R based on the rhodopsin structure, viewed from the extracellular space. The functional groups of E100 (red), D122 (red), and C130 (yellow) are highlighted in CPK rendering.

number of reports have appeared in the literature on non-peptide MC4R agonists. Furthermore, most of the disclosures to date have been in the patent literature, and therefore, details regarding potency and efficacy toward MC4R, pharmacokinetics, possibly brain penetration, and in vivo antiobesity efficacy information are not available. Pyridazinone MC4R agonist **15** (MC4R EC_{505} = 177 nM) was disclosed recently by Ujjainwalla and coworkers.[58] Also, thiadiazole compound **16** (MC4R binding IC_{50} = 22 nM) demonstrated excellent binding affinity toward MC4R.[59] Compound **16** was reported to be an agonist, but functional data were not reported. Among the most significant disclosures in the patent literature are guanidine-based MC4R agonists, as exemplified in **17** and **18**.[60,61] An undisclosed member in the guanidine series (MC4R EC_{50} = 23 nM) reduced food intake in a dose-dependent manner when it was administered to obese mice at 10, 30, and 60 mg kg^{-1} orally.[43] In a separate patent application, approximately 15-fold greater efficacy was noted when the compound was dosed intranasally instead of via the oral route.[62] Other noteworthy disclosures from the patent literature include *N-tert*-butylpyrrolidines **19** and **20**, respectively, and imidazopyridine **21**.[63−65] Finally, a number of institutions and other companies have claimed small molecules as MC4R agonists.[66]

Structure−Function Relationship of MC4R

The structure−function relationship of MC4R has been investigated by mutational analysis. Most of the studies focused primarily on receptor mutants and how the mutations affected receptor function.[67−69] While these studies provided an initial guidance for further studies, demonstrating an effect on receptor function by a mutation is not sufficient to propose a direct interaction between the ligand and a specific residue. Hence, attempts have been made to develop ligand binding site models by elucidating the binding motif for the peptides.[67] The MC4R and other melanocortin receptors contain three unique acidic residues (E100, D122, and D126 in MC4R) in the transmembrane (TM) domain that have been hypothesized to interact with the arginine side chain of α-MSH. Substitution of these acidic residues reduces binding affinity and activation potency. Using a complementary substitution approach in which the D122A mutant and wild-type receptor were analyzed with peptides in which various side chains were substituted systematically, it was demonstrated that the D122 residue is more likely to be in direct interaction with the Arg side chain of α-MSH because the D122A mutation does not affect the binding affinity of the Nle8 analogue of NDP-α-MSH.

Another approach to studying ligand−receptor interaction is by combining chemical modification with receptor mutagenesis. A recent study indicated that a sulfhydryl reactive agent can inhibit ligand binding. The C130A mutant of MC4R, however, completely prevented the inhibitory effect of the sulfhydryl reactive agent, hence suggesting that the C130 residue is probably in the vicinity of the peptide binding site.[70] Both D122 and C130 are within TM3, separated by two helical turns and facing the interior of the TM domain. The ligand binding site of MC4R thus contains at least the interior part of TM3. The available data also support a model that D122 is more critical for the binding of Arg-containing peptides while C130 is clearly accessible to small molecules and can potentially form a direct contact with a small-molecule agonist or antagonist (Figure 6).

Neurophysiology of MC4R

Recent advances in slice preparations have allowed the functional characterization of melanocortin receptors in rodent brain. Using nonselective agonists, early studies indicated that **1** increases the inhibitory GABA synaptic transmission while NPY inhibits GABA transmission in the PVN of rat hypothalamus.[71] Since the synaptic modulation by **1** is relatively small (up to 25% increase), additional neurophysiological effects of MC4R activation are expected. A more substantial inhibition of spontanenous firing rate was documented for α-MSH and MC4R selective agonists.[72] These studies clearly indicate that MC4R activation can exert an inhibitory effect on hypothalamic neurons through inhibition of neuronal firing rate and facilitation of GABA transmission.

As expected for any pathway in the brain, there is significant interaction between the melanocortin system and other systems. At the single-cell level, coexpression of neuropeptide Y1 (NPY1) receptor and MC4R in specific neurons of the paraventricular nucleus and amygdala supports the opposing biological effects of Y1R and MC4R in controlling feeding behavior.[73] Coexpression of two receptors in the same neuron is not the only way to produce synergy because system level interaction can still produce physiological antagonism or synergy. Opioid antagonists have been shown to reverse AGRP-induced hyperphagia.[74,75] Consistent with the physiological antagonism of the

opioid and melanocortin pathways, chronic treatment with morphine led to down-regulation of MC4R mRNA in the striatum and periaqueductal gray.[76] Similarly, *d*-fenfluramine, which activates the 5HT2c receptor, is less efficacious in the yellow agouti Ay mice than the wild-type mice. Furthermore, the melanocortin receptor antagonist **2** counteracts the food intake effect of *d*-fenfluramine.[77]

The melanocortin pathway is partially responsible for the orexigenic activity of the stomach-derived hormone ghrelin. While AGRP or MC3R-MC4R gene deletion partially attenuates the efficacy of a ghrelin agonist, NPY and AGRP double KO mice do not respond to a synthetic ghrelin agonist.[78] Independent electrophysiological and anatomical studies also indicate that ghrelin increases NPY neuron firing rate and inhibits POMC neuron firing rate, and ghrelin immunoreactive terminals are close to NPY- and POMC-immunoreactive neurons.[79] Taken together, the available evidence suggests that MC4R can act downstream from ghrelin and can act in parallel with many other pathways including NPY, opioid, and serotonin pathways.

Role of Melanocortins in Sexual Function

Opportunities may exist for the MC4R-mediated treatment of diseases in addition to obesity. Recently, melanocortin receptor agonists have gained support for the treatment of male and female sexual dysfunction.[80] In humans, **1** increased proerectile events in men with organic and psychogenic erectile dysfunction following subcutaneous administration.[81] Recently, **3** was reported to be effective at doses as low as 4 and 6 mg, sc, respectively, in ED patients with inadequate responses to a PDE5 inhibitor.[5] In a phase II trial, peptide **3** caused statistically significant increases in erectile responses with a 60−80% response rate. Pharmacokinetics/pharmacodynamics analysis showed that efficacy was noted at C_{max} values of >125 nM. Since **1** and **3** are pan-MC-receptor agonists, it is not known which melancortin receptor modulates erectile activity in humans. However, in rodent assays, activation of MC4R modulates erectile activity.[82,83] In anesthetized mice, MC4R agonists such **4** and **10** potentiate erectile activity as measured by increases in intracavernosal pressure (ICP) following electrical stimulation of the cavernous nerve.[53] Furthermore, the proerectile activity of **4** is mediated by MC4R because erectile activity was increased only in mc4r$^{+/+}$ mice cohorts in a dose-dependent manner and no activity was seen in mc4r$^{-/-}$mice.[83] Melanocortin agonists are also active in rat models of erectile function. For example, MC4R agonist **4** increased the number of penile erections in an ex copula conscious rat model in a dose-responsive manner following intravenous administration.[82] Peptide **3** also increased the erectile frequency in rats following dosing via the intravenous and intranasal routes. In conscious rats, **1** was shown to induce penile erection via activation of brain and spinal chord melanocortin receptors.[84] Pfaus and co-workers recently reported that **3** increased proceptive behaviors in a female rat model.[6] Hence, the authors suggested that melanocortin agonists may have utility in treating female hypoactive sexual desire disorder. As described above, preclinical data strongly support the involvement of MC4R in sexual function. Whether MC4R agonists will be useful for treating male and female sexual dysfunction can only be determined by testing compounds in humans. If MC4R agonists induce spontaneous penile erections in men, this would represent a significant impediment to the development of compounds to treat obesity.

The nonselective melanocortin agonists, **1** and **3**, have nausea and vomiting as adverse side effects when administered to humans either subcutaneously or intranasally.[5,81,85] Attempts to study whether the effects of these two structurally related molecules are mechanism-based have been of limited utility. Most studies have been done with rodent models, which can only address this question indirectly because rodents do not vomit and the behavioral assays to address potential nausea are nonspecific and can encompass other behavioral changes in their readouts. Therefore, as MC4R agonists progress into clinical development, it will be important to determine if mechanism-based nausea and emesis can be avoided.

Future Directions

In the clinic, MC4R agonists present a number of therapeutic opportunities. However, it is unclear at this time what type of pharmacodynamic profile will result in optimal efficacy. While sustained weight loss is achieved upon repeated dosing with MC4R agonists in rodent models, whether tachyphylaxis will occur in humans remains an open question. Furthermore, there is little information on target engagement biomarkers and/or surrogate markers that correlate with efficacy. Hence, it will be difficult to assess the extent to which the target was engaged following dosing with an MC4R agonist. Consequently, longer term phase II trials will most likely be required to determine the extent of weight loss with a MC4R agonist. In addition, consideration must be given for unwanted but potential mechanism-based side effects that could include modulation of the cardiovascular system, the immune system, and CNS (motor activity, sexual behavior, pain).

The AGRP tone at synapses that express MC4R may affect efficacy also. As described above, AGRP is an endogenous antagonist of both MC3R and MC4R,[86,87] and while the exact level in the human brain is not known, AGRP levels are increased by fasting in rodent brains.[88] At least in mice, an RNAi study has indicated that reducing the mRNA expression level of AGRP by 50% can lead to reduced body weight compared to a control siRNA vector.[89] Hence, it is conceivable that the endogenous AGRP can lead to a net reduction of MC4R agonist efficacy, and manipulation of AGRP provides a novel approach to reverse the endogenous AGRP inhibition which can then be expected to enhance the MC4R agonist efficacy.

A low level of constitutive activity in MC4R has been reported on the basis of heterologous expression where the constitutive activity can be inhibited by AGRP.[68,90−92] While the degree of MC4R constitutive activity is much lower than that observed for Gi-coupled GPCRs and the constitutive activity remains to be demonstrated in ex vivo preparations, it does raise the theoretical possibility of allosteric activators that can increase constitutive activity without binding to the α-MSH binding site. Such an allosteric activator can potentially be combined with MC4R agonist to achieve clinical efficacy.

Current therapeutics for obesity produce limited weight loss; sibutramine treatment typically achieves a weight loss of ∼5−10% and orlistat a weight loss of 3%.[93] *d*-Fenfluramine, no longer on the market, resulted in a weight loss of up to 10−15%. While this is clearly a sufficient body weight loss to provide therapeutic value in decreasing obesity-related comorbidities such as type 2 diabetes, dyslipidemia, and hypertension, it will not normalize body weight in most circumstances. Simultaneous modulation of multiple pathways holds out the best hope for better normalization of weight, similar to the need to provide multiple medications for treatment of other chronic diseases such as type 2 diabetes, dyslipidemia, and hypertension. To date, maximal suppression of body weight of 10−15% is observed with either nonselective melanocortin agonists or

MC4R selective agonists in rodents. Similar values have been seen in rodents with dexfenfluramine. Hence, if rodent efficacy is predictive of weight loss in humans, MC4R agonists may also afford significant body weight reductions in humans also. Cannabinoid 1 receptor (CB1R) inverse agonists are approaching the marketplace and appear to have similar efficacy to that seen with dexfenfluramine in humans. In rodent studies, there are synergistic effects in the acute suppression of food intake with the endogenous melanocortin, α-MSH, and a cannabinoid inverse agonist,[94] thus suggesting that the combination of a melanocortin agonist and a cannabinoid 1R inverse agonist may provide therapeutic benefit. Other transmitter systems shown to interact with the melanocortin pathways in preclinical studies both pharmacologically and in gene deletion studies include ghrelin,[78] corticotrophin releasing factor,[95] serotonin,[77] and cholecystokinin.[96] The extent to which combination therapies will be of use awaits the appropriate individual therapeutics to come to market.

In addition to obesity treatment, melanocortin agonists may have utility in other indications. Melanocortin agonists such as **1** have been shown to reduce voluntary alcohol intake in rodents,[97,98] and such an inhibitory effect can be blocked by the MC3R and MC4R antagonist AGRP. Furthermore, the effect of **1** was absent in MC3R KO mice, suggesting that MC4R probably mediates the inhibitory effect of **1** on alcohol intake.[99] Therefore, it can be expected that MC4R agonist may be useful in the treatment of alcoholism.

Conclusion

Since the first series of publications describing the role of MC4R in energy homeostasis in the late 1990s, considerable progress has been made in developing small-molecules that mimic the actions of the peptides α-MSH and **1** and that do so by selectively agonizing MC4R. The design of these peptidomimetics is interesting because they are receptor agonists. There has also been significant progress in MC4R biology, and this has been facilitated by the availability of tools such as MC4R selective agonists and antagonists. MC4R agonists present a number of opportunities for therapeutic intervention, and while scientific and medical challenges to the developments of MC4R agonists remain, their therapeutic potential is tantalizing. The coming few years will be an exciting time for MC4R agonists because it is anticipated that compounds will progress into clinic trials and human pharmacological proof-of-concept data will be available for the obesity indication.

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