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Review Article

The MC_4 receptor as a therapeutic target

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CONTENTS

Abstract

Melanocortins, which are derived from pro-opiomelanocortin (POMC) by enzymatic processing, are involved in a wide range of physiological events. The melanocortins exert their effects by binding to melanocortin receptors. To date, five receptor subtypes, MC_1-MC_5, all of which are G-protein-coupled receptors, have been cloned. Of these, the MC_4 receptor, the expression of which is restricted to the central nervous system (CNS), is of interest in terms of the central regulation of feeding behavior and energy homeostasis. Recent findings on the distribution of the receptor in the brain and studies with selective agonists/antagonists have underscored its role in stress responses, the development of addiction, nociception and sexual function. The MC_4 receptor may therefore be an attractive target for the treatment of many CNS-related disorders, such as obesity, cachexia, depression/anxiety, drug addiction, pain and sexual dysfunction.

Introduction: the melanocortin system

Melanocortins, *i.e.,* adrenocorticotropic hormone (ACTH) and α-, β- and γ-melanocyte-stimulating hormone (α-, β- and γ-MSH), are derived from pro-opiomelanocortin (POMC) by enzymatic processing, and are involved in a wide range of physiological functions. The POMC gene is expressed primarily in the central nervous system (CNS) and the pituitary. In the brain, POMC cell bodies are predominantly found in the arcuate nucleus of the hypothalamus and in the nucleus of the solitary tract in the brain stem (1). Melanocortinergic terminals are found in various hypothalamic regions, such as the paraventricular nucleus (PVN), the dorsomedial hypothalamic nucleus and the lateral hypothalamic regions (2). In the PVN, the majority of immunoreactivity for α-MSH and ACTH is found in the parvocellular and magnocellular subdivisions (3), where corticotropin-releasing factor (CRF) and arginine-vasopressin (AVP) are synthesized. POMC mRNA is also detectable in the spinal cord and dorsal root ganglia (DRG) (4, 5). Likewise, ACTH and α-MSH immunoreactivity is detected in the dorsal horn of the spinal cord (4, 6). POMC mRNA and immunoreactivity have also been reported in a number of peripheral tissues, including the genitourinary tract, gastrointestinal tract, adrenal gland, spleen, lung and thyroid, and in cells of the immune system (7). POMC mRNA has further been detected in cutaneous keratinocytes and melanocytes (7).

The melanocortin system has two endogenous melanocortin receptor antagonists, agouti (8) and agouti gene-related protein (AGRP) (9). Agouti was described as a genetic locus controlling skin pigmentation (10), and is expressed primarily in peripheral tissues (11). AGRP was cloned on the basis of its homology with agouti (25% identity with human agouti) (9). AGRP has a very distinct distribution in the CNS, as it is expressed in neuronal cell bodies of the posterior hypothalamus in close vicinity to POMC-expressing neurons (12).

To date, five subtypes of melanocortin receptors (MC_1-MC_5) have been reported (13-17). These receptors belong to the G-protein-coupled receptor superfamily and are linked to cAMP generation via the stimulatory G-protein Gs and adenylate cyclase.

All MC receptors are activated by ACTH and all but the MC_2 receptor are activated by MSH. The MC_2 receptor differs pharmacologically from the other MC receptor subtypes in having no affinity for α-, β- or γ-MSH. α-MSH and ACTH exhibit higher affinity for MC_1, MC_4 and MC_5 receptors than β- and γ-MSH, while all of the melanocortins are roughly equipotent at MC_3 receptors. Agouti is a competitive antagonist at MC_1 and MC_4 receptors, but

does not bind to MC_3 and MC_5 receptors (8). In contrast, AGRP binds to and antagonizes MC_3 and MC_4 receptors (9), and also acts as an inverse agonist at MC_4 receptors (18).

The MC_1 receptor was first identified as the α-MSH receptor, and is expressed by cutaneous melanocytes, where it plays a key role in determining skin and hair pigmentation (13). It has also been reported to be expressed on macrophages and monocytes (7), and the pattern of expression of the MC_1 receptor appears to be related to the antiinflammatory actions of melanocortins. The MC_2 receptor was originally identified as the adrenocortical ACTH receptor. It is highly expressed in the zona reticularis and zona fasiculata of the cortex of the adrenal gland, where it mediates the effect of ACTH on steroid secretion (15). The MC_3 receptor is expressed in the brain and in several peripheral tissues, including the gastrointestinal tract and placenta (16), while the MC_4 receptor is expressed predominantly in the CNS (17). In the brain, MC_3 receptor mRNA has a quite restricted distribution, with the highest densities in the hypothalamus and limbic system (19). The MC_5 receptor was initially demonstrated in the brain with a very limited distribution. In subsequent studies, it was found to be expressed ubiquitously, being detected in numerous human peripheral tissues, including the adrenal gland, adipocytes, leukocytes and many others (20).

The MC_4 receptor

The human MC_4 receptor is a 332-amino-acid protein encoded by a single exon of 999 nucleotides (17). The rat homologous gene is 95% identical to the human gene (21). The human MC_4 receptor is structurally most similar to the MC_3 receptor, with which it exhibits 58% and 76% overall amino acid identity and similarity, respectively (17), and the rat MC_4 receptor is most similar to the MC_5 and MC_3 receptors (77.4% and 76.1% amino acid similarity, respectively) (21). The gene encoding the MC_4 receptor has been localized to chromosome 18 (q21.3) (17).

MC_4 receptor mRNA is much more widely expressed than MC_3 receptor mRNA in the brain, with multiple sites of expression including the cortex, thalamus, hypothalamus, brainstem and spinal cord (22, 23). In contrast, the MC_4 receptor was not detected in peripheral tissues in studies covering 20 human organs (24). The MC_4 receptor, unlike the MC_3 receptor, is found in both the parvocellular and magnocellular regions of the PVN of the hypothalamus, suggesting that it plays a role in the regulation of the activity of the hypothalamic-pituitary-adrenocortical (HPA) axis via AVP and CRF neurons (23). Furthermore, extensive to moderate labeling is found in the limbic system, *i.e.*, in several nuclei of the amygdala, including the central and basolateral nuclei, as well as the lateral septal nucleus, hippocampus and enthorhinal cortex (23). There are also overlaps between the expression of the MC_4 receptor and the monoaminergic systems. The MC_4 receptor is densely expressed in the caudate putamen, core and shell of the nucleus accumbens, as well as in the ventral tegmental area and substantia nigra. The MC_4 receptor is thus expressed in dopaminergic nuclei as well as in the main dopaminergic projection areas. MC_4 is abundantly expressed in both the spinal cord and DRG, while the MC_3 receptor is scarcely detectable in the spinal cord and is not present in the DRG (22, 23, 25).

MC_4 receptor agonists and antagonists

In the last couple of years, small-molecule MC_4 receptor agonists and antagonists, both peptidomimetic and nonpeptide compounds, have been identified, as shown in Figure 1, and the binding affinities of these compounds are summarized in Table I. Based on structural characteristics, the agonists can be classified into four groups: peptidomimetics containing a D-Tic-D-(*p*-Cl)-Phe (Tic: 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) core (compounds **1**, **2** and **3**), peptidomimetics containing a *cis*-1,4-diaminocyclohexane core (compound **4**), peptidomimetics containing a succinamide core (compound **5**) and nonpeptide thiadiazoles (compound **6**). The antagonists can be classified into three groups: nonpeptide compounds containing a piperazinylethylpiperazine core (compounds **7** and **8**), peptidomimetics containing a succinamide core (compound **9**) and nonpeptide compounds containing a phenyl-4,5-dihydro-1*H*-imidazole core (compound **10**), also based on structural characteristics.

Compound **1** was the first small-molecule MC_4 receptor agonist designed from a growth hormone secretagogue peptidomimic based on close homology between growth hormone secretagogue peptide (GHRP-6; Trp-Ala-D-Trp-His) and MC_4 (Trp-Arg-D-Phe-His) pharmacophores. Compound **1** exhibits selectivity and high affinity for MC_4 receptors and also displays selective functional activity at human and rat MC_4 receptors over human MC_1, human MC_3, human MC_5, rat MC_3 and rat MC_5 receptors, as determined by cAMP accumulation. Furthermore, compound **1** has acceptable pharmacokinetic profiles in rats and dogs, with a bioavailability of 14% and 16%, respectively (26).

Compound **2,** derived by coupling D-Tic-D-(*p*-Cl)-Phe with arylpiperazine, is an MC_4 receptor full agonist with an EC_{50} of 24 nM, and exhibits high selectivity for the MC_4 receptor over other MC receptors (27).

Compound **3** is also comprised of D-Tic-D-(*p*-Cl)-Phe and an arylpiperazine but exhibits low or moderate selectivity for the MC_4 receptor over MC_1, MC_3 and MC_5 receptors, in terms of both affinity and agonist activity. Compound **3** also has a good pharmacokinetic profile in rats, with an oral bioavailability of 30% (28).

Compound **4**, with a 1,4-cyclohexyldiamine moiety, was designed based on the MC receptor agonist Ac-Nle-cyclo[Asp-Pro-D-Phe-Arg-Trp-Lys]-NH_2, using distance restraints determined from ^{1}H-NMR spectroscopy. It exhibits high-affinity agonist activity at MC_4 receptors,

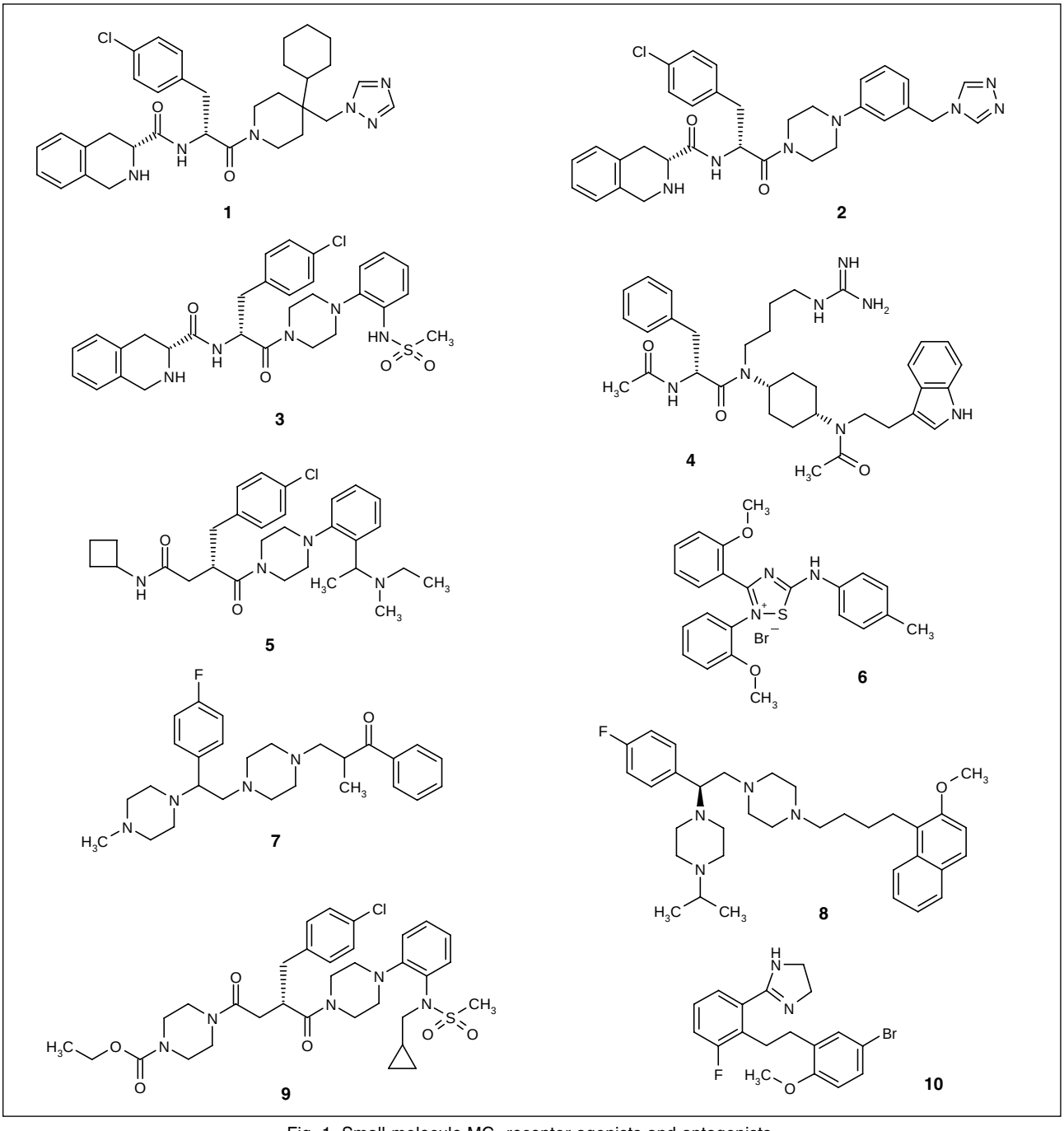

Fig. 1. Small-molecule MC_4 receptor agonists and antagonists.

but its selectivity for MC_4 receptors over MC_1 and MC_3 receptors is rather low (29).

Compound **5**, containing a succinamide core, was designed by replacing the CO-NH amide bound of D-Tic-D-(*p*-Cl)-Phe with a CO-CH_2 component. Compound **5** exhibits high affinity and selectivity for MC_4 receptors and also exerts potent agonist activity (30).

Compound **6** is a unique MC_4 receptor agonist with a 2,3-diaryl-5-anilino[1,2,4]thiadiazole skeleton, discovered using high-throughput screening (HTS). Compound **6** does not have a D-Phe moiety while other MC_4 receptor agonists (**1-5**) have D-(*p*-Cl)-Phe, D-Phe or its isostere in the center of each structure (31).

Compound **7**, which has a 1,2-bispiperazinylethane core, inhibits AGRP binding to the MC_4 receptor (IC_{50} = 52 nM) more potently than NDP-MSH binding (IC_{50} = 217 nM) (32).

Table I: Affinity, selectivity and functional activity of MC4R agonists and antagonists.

Agonists

	Binding Affinity[a] IC_{50} or K_i (nM)								
Compd.	hMC1R	hMC3R	hMC4R	hMC5R	mMC1R	rMC3R	rMC4R	rMC5R	Ref.
1	2067	761	1.2	326		1883	0.6	1575	1
2									2
3	12000[b]	8800[b]	220[b]	1200[b]					3
4		280	7.7		51				4
5		>10000	5.8		>600				5
6			4.4						6

Antagonists

	Binding Affinity[a] IC_{50} or K_i (nM)								
Compd.	hMC1R	hMC3R	hMC4R	hMC5R	mMC1R	rMC3R	rMC4R	rMC5R	Ref.
7			217						7
8	>10000[b]	>10000[b]	7.9[b]						8
9			1.4						5
10			160[b]						9

Agonists

	Agonist Potency[a] IC_{50} (nM)							
Compd.	hMC1R	hMC3R	hMC4R	hMC5R	rMC3R	rMC4R	rMC5R	Ref.
1	2850	2487	2.1	737	1325	2.9	>3000	1
2	>3000	>3000	24	>3000		38		2
3		2000	16					3
4			4					4
5			15					5
6								6

Antagonists

	Antagonist Potency[a] IC_{50} (nM)							
Compd.	hMC1R	hMC3R	hMC4R	hMC5R	rMC3R	rMC4R	rMC5R	Ref.
7								7
8								8
9								5
10			103					9

[a]IC_{50} or K_i values determined by radioligand binding assay using [^{125}I]-NDP-α-MSH and receptors expressed in CHO cells, in HEK 293 cells, in Bowes melanoma cells or in COS-1 cells [b]K_i values.

Compound **8** (MCL0129), which also has a 1,2-bispiperazinylethane core, exhibits high affinity for MC_4 receptors but no affinity for MC_1 and MC_3 receptors. It also exhibits antagonist activity, as determined by α-MSH-induced cAMP formation in MC_4 receptor-expressing cells. Furthermore, compound **8** has no apparent affinity for other receptors, transporters and ion channels at 1 μM, with the exception of moderate affinities for σ_1 receptors (IC_{50} = 69.8 nM), serotonin transporters (IC_{50} = 383 nM) and α_1-adrenoceptors (IC_{50} = 630 nM) (33).

Compound **9**, which has a succinamide core like the MC_4 receptor agonist **5**, exhibits high affinity for MC_4 receptors but no agonist activity at 10 μM, unlike compound **5** (30). This finding suggests that the substituents coupled at both terminals of succinamide play a role in determining MC_4 receptor-agonist or -antagonist activity.

Compound **10** (ML-00253764) was discovered by optimizing certain benzamidines. It exhibits moderate affinity and antagonist activity at MC_4 receptors and achieved AUC values of 29,900 nM/h and 8800 nM/h for

brain and plasma, respectively, after a dose of 30 mg/kg s.c. to mice (34).

Potential therapeutic indications for MC_4 receptor ligands

Obesity and eating disorders

Obesity is common in industrialized countries and the incidence has dramatically increased over the past 20 years worldwide. Obesity is a major risk factor for type 2 diabetes, hypertension, coronary artery disease and certain cancers. Obesity is attributable to an imbalance between energy intake and expenditure. Optimal treatment of obesity should therefore involve both suppression of food intake and increase in energy expenditure.

There are several lines of evidence indicating that the MC_4 receptor is implicated in feeding behavior and energy expenditure. Ac-cyclo[Nle-Asp-His-D-Phe-Arg-Trp-Lys]-NH_2 (MT II), a preferential MC_4 receptor agonist, potently inhibits food intake in four models of hyperphagia (fasted mice, *ob/ob* mice, A^y mice, neuropeptide Y-induced hyperphagia), while Ac-cyclo[Nle-Asp-His-D-Nal-Arg-Trp-Lys]-NH_2 (SHU-9119) increases nocturnal food intake (35). Targeted disruption of the MC_4 receptor causes maturity-onset obesity, with symptoms of hyperphagia, hyperinsulinoma and hyperglycemia, similar to those associated with the agouti obesity syndrome (36). It has been reported that highly selective peptidomimetic MC_4 receptor agonists inhibit food intake in rodents (37, 38). Conversely, Kask *et al.* (39, 40) reported that a series of selective MC_4 receptor antagonists stimulated food intake. Thus, MC_4 receptor stimulation potently decreases food intake in animals.

Leptin increases the expression of POMC mRNA in the arcuate nucleus (41). Leptin signaling in the arcuate nucleus appears to be directed to the PVN of the hypothalamus, since administration of leptin increases c-fos expression in that area, and this increase is blocked by SHU-9119 (42). Moreover, the selective MC_4 receptor antagonist Ac-cyclo[Cys-Glu-His-D-Nal-Arg-Trp-Gly-Cys]-Pro-Pro-Lys-Asp-NH_2 (HS-014) attenuates feeding inhibition and the loss of body weight induced by leptin in rats (40). Thus, it is suggested that POMC neurons originating in the arcuate nucleus contact MC_4 receptor-containing neurons synaptically, presumably in the PVN, and that MC_4 receptor signaling is downstream of the leptin signaling involved in feeding behavior.

The MC_4 receptor is also implicated in energy expenditure. Central administration of MT II increases uncoupling protein 1 (UCP1) gene expression in brown adipose tissue, which mediates increased sympathetic outflow (43). A significant increase in oxygen consumption is observed after MT II infusion (44).

Recent clinical studies in obese patients carrying MC_4 receptor variants have provided further evidence for the involvement of MC_4 receptors in the control of energy balance. It has been estimated that MC_4 receptor mutations are present in 4% of severely obese French individuals (45). Furthermore, many MC_4 receptor variants have been found in obese patients, including patients with binge eating (46, 47). Based on these findings, MC_4 receptor agonists are though to have potential utility in the treatment of obesity.

Anorexia nervosa is a life-threatening disorder primarily affecting adolescent women. It is a dramatic psychiatric syndrome accompanied by severe weight loss, hyperactivity and neuroendocrine changes. Currently, only ineffective and costly psychological and behavioral therapies exist for the treatment of this disorder. It has been reported that the selective MC_4 receptor antagonist HS-014 blocks immobilization stress-induced anorexia in rats (48), and we have recently reported that a newly synthesized peptidomimetic MC_4 receptor antagonist, Ac-D-2Nal-Arg-2Nal-NH_2 (MCL0020), prevents immobilization stress-induced reduction of food intake in rats without affecting basal food intake (49). The anorectic mutant (*anx/anx*) mouse is deficient in AGRP projections to hypothalamic centers associated with feeding and satiety (12), suggesting that deficient inhibitory input to MC_4 receptor activity may play a role in the development of anorexia. Moreover, certain single nucleotide polymorphisms in the human AGRP gene are found with higher frequency in anorexia nervosa patients than in controls (50), possibly due to defective suppression of the MC_4 receptor by variant AGRP. Thus, blockade of the MC_4 receptor may be a useful approach to the treatment of anorexia nervosa.

Cachexia

Cachexia is a common pathological syndrome associated with cancer and other chronic illnesses that encompasses both the loss of appetite and the inability to conserve energy (51). Ultimately, there is loss of fat and lean body mass, which is the hallmark of the disorder, contributing to morbidity, mortality and reduced quality of life in such patients. Malnutrition and loss of lean body mass compromise recovery by decreasing tolerance to therapy and increasing postsurgical complications. Existing drug treatment for cachexia has met with limited success, and this disorder of energy homeostasis is poorly understood. Previous studies have demonstrated that cytokines released during inflammation and malignancy act on the CNS to alter the release and function of certain neurotransmitters, thereby altering both appetite and metabolic rate (52, 53), and that proinflammatory cytokines may activate central melanocortin release (54) which may act via MC_4 receptors to produce inhibition of food intake.

Blockade of central MC_4 receptors by i.c.v. injection of AGRP prevents sarcoma-induced or lipopolysaccharide (LPS)-induced loss of lean body mass, and maintains normal circadian activity patterns during tumor growth (55). Likewise, MC_4 receptor-deficient mice are relatively resistant to sarcoma- or LPS-induced anorexia and weight loss, even with continued tumor progression (55). In contrast, MC_3 receptor knockout mice exhibit

illness-induced anorexia and weight loss with LPS and cytokine administration (56), suggesting that the MC_3 receptor may not play a role in the development of cachexia. Involvement of the MC_4 receptor is further supported by the recent finding that the selective MC_4 receptor antagonist MBP-10 (57) significantly reversed the anorexia induced by IL-1β (58). Moreover, SHU-9119 completely reverses anorexia in rats bearing prostate carcinoma, unlike either ghrelin or NPY (54), and ML-00253764, a newly synthesized nonpeptide MC_4 receptor antagonist, effectively reduces tumor-induced weight loss (34). These studies suggest that MC_4 receptor antagonists could improve the debilitating effects of cachexia in human diseases such as cancer, heart failure, Alzheimer's disease and AIDS.

Sexual dysfunction

Central administration of ACTH and α-MSH has been shown to elicit erectile activity in rodents (59). Moreover, a highly selective nonpeptide MC_4 receptor agonist augments erectile activity initiated by electrical stimulation of the cavernous nerve in wild-type but not MC_4 receptor-null mice, and copulatory behavior is enhanced by administration of a selective MC_4 receptor agonist (60), suggesting the involvement of the MC_4 receptor in the modulation of penile erectile function. MC_4 receptor mRNA was found to be expressed in tissues that modulate erectile function, including the spinal cord, hypothalamus and pelvic ganglion (major autonomic relay center to the penis) of rats, and nerve fibers and mechanoreceptors in the glans of the penis of both rats and humans (60). Thus, MC_4 receptor agonist modulation of erectile and sexual function may derive from both peripheral and central effects. In small, double-blind, placebo-controlled, crossover studies, Wessells *et al.* (61, 62) reported erectile activity upon administration of MT II to human subjects. Subcutaneous doses resulted in transient erections in 8 of 10 men with psychogenic erectile dysfunction and in 9 of 10 of those studied with organic dysfunction.

Depression and anxiety

Stress is known to play a pivotal role in mental disorders such as depression and anxiety, as both a causal factor and an outcome of disordered thought and disrupted interpersonal relationships, and hypothalamic neuropeptides have been considered attractive targets for the treatment of depression and anxiety in light of their role in stress responses (63). MC_4 receptor agonists induce grooming behavior in rats, a behavioral response of rodents to stressful situations, and SHU-9119 attenuates MC_4 receptor agonist-induced grooming, as well as novelty-induced grooming (64). Intracerebroventricular injection of ACTH increases plasma ACTH by stimulating MC4 receptors, while Lys-γ2-MSH (a selective MC_3 receptor agonist) has no effect on the HPA axis (65). Moreover, α-MSH injection into the PVN increases plasma ACTH and corticosterone levels, and α-MSH increases the release of CRF and AVP, both of which are potent stimulators of the HPA axis, from hypothalamic explants (66). MC_4 receptor mRNA is expressed in the parvocellular subdivision of the PVN, in which CRF and AVP neurons are predominantly located (22, 23, 67-69), and CRF neurons in the PVN are innervated by α-MSH neuronal terminals (70, 71). Thus, MC_4 receptors may mediate responses to stress. Of note, it has been reported that POMC mRNA levels in the arcuate nucleus are increased by restraint stress (72, 73), and that POMC and MC_4 receptor mRNA levels in the hypothalamus and amygdala are increased by foot shock stress (74).

Melanocortins have been reported to elicit anxiogenic-like effects and α-MSH and ACTH have been shown to inhibit punished responding in the Vogel conflict test in rats (75). We also reported that α-MSH, as well as MT II, dose-dependently and significantly reduced the number of licking periods in the rat Vogel conflict test (49). α-MSH reduced time spent in the open arms in the elevated plus-maze test (76). Injection of α-MSH into the ventromedial nucleus significantly increases aggressive behavior (76). ACTH increases isolation-induced distress vocalization in domestic chicks (77). Moreover, ACTH inhibits social contacts in the social interaction test in rats (78). Therefore, stimulation of brain MC receptors, presumably MC_4 receptors, appears to cause anxiety.

We have recently reported that newly synthesized MC_4 receptor antagonists (MCL0020, MCL0129) have anxiolytic and antidepressant effects in rodents (33, 49). MCL0020 and MCL0129 prevented swim stress-induced anxiety-like behavior (reduction in the time spent in the light area) in the mouse light/dark exploration test. Consistent with this observation, MCL0129 reversed swim stress-induced anxiety-like behavior (reduced time spent in the open arms) in the rat elevated plus-maze test. Under nonstressful conditions, MCL0129 also showed a significant anxiolytic-like effect in the mouse light/dark exploration test, although the anxiolytic-like effects of the MC_4 receptor antagonist were more pronounced in stressful than in nonstressful conditions, consistent with the hypothesis that the MC_4 receptor mediates stress responses. MCL0129, like selective serotonin reuptake inhibitors (SSRIs), suppressed marble-burying behavior, which is predictive of clinically relevant anti-impulsive properties, and this effect is of interest in view of the increasing utility of SSRIs in the treatment of subjects with obsessive-compulsive disorders (79). As for antidepressant-like effects, MCL0129 shortened immobility time in the forced swim test, and reduced escape deficit in the rat learned helplessness test upon acute administration. We reported that under the same conditions as used in the learned helplessness test, imipramine and fluvoxamine exhibited antidepressant-like effects only when administered subchronically for 8 days (80). These results suggest that MC_4 receptor antagonists may have antidepressant-like potential, with an earlier onset. Importantly, MCL0129 neither produced

severe sedation nor impaired motor coordination at doses much higher than pharmacologically active levels. This should represent an advantage, as many drugs that act on the CNS often have unwanted side effects such as sedation and impairment of motor coordination. Thus, MC_4 receptor antagonists may prove effective for treating subjects with stress-related disorders such as depression and/or anxiety.

Drug addiction

Drug addiction can be defined as the loss of control over drug use or the compulsive seeking and taking of a drug regardless of the consequences, and is increasingly recognized as a leading cause of death, morbidity and loss of productivity in the USA. There are some reports suggesting that MC_4 receptors play a role in addiction. For example, chronic morphine administration has been shown to result in a decrease in MC_4 receptor mRNA level in the nucleus accumbens and the periaqueductal gray, brain regions involved in reward and the reinforcing properties of drugs of abuse (21). The MC_4 receptor mRNA level is decreased by acute morphine administration in the amygdala, while it is gradually increased after chronic morphine administration (81). Moreover, intra-amygdalar injection of the MC_4 receptor antagonist SHU-9119 reverses morphine tolerance, suggesting that MC_4 receptors in the amygdala play an important role in morphine tolerance (81).

Intracerebroventricular injection of MT II augments the threshold-lowering effect of amphetamine for lateral hypothalamic self-stimulation, indicating that stimulation of the MC_4 receptor potentiates the rewarding effect of amphetamine (82). It was reported that chronic cocaine treatment increases the expression of MC_4 receptor mRNA in the striatum, and that administration of a melanocortin antagonist blocks the rewarding effect and hyperlocomotion induced by cocaine (83, 84). Consistent with alteration of MC_4 receptor level, cocaine treatment increases behavioral responses to a melanocortin agonist (83), indicating that brain MC_4 receptors mediate the behavioral effects of cocaine. Moreover, the MC_4 receptor antagonist Ac-cyclo[Cys-Gly-D-Nal-Arg-Trp-Cys]-NH_2 (HS-131) reduces dopamine release in the nucleus accumbens (85). Based on these findings, blockade of the MC_4 receptor has been proposed for the treatment of subjects with drug addiction.

Neuropathic pain

Neuropathic pain in man can occur following injury to the peripheral or central nervous system arising from multiple causes, including chemotherapy, traumatic injury and herpes zoster infection. These neuropathies may be persistent and are particularly problematic because they are often poorly managed by conventional opioid analgesics (86) and nonsteroidal antiinflammatory drugs (87). Central administration of melanocortins such as ACTH and α-MSH causes hyperalgesia in various pain tests (88, 89). Components of the melanocortin system, including POMC mRNA, immunoreactivity for ACTH and α-MSH and MC_4 receptor mRNA, have been demonstrated in the spinal cord and DRG (4-6, 23). Vrinten *et al.* (90) reported that chronic constriction injury (CCI) of the rat sciatic nerve, a lesion that produces neuropathic pain, results in an increase in [^{125}I]-NDP-α-MSH binding to the dorsal horn, and that MC_4 and POMC transcript levels are upregulated in the spinal cord of the CCI model (25). Furthermore, they demonstrated that intrathecal administration of SHU-9119 has a profound antiallodynic effect in the CCI model, while MT II and D-Tyr-MT II (a more selective MC_4 receptor agonist), but not Nle-γ-MSH (an MC_3 receptor agonist), increase sensitivity to mechanical and cold stimulation, suggesting that MC_4 receptors, but not MC_3 receptors, are implicated in neuropathic pain. It has also been reported that the MC_3 receptor mRNA level in the spinal cord is unaffected in the CCI model (25). Moreover, chronic administration of SHU-9119 in the cisterna magna produces profound cold and mechanical antiallodynic effects, while MT II increases mechanical allodynia (91). These findings suggest that antagonism of spinal MC_4 receptors may be a new approach to the treatment of neuropathic pain.

Conclusions

Since the cloning of MC_4 receptor cDNA in 1993, progress has been made in understanding the physiological roles of this receptor. In particular, since the finding of the involvement of the MC_4 receptor in feeding and energy homeostasis in 1997 using genetic animal models and selective agonists/antagonists, this receptor has become the focus of much attention, and many pharmaceutical companies are developing selective MC_4 receptor agonists and antagonists. It now appears that the MC_4 receptor is involved not only in feeding behavior and the regulation of body weight, but also sexual function, regulation of the activity of the HPA axis, emotional states, pain and drug addiction. Thus, the MC_4 receptor may serve as an attractive target for the treatment of diseases involving abnormalities of these functions. The most important forthcoming issue will be the development of selective small molecules and evaluation of these compounds in both preclinical and clinical studies, which should lead to a better understanding of the physiological functions of the MC_4 receptor and provide better drug therapy for diseases for which adequate medications are not available.

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