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Review Article

The MC_4 receptor as a drug discovery target

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CONTENTS

Abstract

Molecular and pharmacological studies have provided valuable information for elucidating the biological functions of the central melanocortin system, which includes the MC_3 and MC_4 receptor subtypes and their endogenous ligands. Compelling evidence demonstrates that the MC_4 receptor plays an important role in feeding behavior, energy homeostasis, sexual function, nociception, drug addiction and stress responses. Potent and selective MC_4 receptor ligands are vital to define the physiological roles of this receptor. Preclinical studies have proven that selective MC_4 receptor agonists reduce food intake and body weight and increase erectile activity. Selective MC_4 receptor antagonists alleviate cachectic syndrome, neuropathic pain, the motivational effects of addictive drugs and stress-induced depression and anxiety. Clinical studies have shown that the neural MC_3/MC_4 receptor dual agonist MTII elicits erectile responses in healthy volunteers and in patients with erectile dysfunction. In this review, we summarize the recent development of small-molecule MC_4 receptor ligands and evidence concerning the involvement of this receptor in various physiological functions. Within this context, we attempt to provide an insight into the potential therapeutic uses of MC_4 receptor ligands.

Introduction

The melanocortin system

The melanocortin (MC) system is comprised of MC receptors and endogenous peptide ligands functioning as agonists and antagonists. The endogenous MC agonists include α-, β- and γ-melanocyte-stimulating hormones (α-, β- and γ-MSH) and adrenocorticotropin (ACTH), collectively known as MC peptides. These endogenous MC peptides are derived from the posttranslational modification of the prohormone pro-opiomelanocortin (POMC; Fig. 1) (1). POMC itself is a functionally inert polypeptide that is expressed in the pituitary gland, skin, immune system and brain.

MC peptides participate in diverse physiological functions in mammalian species, including humans. For example, POMC knockout mice are hyperphagic and develop severe obesity (2). They also have adrenal insufficiency due to the loss of ACTH and grow a yellow coat, as compared to the brown fur of wild-type mice. Human polymorphisms of the POMC gene result in similar abnormalities, which include early-onset obesity, adrenal insufficiency and red hair (3, 4).

The MC system also contains endogenous antagonists (inverse agonists), namely Agouti and Agouti-related protein (AGRP) (5). Agouti is a paracrine signaling factor that acts within the hair follicle microenvironment to block MC peptide action at the MC_1 receptor. In contrast, AGRP shows a very distinct expression in the CNS and regulates energy homeostasis via its antagonist effect on central MC receptors (5). Postembryonic ablation of AGRP neurons in mice leads to a lean, hypophagic phenotype (6). Humans with polymorphisms in the AGRP gene are leaner than wild-type (WT) individuals and resis-

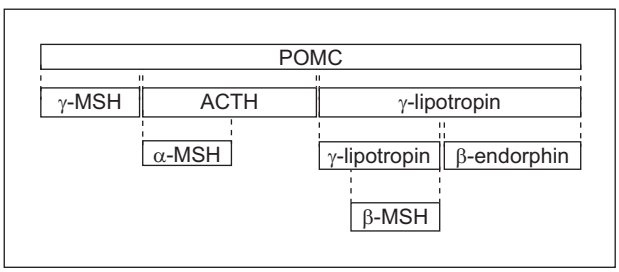


Fig. 1. Illustration of the posttranslational processing of POMC.

tant to late-onset obesity (7). It is believed that AGRP counteracts the effects of α-MSH in the brain to regulate body weight, thus providing a means of fine-tuning physiological processes (5, 8).

Five MC receptor subtypes, named MC_{1-5} receptors, have been identified and cloned to date (9). They all belong to the superfamily of 7-transmembrane G-protein-coupled receptors (GPCRs). These receptors couple to adenylyl cyclase and mediate their functions primarily by activating a cAMP-dependent signaling pathway. MC receptors show high sequence homologies, ranging from 60% identity between the MC_4 and the MC_5 receptor, to 55% identity between the MC_4 and the MC_3 receptor, and 38% identity between the MC_4 and the MC_2 receptor (10, 11). On the other hand, MC receptors exhibit different tissue distributions and have diverse physiological roles. Table I lists the main sites of expression and physiological roles of MC receptor subtypes (11).

The MC_4 receptor is mainly expressed within the neuroendocrine system, with particularly dense expression in the hypothalamus (12). Conversely, the MC_4 receptor was not detected in peripheral cells in an extensive study including 20 different tissues (13). The distribution of the MC_4 receptor is consistent with its involvement in autonomic and neuroendocrine functions. Thus, this subtype has been found to participate in the regulation of feeding behavior, energy homeostasis, erectile function, drug addiction, nociception and stress responses (14).

The MC_4 and the MC_3 receptors are commonly referred to as central melanocortin receptors (10). MC_4 receptor mRNA is more widely expressed than MC_3 receptor mRNA. However, both of these receptors have been found in the same areas, such as the hypothalamus and spinal cord. To differentiate the physiological functions of the MC_3 and MC_4 receptors, knockout animal models are often used (14). Selective MC_3 and MC_4 receptor agonists and antagonists are also employed as powerful tools to investigate the functions of these two receptors (*vide infra*).

Peptide-based melanocortin receptor ligands

All the endogenous melanocortin agonists contain a core His-Phe-Arg-Trp tetrapeptide sequence, which is important for MC receptor recognition and stimulation (15). Based on this pharmacophore, many MC peptide analogues have been prepared in the past decade and substances displaying valuable properties, such as antagonism, high activity and selectivity, were discovered (16). Table II lists several synthetic peptides that have been extensively used in elucidating the physiological functions of MC receptors. The Phe in the core sequence plays a prominent role in enhancing binding and functional potencies, as demonstrated in cyclic peptides MTII (17) and PT-141(18), MC_4 receptor agonists in which the Phe is replaced with its enantiomer D-Phe, and SHU-9119 (19) and HS-014 (20), MC_4 receptor antagonists in which the Phe is replaced with the naphthalene derivative D-Nal. Detailed discussions regarding peptide-based MC receptor ligands can be found in a recent review (21).

Recent development of MC_4 receptor ligands

From melanocortin peptides to small-molecule MC_4 receptor agonists

Based on the structure and conformation of MC peptides, one can create a pharmacophore model for designing small-molecule MC_4 receptor ligands (23, 25). A well-defined pharmacophore identifies the minimal chemical feature necessary for activity, and provides relative positions of peptide side-chains (27). The cyclic peptide MTII and its analogues have been extensively used in this regard because of their conformational rigidity (23-25).

Table I: The melanocortin receptor subtypes, their location and physiological functions (11).

MC receptor subtype	Prevalent expression sites	Functions
MC_1	Melanocytes	Skin pigmentation
MC_2	Adrenal cortex	Steroid production and release
MC_3	Hypothalamus, amygdala, hippocampus, placenta, heart, pancreas	Autonomic functions, energy homeostasis
MC_4	Hypothalamus, amygdala, spinal cord, dorsal root ganglia	Feeding behavior, erectile function, nociception, stress response
MC_5	Sebaceous gland	Glandular lipid production

Table II: Important endogenous and synthetic melanocortin receptor peptide agonists and antagonists.

Peptide	Structure	K_i (nM)		
		MC_3	MC_4	MC_5
α-MSH	Ac-Ser-Tyr-Ser-Met-Glu-His-**Phe**-Arg-Trp-Gly-Lys-Pro-Val-NH2	31	660	5700
MTII	Ac-Nle-c[Asp-His-**D-Phe**-Arg-Trp-Lys]-NH2	34	6.6	46
SHU-9119	Ac-Nle-c[Asp-His-**D-Nal**-Arg-Trp-Lys]-NH2	1.2	0.36	1.1
PT-141	Ac-Nle-c[Asp-His-**D-Phe**-Arg-Trp-Lys]-OH	NA	15	NA
HS-014	Ac-c[Cys-Glu-His-**D-Nal**-Arg-Trp-Gly-Cys]-Pro-Pro-Lys-Asp-NH2	54	3.2	690

All K_i values are for human melanocortin receptor subtypes and are from Ref. 22, except for PT-141, which is from Ref. 18. NA: not available.

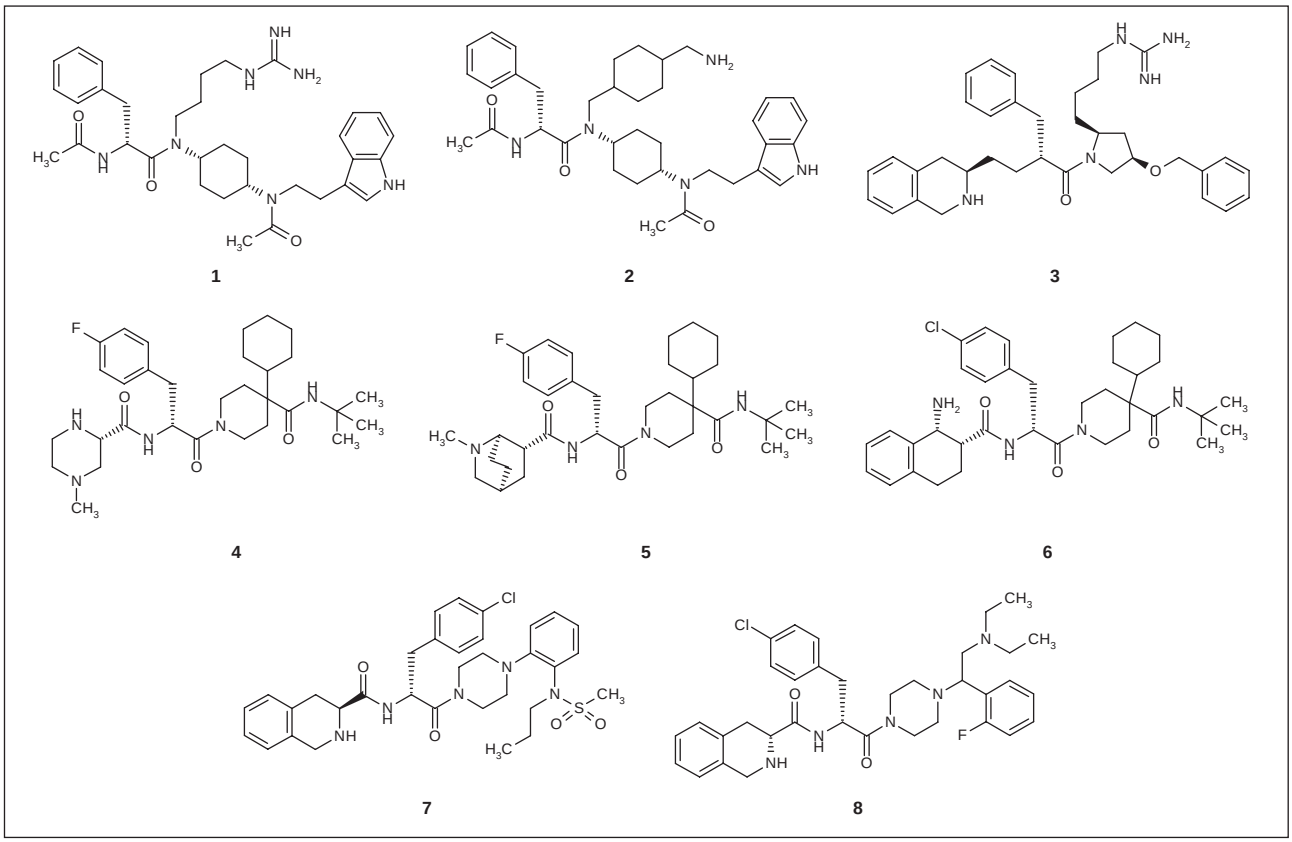


Fig. 2. Small-molecule MC_4 receptor agonists.

Compounds **1-3** in Figure 2 are MC_4 receptor agonists designed by mimicking the structures of MC peptides.

Fotsch *et al*. established a pharmacophore model based on the solution structures of a potent MC receptor agonist, Ac-Nle-cyclo[Asp-Pro-D-Phe-Arg-Trp-Lys]-NH_2 (25). Accordingly, the authors were able to design the *cis*-cyclohexyl analogue (**1**) to mimic the spatial arrangement of the critical tripeptide region D-Phe-Arg-Trp found in the cyclic peptide. Compound **1** is a potent human MC_4 receptor agonist with IC_{50} and EC_{50} values below 10 nM in binding and functional assays. The selectivity profile of compound **1** is improved as compared to that of MTII, showing 36- and 7-fold selectivity *versus* the MC_3 and MC_1 receptors, respectively (see Table III) (25). Brief structure-activity relationship (SAR) studies based on compound **1** indicated that subtle changes in cyclohexane stereochemistry and removal of any of the functional groups led to analogues with substantial loss of affinity for the MC receptors. Interestingly, the guanidine side-chain can be replaced with a conformationally constrained 1,3-cyclohexanebis(methylamine) moiety (26). The resulting amino analogue (**2**) existed as a mixture of diastereomers, is equipotent to compound **1** at the MC_4 receptor and exhibits > 140- and 2-fold selectivity *versus* the MC_3 and MC_1 receptor, respectively, in binding affinity.

The *cis*-cyclohexyldiamine core was also modified to a 5-membered scaffold, as represented by a pyrrolidine analogue, which was recently disclosed by Tian and coworkers (28, 29). Compound **3** has a K_i of 76 nM at the MC_4 receptor, with 5-fold selectivity *versus* the MC_3 receptor.

From GPCR privileged structures to MC_4 receptor agonists

Potent and selective MC_4 receptor ligands were developed based on GPCR privileged structures (30). The so-called GPCR “privileged structures” are substructures that can generate high-affinity ligands for more than one type of GPCR (31). The spiroindanylsulfonamide (32), arylpiperazine (33, 34) and cyclohexylpiperazine (32, 35-38) scaffolds are examples of privileged structures that have been successfully used in MC_4 receptor ligands.

The close homology between the message sequence in the MC peptides and the active core of the growth hormone secretagogue peptide GHRP-6 enabled scientists at Merck to access a series of MC_4 receptor agonists, as represented by compounds **4-6** in Figure 2 (32, 35-38). Compound **4** (35) shows potent activity at the human MC_4 receptor, with an IC_{50} of 16 nM and an EC_{50} of 11 nM. At the human, rat and mouse MC_4 receptors, compound **4** is > 100-fold selective *versus* the MC_1, MC_3 and MC_5 receptor in binding affinity, and > 32-fold selective in functional potency (Table III). However, compound **4** has low oral bioavailability (F < 20%) in rats and shows extensive

Table III: Binding affinity and functional activity of MC_4 receptor agonists at human melanocortin receptor subtypes.

Compound	Binding affinity[a] (IC_{50} or K_i, nM)			Functional potency[b] (EC_{50}, nM)		
	MC_3	MC_4	MC_5	MC_3	MC_4	MC_5
1	280	7.7	NA	NA	4	NA
2	982	7.0	NA	NA	8.6	NA
3	382[c]	76[c]	NA	NA	142	NA
4	1600	16	2400	(11)[d]	11	1900
5	942	8	945	(8)[d]	11	850
6	120	0.37	64	(4)[d]	1.9	250
7	NA	25	NA	> 10,000	0.4	210
8	NA	20[c]	NA	NA	NA	NA

[a]IC_{50} or K_i values at human melanocortin receptors stably transfected in CHO or HEK-293 cells, using the radiolabeled ligand ^{125}I-NDP-α-MSH. [b]Concentration of compound at 50% maximum cAMP accumulation. [c]K_i values. [d]Percentage of cAMP accumulation relative to α-MSH at 10 μM compound concentration.

covalent binding in rat and human liver microsome stability studies (39).

Consequently, the isoquinuclidine derivative (**5**) (37) was prepared to address the bioactivation issue found in **4**. Indeed, the sterically hindered piperazine analogue (**5**) showed low bioactivation potential as measured by covalent binding in microsome preparations. Compound **5** is a selective human MC_4 receptor agonist, displaying 120-fold selectivity *versus* both the MC_3 and MC_5 receptors in binding affinity (see Table III). It has excellent activity at both the mouse and rat MC_4 receptors (IC_{50} < 10 nM). Also, compound **5** has better oral bioavailability in rats (F = 32%) than **4**.

The 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) is important for enhancing the binding to and efficacy at the MC_4 receptor (32-34). Attempts to modify the Tic group led to the 1-amino-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid analogue (**6**) (38). Optically pure compound **6** has an IC_{50} of 0.37 nM and an EC_{50} of 1.9 nM at the human MC_4 receptor and is over 170-fold selective *versus* the MC_1, MC_3 and MC_5 receptors in binding affinity, and > 130-fold selective in functional potency (Table III).

Other privileged structures have also been incorporated into MC_4 receptor agonists. For example, Fotsch *et al*. reported the arylsulfonamide **7** as a potent and selective MC_4 receptor agonist (33). Conversely, compound **7** is a weak agonist for the MC_3 and MC_5 receptors. Compound **7** also exhibits excellent potency at the mouse MC_4 receptor, with IC_{50} and EC_{50} values below 1 nM. Piperazine analogues with various substitutions at the aryl *ortho* position were also identified as MC_4 receptor agonists (40, 41). More recently, Fisher *et al*. reported the benzylic piperazine analogue **8** as an MC_4 receptor agonist (41). This compound, tested as a mixture of diastereomers, displays a K_i of 20 nM at the MC_4 receptor, indicating that substituted benzylic piperazines can also serve as privileged structures when coupled to the D-Phe-Tic moiety.

From agonists to antagonists

Structural modifications of MC_4 receptor agonists can lead to antagonists (42). Utilizing information derived from SAR studies of MC_4 receptor agonists and mutagenesis results, scientists at Neurocrine Biosciences developed a series of structurally diversified arylpiperazine analogues as MC_4 receptor antagonists (Fig. 3) (43-47). One example is the 2,4-dichlorophenylalanine derivative (**9**), which possesses a K_i value of 1.8 nM in the binding assay (Table IV). Schild analysis showed that **9** was a competitive functional antagonist, with a pA_2 value of 7.9 for inhibition of α-MSH-stimulated cAMP accumulation (43). Changing the β-alanine moiety in compound **9** to a carbamate group resulted in another functional antagonist (**10**), which displays low nanomolar binding affinity at the human MC_4 receptor but weak affinity at the human MC_3 receptor, as shown in Table IV (47).

Modification of the arylpiperazine portion in **9** leads to cyclohexylpiperazine analogues (**11** and **12**), which are functional antagonists at the MC_4 receptor (see Table IV) (48, 49). Compound **11** contains a *para*-methoxyphenyl acetamide side-chain and displays binding affinity of 4.2 nM at the MC_4 receptor (48). Compound **12** is an ester analogue and has K_i values of 6.3, 2800, 460 and 310 nM at the MC_4, MC_1, MC_3 and MC_5 receptors, respectively. This corresponds to > 50-fold selectivity for the MC_4 receptor *versus* all the other MC receptor subtypes. Compound **12**, however, has a low oral bioavailablity of 12% in rats (49).

Potential therapeutic uses for MC_4 receptor ligands

Obesity

Compelling evidence supports the notion that the MC_4 receptor regulates food intake and energy homeostasis, and that MC_4 receptor agonists reduce food intake and body weight in animals (50, 51). Human genetic studies showed that mutations in the MC_4 receptor gene are the most common monogenic form of obesity in human. For example, results from examination of 750 men with juvenile-onset obesity showed a 2.5% carrier frequency of pathogenic mutations in the MC_4 receptor gene (52). These mutations, absent in normal-weight individuals, are clear evidence that defects in the MC pathway are related to severe obesity (53).

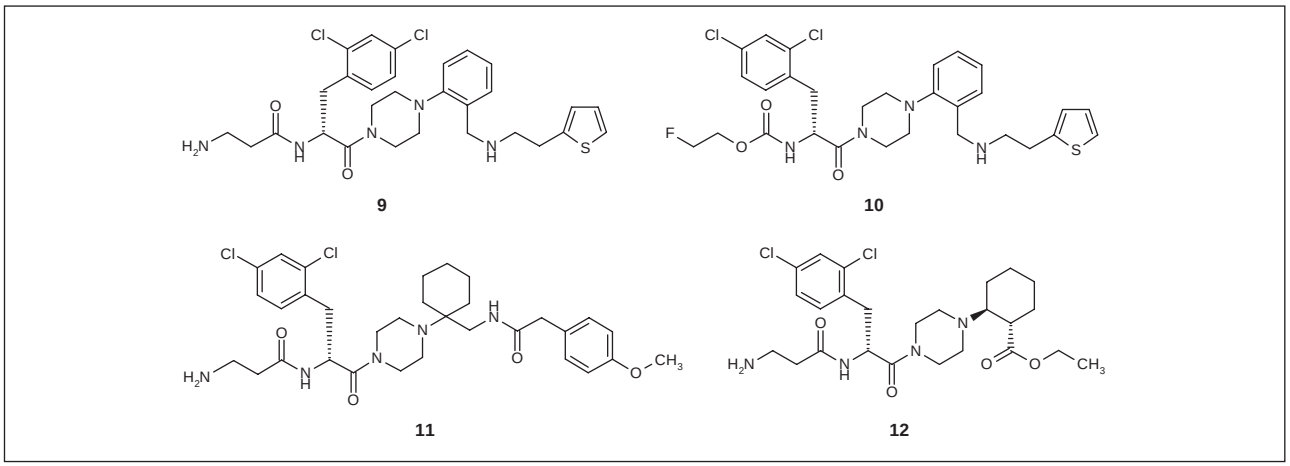


Fig. 3. Small-molecule MC_4 receptor antagonists.

Table IV: Binding affinity of MC_4 receptor antagonists at the human melanocortin receptor subtypes.

Compound	Binding affinity[a] (K_i, nM)			Compound	Binding affinity[a] (K_i, nM)		
	MC_3	MC_4	MC_5		MC_3	MC_4	MC_5
9	640	1.8	280	**11**	1100	4.2	540
10	790	3.2	560	**12**	460	6.3	310

[a]Binding affinity at human melanocortin receptors stably expressed in HEK-293 cells, using the radiolabeled ligand ^{125}I-NDP-α-MSH

The roles of the MC_4 receptor in obesity were also demonstrated in animals (54, 55). Mice that are engineered to be MC_4 receptor-deficient have a characteristic obese phenotype typified by hyperphagia, increased linear growth and metabolic defects (54). Recently, Hansen *et al*. examined the feeding responses of Sprague-Dawley (SD) rats to MTII and the selective MC_4 receptor antagonist HS-014 (55). Rats were fed a high-fat cafeteria diet (30% fat) or chow (5% fat) before the peptide was administered through an implanted intracerebroventricular (i.c.v.) cannula. Chronically overfed animals had a profound inhibitory feeding response after MTII injection and lost more body weight (15 g) compared to the control rats (4 g). Daytime administration of HS-014 significantly increased food intake in all rats to the same extent (55).

Newly developed small-molecule MC_4 receptor agonists, *i.e.*, compounds **4** and **5** (Fig. 2), were evaluated in a rat obesity model and a fasting-induced re-feeding paradigm (35, 37). When **4** was administered orally (20 mg/kg b.i.d.) to rats raised on an energy-dense diet, the body weight of the rats showed an overall decrease of –7 ± 5 g over 4 days. This is in contrast to the body weight changes observed in vehicle-treated rats (+15 ± 2 g) and in rats treated with dexfenfluramine (3 mg/kg b.i.d.), a known drug that reduces food intake (–16 ± 3 g) (35). Moreover, when **5** was dosed orally at 20 mg/kg to rats that were fasted for 18 h, a significant reduction in food intake was observed at 8- and 18-h time points as compared to vehicle-treated rats. In an overnight food intake study, oral administration of **5** resulted in a significant reduction in food intake in wild-type mice, but produced no effects in $MC_{3/4}$ receptor knockout mice (37).

In a recent publication, both the MC_3 and MC_4 receptors were implicated in the anorectic and metabolic responses to leptin, a hormone known to regulate human energy homeostasis (56). Leptin was administered to rats over a 3-day period by either the i.p. or the i.c.v. route. The absence of MC_4 receptors in rats hindered leptin's ability to increase the mRNA of UCP1 (uncoupling protein 1; a protein involved in nonshivering thermogenesis) in both brown and white adipose tissue, but not its ability to reduce food consumption. In contrast, the deletion of the MC_3 receptor compromised leptin's ability to reduce food intake, but did not have effects on adipose tissue. In both genotypes, modest increases in the fat content of the diet from 4% to 11% accentuated fat deposition and produced a rapid and comparable 10-12% increase in the percentage of body fat. These results indicate that the anorectic and metabolic responses to leptin are dependent on integrated but separable inputs from the MC_3 and MC_4 receptors (56).

These molecular and pharmacological results demonstrate that the central MC signaling pathway is a critical element in the control of mammalian energy balance. Accumulating evidence indicates that the MC_4 receptor is a vital player in the regulation of food intake. Undoubtedly, further understanding of the roles played by the neural MC_3 and MC_4 receptors will facilitate the development of MC receptor ligands to treat human obesity.

Cachexia

Cachexia (involuntary weight loss) is a wasting syndrome associated with many chronic diseases, including cancer, late-stage HIV infection, heart and liver failure. It

is believed to be a major contributor to morbidity and mortality in these chronic diseases. However, there are currently no effective treatments available for reversing the progressive loss of lean body mass in cachectic patients.

Evidence from animal models suggests that central MC signaling through the MC_4 receptor modulates cachexia and that MC_4 receptor antagonists attenuate the cachectic syndrome (57, 58). To demonstrate the involvement of the MC_4 receptor in cachexia, Cheung *et al*. established a uremia-induced cachexia model through either subtotal nephrectomy or sham operation (as controls) in wild-type and MC_4 receptor knockout mice (59). Nephrectomized wild-type mice showed a classic syndrome of cachexia characterized by decreased food intake, increased metabolic rate and loss of lean body mass. On the contrary, MC_4 receptor knockout mice resisted the cachectic effects of uremia on weight loss, metabolic rate and body composition. Treatment of nephrectomized wild-type mice with intracranial injection of AGRP reversed the cachectic syndrome (59).

Small-molecule MC_4 receptor antagonists increase food intake and prevent body weight loss in a tumor-induced model of cachexia (60, 61). Markison *et al*. recently evaluated their selective MC_4 receptor antagonist **12** (Fig. 3) in tumor-bearing cachectic mice (61). When administered i.p., compound **12** reversed the reduction in food intake in the cachectic mice as compared to vehicle. Compound **12** also protected the animals from loss of body weight and lean mass. The tumor size, on the other hand, was not affected by the drug. In addition, the basal metabolic rate, as measured by oxygen consumption, decreased in the drug-treated normal mice (61). Thus, MC_4 receptor antagonists represent an attractive therapeutic approach to ameliorate the cachectic syndrome in humans.

Sexual dysfunction

In the first clinical study designed to target the MC_1 receptor for skin tanning, the MC receptor agonist MTII showed the unexpected side effect of enhancing erectile activity (62). Palatin Technologies subsequently conducted clinical trials with PT-141 (the acid form of MTII) for male and female sexual dysfunction (63). Clinical data showed a statistically significant erectile response following intranasal or subcutaneous administration of PT-141 in healthy male subjects (64, 65). A statistically significant erectile response was also observed in patients with erectile dysfunction (ED) who were either responsive or unresponsive to sildenafil (65). However, the role of the MC_4 receptor in erectile function was not apparent from these clinical trials, since PT-141 is a dual neural $MC_{3/4}$ receptor agonist (64).

Evidence from animals administered selective MC_4 receptor agonists has shed light on the involvement of the MC_4 receptor in erectile function (35, 37). For example, the erectogenic activity of the selective MC_4 receptor agonists **4** and **5** was evaluated using an established rodent model. In the model, each rat served as its own control, where the erectogenic effect of the compound was compared to that produced by vehicle (66). When administered at a dose of 3 mg/kg i.v. to rats, compound **4** significantly increased the number of penile erections induced by electrical stimulation over a 15-min time period. Orally dosed **4** (5-40 mg/kg) also augmented the erectile activity in a dose-dependent manner (35). The selective MC_4 receptor agonist **5** enhanced the magnitude and duration of electrically stimulated erectile activity in mice when injected i.v. (1 mg/kg). Notably, administration of **5** in mice did not elicit erectile activity in the absence of electrical stimulation (37).

MTII has been shown to initiate erections in rodents and humans without sexual stimulation (63, 67, 68), in contrast to selective MC_4 receptor agonists such as compound **5** (37). This leads to the hypothesis that both the MC_3 and MC_4 receptors are necessary for complete proerectile actions (69). Additional evidence implying the involvement of the neural MC_3 receptor in enhancing erectile activity includes the finding that the selective MC_4 receptor antagonist HS-014 did not fully abolish α-MSH- and ACTH-induced penile erections (70), and HS-014 showed no influence on sexual behavior in male rats (71). Nonetheless, further investigations are necessary to fully elucidate the putative receptor subtypes, pathways and mechanisms implicated in mediating the proerectile effects of melanocortins (62, 69).

Neuropathic pain

In humans, damage to the nervous system can lead to a pain state referred to as neuropathic pain. This disorder remains one of the most difficult forms of pain to treat. The wide variety of drugs currently used in its treatment often do not provide adequate pain relief. Published data suggest that the MC_4 receptor plays a role in nociception and that MC receptor antagonists produce an antiallodynic effect in neuropathic rats (72). For example, the MC_4 receptor transcript is expressed in the spinal cord and dorsal root ganglia (DRG), which suggests its involvement in presynaptic regulation of nociceptive input (73). In rats with chronic constriction injury (CCI), MC_4 receptor mRNA in the DRG is downregulated, which parallels the antiallodynic or proallodynic efficacy of MC receptor ligands (74). In contrast, MC_3 receptor mRNA is found only in the spinal cord at low levels (75), and administration of a selective MC_3 receptor agonist does not elicit a nociceptive action (76). These data support the hypothesis that the MC_4 receptor rather than the MC_3 receptor modulates nociception.

The effects of MC receptor agonists and antagonists in CCI rats were also examined. Bertorelli *et al*. demonstrated that intrathecal injection of the antagonist AGRP or SHU-9119 to CCI rats produced a profound cold and mechanical antiallodynic effect (77, 78). On the other hand, administration of MTII and the more selective MC_4 receptor agonist D-Tyr-MTII increased the sensitivity to cold and mechanical stimulation in CCI rats. These

effects were observed in both acute and chronic administration paradigms (79).

Starowicz and colleagues recently showed that co-administration of SHU-9119 and morphine had an additive antiallodynic effect (80). A single intrathecal injection of SHU-9119 restored morphine analgesic potency in morphine-tolerant rats, and repeated administration of SHU-9119 prevented the development of morphine tolerance. Thus, administration of MC_4 receptor antagonists or combined treatment with opioids might provide a promising tool in the treatment of neuropathic pain (81).

Drug addiction

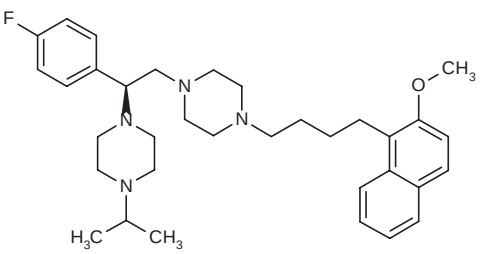
A growing body of literature suggests that the MC axis modulates neurobiological responses to drugs of abuse (82-85). Molecular studies with rodents showed that the MC_4 receptor and melanocortins are expressed in dopamine-rich regions such as the nucleus accumbens, where the rewarding actions of cocaine and other drugs of abuse are implicated (82). Chronic cocaine treatment resulted in a 2-3-fold induction of MC_4 receptor mRNA in the striatum and a smaller increase in the hypothalamus and hippocampus (83). These results suggest the involvement of the MC_4 receptor in modulating drug addiction.

MC receptor antagonists block the motivational effects of addictive drugs. The reward response (as measured by cocaine self-administration) and locomotor-activating effects of cocaine were used as behavioral models to assess the actions of cocaine (83). Intra-accumbens infusion of SHU-9119 to SD rats blocked the reinforcing, motivational and locomotor-sensitizing effects of cocaine. This is consistent with the observation that i.c.v. infusion of MTII to rats potentiated the acute threshold-lowering effect of amphetamine for lateral hypothalamic self-stimulation (84). Moreover, chronic i.c.v. infusion of MTII (12 days) increased the sensitivity to amphetamine during the postinfusion period (24 days) (85). Obviously, neural $MC_{3/4}$ receptor stimulation reinforces the rewarding effect of amphetamine.

Hsu *et al*. also reported that the locomotor-activating effects of cocaine were completely blocked in MC_4 receptor-null mutant mice and reduced in Agouti mice (animals that overexpress Agouti in the brain) (83). Moreover, intra-accumbens injection of α-MSH to cocaine-treated rats induced a significant increase in locomotor activity. Clearly, the rewarding and locomotor-activating effects of cocaine are critically dependent on the activity of the MC system in the nucleus accumbens. Thus, modulation of the rewarding effects through the MC system could be useful for the treatment of drug addiction.

Depression and anxiety

In the course of studying the MC system, excessive grooming behavior in rats was observed when the animals were given α-MSH and ACTH (70). Since grooming is one way rodents respond to stressful situations, it was postulated that the neural MC_4 receptor was involved in emotional stress and that MC_4 receptor antagonists might alleviate stress-induced symptoms such as depression and anxiety. Further evidence to support this assertion included: 1) the expression of MC_4 receptor mRNA in the amygdala and hypothalamus was significantly increased during electrical foot shock stress (86); 2) SHU-9119 attenuated the effect of MC_4 receptor agonist-induced grooming (87); 3) the selective MC_4 receptor antagonist HS-014 blocked immobilization stress-induced anorexia in rats (88); and 4) MTII dose-dependently and significantly reduced the time mice spent in social interaction, while administration of a selective, small-molecule MC_4 receptor antagonist, MCL-0129 (**13**), to mice for 1 week significantly and dose-dependently increased the time spent in social interaction, without affecting locomotor activity (89).



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Recently, Kokare *et al*. studied the interaction of neuropeptide Y (NPY), an endogenous neuropeptide known to modulate mood disorders, and α-MSH in the amygdala (90). The peptides were injected unilaterally into the right amygdala either together or separately. The authors found that rats spent less time in an elevated plus-maze when they were treated with α-MSH. Also, α-MSH reduced the anxiolytic-like effect of NPY. Co-administration of HS-014 and NPY at subeffective doses evoked synergistic anxiolysis in rats (90). Therefore, blockade of the MC_4 receptor may be useful for treating subjects with stress-related disorders such as depression and anxiety.

Conclusions

Molecular and pharmacological studies have shown that the MC_4 receptor is involved in the regulation of feeding behavior, energy homeostasis, sexual function, nociception, motivational effects of addictive drugs and stress responses. Results from preclinical studies illustrate the potential usefulness of MC_4 receptor ligands to treat obesity, cachexia, ED, neuropathic pain, drug addiction, depression and anxiety. The encouraging results from clinical trials with the agonist MTII for ED further underscore the possibility of modulating $MC_{3/4}$ receptor activity in humans for therapeutic use. Current evidence supports the hypothesis that the central MC system, which includes MC_3 and MC_4 receptors and their endogenous ligands, functions in a cooperative or integrative fashion to optimize its regulatory activities. Nonetheless, the puta-

tive receptor subtypes, pathways and mechanisms of the central MC system implicated in the above-mentioned disorders remain to be fully elucidated.

Tremendous progress has been made during the past decade in the discovery of small-molecule MC_4 receptor ligands, and in evaluating these compounds in animal models. Most of these ligands display excellent potency and selectivity at the MC_4 receptor and are effective in animal disease models. However, their pharmacokinetic properties, such as the ability to penetrate the brain and oral bioavailability, as well as metabolic stability, require further optimization for chronic oral administration. This is an important issue since prolonged drug administration is desirable for treating disorders such as obesity and depression. Once the required MC_4 receptor ligands are available, physiological responses through chronic modulation of the MC_4 receptor can then be fully evaluated, particularly in humans.

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