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The Melanin-Concentrating Hormone System and Its Physiological Functions

Yumiko Saito[1] (✉) · Hiroshi Nagasaki[2]

[1]Graduate School of Integrated Arts and Sciences, Hiroshima University, 739-8521 Hiroshima, Japan
yumist@hiroshima-u.ac.jp

[2]Department of Metabolic Medicine, Nagoya University, School of Medicine, 466-8550 Nagoya, Japan

Abstract Melanin-concentrating hormone (MCH) is a neuropeptide that was originally isolated from salmon pituitary where it causes pigment aggregation. MCH is also abundantly present in mammalian neurons and expressed in the lateral hypothalamus and zona incerta, brain regions that are known to be at the center of feeding behavior. MCH binds to and activates two G protein-coupled receptors, MCH1R and MCH2R. Although MCH2R is non-functional in rodents, genetic and pharmacological studies have demonstrated that rodent MCH1R is involved in the regulation of feeding behavior and energy balance. Unexpectedly, some antagonists have provided evidence that MCH signaling participates in the regulation of other processes, such as emotion and stress. The discovery of MCH receptors has extensively promoted the progress of MCH studies and may represent an ideal example of how deorphanized receptors can open new directions toward more detailed physiological studies.

Abbreviations

AGRP	Agouti-related peptide
NPY	Neuropeptide Y
POMC	Pro-opiomelanocortin
CART	Cocaine-amphetamine-regulated transcript

1 Introduction: MCH from Fish Scales

Melanin-concentrating hormone (MCH) was originally isolated from salmon pituitaries where it induces the aggregation of melanin granules in melanophores, thereby resulting in a pale skin color (Kawauchi et al. 1983). This effect is opposite to the pigment-dispersing effects of alpha-melanotropin (α-MSH) found in lower vertebrates. Similar to α-MSH, MCH was found to be a neurohypophysial hormone produced by neurons in the hypothalamus and released from the neurohypophysis of teleosts as a circulating factor. Fish MCH is a cyclic 17-amino acid peptide with a dicysteine bridge at positions 5 and 14 forming a ring structure. Rat MCH was purified from 60 000

hypothalamic fragments using antibodies directed against salmon MCH, and its primary structure was determined (Vaughan et al. 1989). Rat MCH consists of 19 amino acids, and is therefore two amino acids longer than its salmon counterpart (Fig. 1A). The ring structure, which is essential for the biological function in teleost fish, is highly conserved in rat MCH. Furthermore, MCH is identical at the amino acid level in all mammals analyzed to date, including mice, rats, rabbits, and humans. Rat MCH is prominently expressed in neurons in the lateral hypothalamus (LHA) and zona incerta, brain regions that are known to be involved in feeding behavior. Unlike teleost MCH-expressing neurons, mammalian MCH-expressing neurons do not extend abundantly to the neurohypophysis but project broadly throughout the central nervous system (CNS) from the olfactory bulb to the spinal cord (Bittencourt et al. 1992). This extensive terminal distribution suggests that the peptide may be involved in many brain functions by acting as a neurotransmitter/neuromodulator. The most active area of research on the MCH system has focused on its role in the regulation of food intake and energy homeostasis, while the characterization of MCH receptors and identification of small-molecule antagonists for MCH receptors has exclusively enhanced our

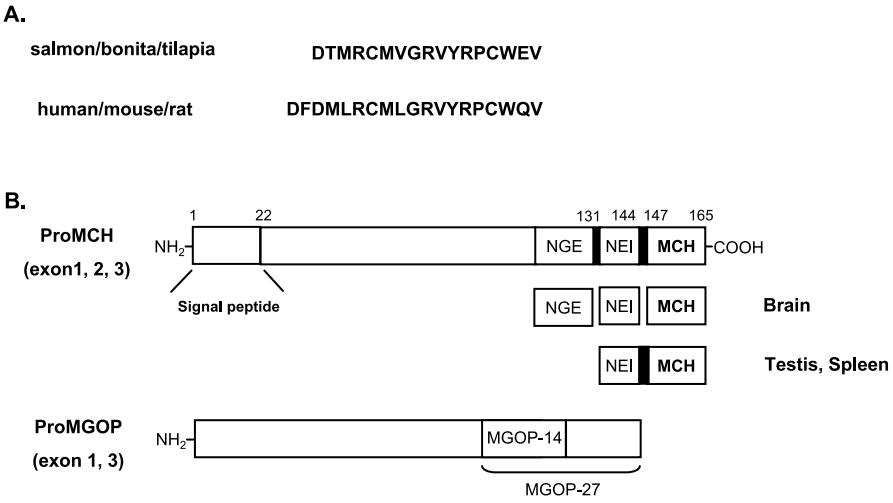

Fig. 1 Structure of MCH and its related peptides. **A** Alignment of rat, mouse and human MCH peptides with fish MCH. Fish MCH lacks two amino acids at the N-terminus compared to mammalian MCH. **B** Schematic diagram of the possible peptides derived from the MCH gene detected either in the brain or in other organs. The prepro-MCH precursor is composed of three exons and includes additional peptide sequences designated NGE and NEI (*above*). An alternative splicing variant, which contains exons I and II, encodes two putative peptides designated MGOP-14 and -27 (*below*). In the rat and human brain, mature peptides, cyclic MCH and amidated NEI, were found, while in mature MCH and NEI were not found in peripheral organs but a large MCH-immunoreactive form was identified in human and mouse

understanding of its pharmacology and physiology. This review will introduce recent data concerning genetic and physiological studies that have revealed how MCH and its receptors are involved in the regulation of signaling and various biological functions.

2
MCH Gene and Its Primary Functions in Mammals

A rodent MCH cDNA was first identified in 1989 (Nahon et al. 1989), and revealed that MCH is generated by cleavage at a dibasic amino acid site in the C-terminus of a 165-amino acid precursor. Subsequently, the rat, mouse, and human MCH mRNA sequences were found to show high degrees of homology, with 90% overall nucleotide identity. Analysis of rat mRNA indicated that the MCH transcript encodes a preprohormone containing other neuropeptides, designated neuropeptide E-I (NEI) and neuropeptide G-E (NGE) (Fig. 1B). NEI is indeed present with MCH in hypothalamic neurons, and has been proposed to affect grooming and locomotion (Sanchez et al. 1997) and be involved in regulating stress responses (Blue-Pajot et al. 1995) and suppressing thyrotropin-releasing hormone release (Kennedy et al. 2001). However, it remains unclear whether NGE is liberated from pro-MCH and exists as a functional peptide. Furthermore, an alternative splicing variant of the prepro-MCH mRNA encodes two other potentially bioactive peptides, designated MCH gene-overprinted peptide (MGOP)-14 and -17 (Fig. 1B). No processed MGOP peptides were detected in the rat hypothalamus by Western blot analyses, and MGOP mRNA expression was restricted to MCH-expressing neurons (Toumaniantz et al. 2000; Allaeys et al. 2004). In addition, a large MCH gene-related transcript, designated antisense RNA-overlapping MCH gene (AROM), has been isolated from PC12 rat pheochromocytoma cells (Borsu et al. 2000). AROM appears to be encoded by the opposite strand at the same locus as the MCH gene, and to generate multiple transcripts by alternative splicing. However, the coding sequence of these peptides does not overlap with the MCH cDNA. It is speculated that AROM may be crucial for RNA-binding or protein–protein interaction selectivity.

Humans have two related, but distinct, MCH gene systems involving authentic and variant MCH genes, although only a single MCH gene has been found in rodents. The authentic human MCH gene is mapped on chromosome 12q23, while the variant genes, PMCHL1 and PMCHL2, are localized on chromosomes 5p14 and 5q13, respectively. PMCHL2 does not yield an mRNA, whereas the sense unspliced RNA of PMCHL1 is transcribed in the developing human brain into an 8-kDa putative protein named VMCH-p8 (Viale et al. 2000). Although their putative functions are still puzzling, these human genes offer us the opportunity to examine the molecular mechanisms of gene re-

modelling and selection of functions in the human lineage (Courseaux and Nahon 2001).

MCH has been implicated in the regulation of several behaviors in rodents. Alpha-MSH increases auditory gating by depth recordings in the dorsal hippocampus, whereas MCH has the opposite effect. When MCH was administered prior to α-MSH, the ability of α-MSH to increase auditory gating was blocked (Miller et al. 1993). Regarding grooming, locomotor activity and rearing, MCH did not influence any of these behaviors, and had the opposite effects to α-MSH and NEI (Sanchez et al. 1997). MCH itself has been shown to modulate learning and memory processes. For example, infusion of MCH into the hippocampus, amygdala and entorhinal cortex increased the response latency in a one-trial step-down inhibitory avoidance test in rats (Monzon et al. 1999). Recently, MCH has been implicated in the control of the sleep-wake cycle, since intracerebroventricular (icv) administration of MCH induced a dose-dependent increase in rapid eye movement sleep and slow-wave sleep quantities (Verret et al. 2003). However, the central effect of MCH that has attracted the most attention is its involvement in the regulation of feeding behavior and energy homeostasis in mammals (Fig. 2). A substantial amount of literature involving genetic studies and administration of selective MCH receptor antagonists has been published and reviewed (Pissios and Maratos-Frier 2006; Handlon and Zhou 2006).

Acute icv injections of MCH transiently stimulated food intake in rats (Rossi et al. 1997), while chronic infusion of MCH into the lateral ventricle significantly increased the food intake, body weight, white adipose tissue mass, and liver mass in mice fed a moderately high-fat diet ad libitum (Qu et al. 1996; Della-Zuana et al. 2002; Ito et al. 2003). The observed reduction in brown adipose tissue functions and increased plasma glucose, insulin and leptin levels in these mice indicate that MCH-induced obesity is caused by not only hyperphagia but also regulation of metabolism (Ito et al. 2003). The relevance of the MCH system to the modulation of energy metabolism is also supported by studies on leptin-deficient obese (*ob/ob*) mice. Briefly, RT-PCR differential display analyses revealed that prepro-MCH mRNA was upregulated in *ob/ob* mice (Qu et al. 1996), while MCH mRNA expression was increased by three-fold in fasted *ob/ob* mice compared to four-fold in fasted wild-type mice. Further characterization via genetic approaches indicated the importance of the MCH system as a potential candidate in obesity treatment. MCH-knockout mice revealed an important physical role for MCH (Shimada et al. 1998), since these mice were 24–28% leaner than their control littermates as a result of hypophagia and exhibited a reduction in body fat and low circulating leptin levels. The MCH-knockout mice were resistant to obesity development obesity on a high-fat diet and consumed more oxygen (Kokkotou et al. 2005). In addition, the lean phenotypes of MCH-null mice persisted for up to 90 weeks due to both increased locomotor activity and a higher basal metabolic rate. Furthermore, these mice were resistant to aging-associated

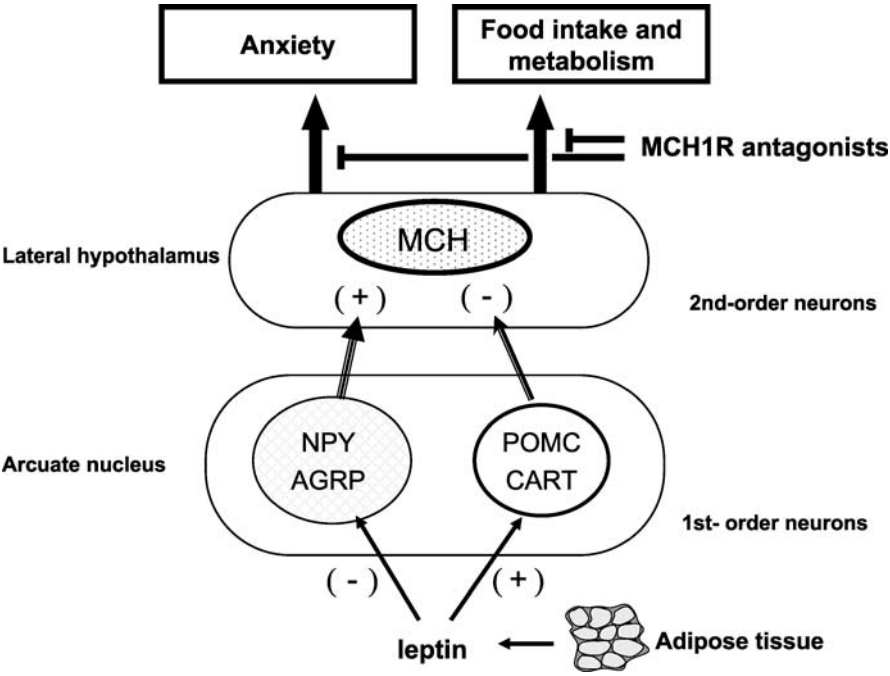

Fig. 2 Schematic representation of MCH circuitry in the hypothalamus. NPY/AGRP and POMC/CART neurons in the arcuate nucleus in the hypothalamus are the first-order neurons that responses to the circulating adiposity signal, leptin. Leptin activates anorectic POMC/CART neurons while inhibits orexigenic NPY/AGRP neurons. These neurons project to the lateral hypothalamus, center of the second-order neurons in the regulation of food intake and energy homeostasis. MCH neurons in the lateral hypothalamus are inhibited by the input from POMC/CART cells, whereas NPY/AGRP neurons exhibit the opposite effect. This anabolic pathway via MCH system can be disrupted by various MCH receptor antagonists. MCH system is also involved in HPA axis, regulating stress and anxiety. The anxiolytic effect of MCH is attenuated by MCH receptor antagonists

glucose intolerance (Jean et al. 2006). Very recently, a toxin-mediated genetic cell ablation strategy using a truncated ataxin-3 has been used to induce apoptosis of MCH-expressing neurons in vivo (Alon and Friedman 2006). MCH/ataxin-3 mice developed a late onset syndrome characterized by leanness, hypophagia and, in males, increased energy expenditure without any obvious changes in the gross histologic appearance of the hypothalamus. These phenotypes are remarkably similar to those of mice with induced mutations of the MCH gene, suggesting that MCH itself is a key molecule that regulates energy balance (Fig. 2).

In contrast to the absence of MCH, overexpression of the MCH gene leads to increased susceptibility to obesity. Transgenic mice overexpressing MCH in the LHA at approximately two-fold higher levels than normal mice were generated. On the original FVB background, the mice were not obese on

a standard diet. However, when the gene was bred to homozygosity, the resulting mice became obese on a high-fat diet. The mice were hyperphagic, hyperleptinemic and had higher blood glucose levels. Furthermore, the mice were also significantly hyperinsulinemic and failed to respond to an insulin challenge (Ludwig et al. 2001).

Leptin treatment can blunt the rapidly induced increases in MCH mRNA in both wild-type and *ob/ob* mice. This implies that the MCH system is targeted by leptin and required for the obesity observed with leptin deficiency. Furthermore, double null animals generated by crossing MCH-knockout mice with *ob/ob* mice revealed attenuated phenotypic manifestations of leptin deficiency (Segal-Lieberman et al. 2003). The marked reduction in weight in these double null mice was secondary to decreased total fat body fat rather than decrease food intake. These mice displayed increased locomotor activity and thermoregulation compared to *ob/ob* mice, but were more hyperphagic than *ob/ob* mice. These observations further indicate that the weight loss induced by the absence of MCH results from increased energy expenditure.

However, pharmacological approaches to MCH research have been hampered due to the lack of suitable selective antagonists for MCH receptors. The discovery of a relevant receptor for MCH in 1999 dramatically changed this situation and offered a new feature for understanding the more diverse physiological roles of MCH (see Sect. 4.2).

3
MCH Receptors and Receptor Signaling

3.1
Discovery of MCH Receptor Through Orphan Receptor Strategies

Since MCH was originally discovered on the basis of its regulation of skin melanocyte aggregation, initial efforts of identify MCH receptor were performed by binding assays using cell lines such as keratinocytes and melanoma cells (Drozdz et al. 1995). Although various cell lines were found to posses a specific binding site for an MCH analogue, [Phe^{13}, Tyr^{19}]-MCH, the pharmacological profiles and signaling associated with this binding rendered the existence of a functional MCH receptor in these cells questionable (Audinot et al. 2002). However, this aspect may deserve further investigation using other approaches (Eberle et al. 2004).

The first MCH receptor was identified by analyzing orphan G-protein coupled receptors (GPCRs), which are cloned GPCRs that recognize undiscovered natural ligands (Civelli et al. 2001). One of the orphan GPCRs, SLC-1, was originally discovered as an expressed sequence tag exhibiting about 40% homology in its hydrophobic domains to the five human somatostatin receptors (Kolakowski et al. 1996). A subsequently identified rat ortholog was

found to share 91% overall sequence identity to the human SLC-1 receptor, and be 49 amino acids shorter in its N-terminal segment (Lakaye et al. 1998). The existence of a shorter form was later reported in humans (Mori et al. 2001). In 1999, five independent groups, including ours, almost simultaneously reported the identity of the cognate ligand of SLC-1 using orphan receptor strategies (Table 1). Three groups used brain extracts as the starting material and monitored SLC-1 activity via three different second messenger responses, namely increases in intracellular-free Ca^{2+} levels with a chimeric Gα protein in transiently transfected CHO cells (Conklin et al. 1983; Saito et al. 1999), cyclic AMP inhibition assays in stable CHO cells (Shimomura et al. 1999) and G protein-gated potassium channels in *Xenopus* oocytes (Bachner et al. 1999). Two other groups screened large libraries of known bioactive substances as potential activators of SLC-1 (Chambers et al. 1999; Lembo et al. 1999), and monitored SLC-1 reactivity by measuring the intracellular-free Ca^{2+} levels. Finally, each group arrived at the same conclusion, namely that the cognate ligand for SLC-1 was the known peptide MCH. Following this deorphanization, the MCH peptide could be studied from the aspect of the MCH-MCH receptor system. The SLC-1 receptor is hereafter referred to as the MCH-1 receptor, MCH1R.

The highest expression of MCH1R is detected in the brain where high levels of its mRNA expression are observed in most anatomical areas implicated in the control of olfaction, such as the olfactory nerve layer, olfactory

Table 1 Characterization of MCH1R. Orphan receptor strategies have been successful in identifying MCH as the cognate ligand for the orphan GPCR SLC-1. SLC-1 is referred to as MCH1R in this review

Cell system	Transfected cDNA	Assay system	Source	Purification steps	Refs. (1999)
HEK (stable)	Human SLC-1	Calcium influx	Compound library (over 500)		Chamber et al.
HEK (stable)	Rat SLC-1	Calcium influx	Compound library		Lembo et al.
CHO (transient)	Rat SLC-1 + Gq/i3 chimera (1 : 1)	Calcium influx	Rat brain extract (whole)	400 g 6 steps	Saito et al.
CHO (stable)	Human SLC-1	Inhibition of cyclic AMP accumulation	Rat brain extract (whole)	70 brains 6 steps	Shimomura et al.
Xenopus oocytes	Rat SLC-1 + GIRK	GIRK-mediated current	Rat brain extract (whole)	67 g 7 steps	Bachner et al.

GIRK: G-protein-gated inwardly rectifying potassium channel

nucleus and tubercle (Hervieu et al. 2000; Saito et al. 2001a). Strong labeling is also detected in the hippocampal formation, subiculum, basolateral amygdala and nucleus accumbens shell, which are substrates for learning, memory, addiction and motivated behavior. Moderate MCH1R mRNA expression is particularly found in regions that are involved in the neuronal circuitry of feeding, such as the arcuate nucleus, ventromedial hypothalamic nucleus and ZI. These localizations imply a role for the MCH system in the integration of taste and olfaction, as well as in positive reward aspects of feeding and satiety (Saito et al. 2001a).

Studies of MCH1R-deficient mice have provided additional evidence that the MCH system is involved in the regulation of metabolism and activity levels. These mice were lean with decreased fat mass and increased energy metabolism (Marsh et al. 2002; Chen et al. 2002). Consistent with their hyperactive phenotype, the mice showed increased resistance to diet-induced obesity. MCH1R-deficient mice were also resistant to the orexigenic actions of MCH, demonstrating that MCH1R is a physiologically relevant MCH receptor. It has been reported that the hyperactivity of MCH1R-deficient mice may be mediated by the mesolimbic dopamine system (Smith et al. 2005). These mice were also hyper-responsive to dopamine stimulation and showed significant upregulation of dopamine D1 and D2 receptors in the nucleus accumbens shell, olfactory tubercle and ventral tegmental area. Since mesolimbic dopamine signaling has been suggested to underlie the reward system stimuli, MCH signaling may have a role in reinforcement in addition to energy homeostasis.

A second high-affinity receptor for MCH was characterized based on its low homology to human MCH1R (Mori et al. 2001; Sailer et al. 2001; Rodriguez et al. 2001). This receptor, referred to as MCH2R in this review, is positively coupled to the Gαq signaling pathway (Sailer et al. 2001; Rodriguez et al. 2001), while MCH1R is coupled to Gαi, Gαo and Gαq. Notably, MCH2R was found to be a pseudogene in rodent species, but is functional in dogs, ferrets, rhesus monkeys, and humans (Tan et al. 2002). The distribution of MCH2R in brain nearly overlaps with that of MCH1R, but the latter shows much higher relative levels and a wider distribution pattern (Mori et al. 2001). MCH2R is expressed in several human brain areas, including the hippocampus and amygdala, although its distribution in the hypothalamus remains controversial. Specifically, it was reported to be mainly expressed in the arcuate nucleus and ventromedial hypothalamic nucleus in African green monkeys by in situ hybridization (Sailer et al. 2001), while three other reports did not detect its expression in the human hypothalamus by RT-PCR (Mori et al. 2001; Hill et al. 2001) or Northern blot analysis (Rodriguez et al. 2001). The functional importance of MCH2R in obesity remains unknown due to the lack of available animal models. Interestingly, three MCH receptor sequences from zebrafish and two receptor sequences from fugu have been identified in whole genome shotgun datasets (Logan et al. 2003). Zebrafish and fugu have

clear MCH1R and MCH2R orthologues. Phylogenetic analyses of these receptors have suggested that an initial duplication of the MCH receptor occurred early in evolution, giving rise to MCH1R and MCH2R. Further characterization of fish MCH receptors may provide further insights into MCH functions in fish, rodents, and humans.

3.2 Characterization of the MCH1R-Signaling Pathway

In MCH1R-overexpressing CHO or HEK293T cells, the receptor was found to couple with various second messenger systems, including elevation of intracellular Ca^{2+} levels, inhibition of forskolin-stimulated cyclic AMP production and activation of extracellular-signal-regulated kinase 1/2 (ERK1/2) (Chambers et al. 1999; Saito et al. 1999; Lembo et al. 1999; Hawes et al. 2000). The observed EC_{50} values for cyclic AMP inhibition and calcium influx suggested that the coupling to Gαi was stronger than that to Gαq in an exogenous receptor-expression system. A number of mutations have been identified in MCH1R that affect its activity, including its signaling. MCH1R contains three consensus *N*-glycosylation sites and several potential phosphorylation sites in its intracellular loops. Biochemical analyses have shown that an aspartic acid residue (Asp^{123}) in the third transmembrane domain is crucial for ligand binding (MacDonald et al. 2000) and that an asparagine residue (Asn^{23}) in the extracellular N-terminal region is the most important site for *N*-linked glycosylation of MCH1R and cell surface expression (Saito et al. 2003). Thr^{255}, which is located at the junction of intracellular loop 3 and transmembrane domain 6, is also necessary for cell surface expression. A single point mutation, T255A, dramatically reduced the cell surface expression of MCH1R, and resulted in the receptor being retained in the endoplasmic reticulum (Fan et al. 2005). Arg^{155} in the second intracellular loop of MCH1R also has a critical role, since mutation of this basic residue to glutamine or lysine produced 75- and 50-fold higher EC_{50} values for elevation of the intracellular Ca^{2+} levels (Saito et al. 2005). The membrane proximal region of MCH1R is predicted to form an amphiphilic cytoplasmic helix, and two dibasic amino acids (Arg^{319} and Lys^{320}) in this helix are also important for receptor signaling (Tetsuka et al. 2004). On the other hand, the distal portion of the C-tail is necessary for the receptor internalization process (Saito et al. 2004).

The actin- and intermediate filament-binding protein periplakin appears to be coexpressed with MCH1R in the mouse brain and may interact with the intracellular C-terminal of MCH1R to impede MCH1R-initiated signal transduction (Murdoch et al. 2005). Calcium mobilization is inhibited by periplakin, although ERK1/2 phosphorylation is induced normally. Recently, the neurite outgrowth-related factor neurochondrin was identified to interact with the C-terminus of MCH1R (Francker et al. 2006). Neurochondrin interacts with the proximal C-terminus of the receptor and inhibits MCH-

induced signal transduction in a similar manner to periplakin. The physiological significance of these interactions with periplakin and neurochondrin is presently unknown.

Although exogenous receptor-expression cellular systems have provided useful information regarding the function and pharmacology of GPCRs, it is still possible that such systems do not reflect the physiological situation in intact cells. In fact, endogenous MCH1R in human melanoma SK-MEL37 cells and neuroblastoma Kelly cells is associated with a signaling pathway that inhibits forskolin-induced cyclic AMP production and induces ERK1/2 activation in a pertussis toxin (PTX)-sensitive manner, but not a calcium influx (Saito et al. 2001b; Schlumberger et al. 2002). An MCH-signaling pathway that activates ERK1/2 and pp70 S6 kinase is also present on 3T3-L1 adipocytes expressing endogenous MCH1R (Bradley et al. 2002). Treatment of 3T3-L1 adipocytes with MCH acutely downregulates MCH1R, indicating a mechanism for ligand-induced receptor downregulation.

Since both MCH-expressing neurons and MCH receptors are found in the LHA, the cellular actions of MCH-expressing neurons in the LHA have been examined using whole-cell recording in current and voltage clamps (Gao and Van Den Pol 2001, 2002). MCH was found to play a dramatic inhibitory role in the regulation of glutamatergic and GABAergic synaptic transmission in LHA neurons, and this effect is based on a reduction of voltage-dependent calcium currents via PTX-sensitive G-protein pathways, probably the Gαi/o pathway. MCH attenuates L-, N- and P/Q-type calcium channels, with the greatest inhibition found for N-type currents. MCH actions in LHA neurons differ from those in non-neuronal cells that express exogenous MCH1R, since the non-neuronal cells show an MCH-mediated increase in calcium, while the reverse occurs in neurons. Previous reports have also suggested that MCH activates G protein-coupled inwardly rectifying potassium channels in non-neuronal cells (Bächner et al. 1999), but no effects of MCH on voltage-dependent potassium channels were observed in LHA neurons. In the hippocampus, where MCH1R mRNA is highly expressed and MCH fibers are projected, exogenously applied MCH lowers the long-term potentiation thresholds by increasing hippocampal synaptic transmission through an *N*-methyl D-aspartate receptor-dependent pathway (Varas et al. 2003). Although the ventral tegmental area receives dense projections from the LHA, MCH does not affect the firing of dopaminergic or fast-firing GABAergic cells in the area (Korotkova et al. 2003). Further electrophysiological characterization is necessary in other regions that express high levels of MCH1R, such as the nucleus accumbens shell or amygdala.

4
Effects of MCH1R Antagonism on Physiological Responses

4.1
Efficacy of Feeding Behavior and Energy Balance

The strong association of the MCH-MCH1R system with obesity has accelerated the development MCH1R agonist/antagonists and their use in behavioral studies. The effects of more than 50 MCH analogues on MCH1R-expressing cells have been investigated and extensive structure-activity relationships have been clarified (Audinot et al. 2001). Acute central administration of these MCH analogues led to a rapid and significant increase in food intake with a potency that was correlated with the affinity of the agonist for MCH1R (Suply et al. 2001). This study clearly indicated that MCH1R is the mediator of the orexigenic effects of MCH. Furthermore, chronic icv infusion of synthetic MCH1R agonists induced obesity in rodents (Della-Zuana et al. 2002; Ito et al. 2003), and their weight gain was accompanied by hyperphagia, a reduced core temperature, and stimulated lipogenic activity in the liver and white adipose tissue. These observations again suggest that MCH plays an essential role in the development of obesity by modulating energy homeostasis.

T-226296 was the first reported non-peptide MCH1R-selective antagonist. This orally active antagonist effectively blocked the food intake stimulated by icv administration of MCH in rats (Takekawa et al. 2002). T-226296 was reported to suppress spontaneous food intake in diet-induced obese rats by selectively decreasing the sizes of the meals consumed rather than by a generalized behavioral malaise (Kowalski et al. 2004). A second non-peptide antagonist, SNAP7941, has provided the first evidence that chronic oral administration of an MCH1R antagonist can effect sustained reductions in body weight (26% weight loss relative to vehicle-treated rat and food intake, that were greater than the effects elicited by D-fenfluamine, an effective anorectic agent (Borowsly et al. 2002). The third reported antagonist was an MCH-modified peptide, designated compound B, and its chronic icv administration to rats resulted in reductions in appetite, caloric efficiency, body weight gain and body fat gain without any effect on lean mass (Shearman et al. 2003). These findings are consistent with the sustained feeding and body weight effects of SNAP7941 in diet-induced obese rats. Although chronic compound B treatment significantly attenuated body weight in wild-type mice, no effects were seen in MCH1R-knockout mice, indicating that compound B specifically acts by interacting with MCH1R (Georgescu et al. 2005). Moreover, other small-molecule antagonists of MCH1R exhibited efficacy in animal feeding and weight loss in chronic rodent models with no toxicity or adverse behavioral effects (Handlon and Zhou 2006). These consistent findings all support the proposal that MCH1R antagonists will provide promising target strategies for obesity treatment (Fig. 2).

4.2 Efficacy in Anxiety, Depression, and Stress

Since MCH1R is localized in several limbic areas and the nucleus accumbens shell, an area involved in the regulation of emotion, stress, motivation and reward (Hervieu et al. 2000; Saito et al. 2001b), the MCH system appears to be important for the regulation of stress and anxiety-related responses in addition to the crucial roles of MCH in feeding behavior.

The administration of MCH into the medial preoptic area induced anxiety in female rats (Gonzalez et al. 1996), while injection of MCH into the nucleus accumbens shell increased depressive behavior (Georgescu et al. 2005). Regarding its role in stress, the direct injection of MCH into the paraventricular nucleus increased the plasma adrenocorticotropic hormone (ACTH) level (Kennedy et al. 2003). MCH also induced corticotropin-releasing factor (CRF) release from hypothalamic explants, an effect that was sensitive to blockade by an MCH1R antagonist (Kennedy et al. 2003), while increases in plasma ACTH following icv injection of MCH were prevented by an anti-CRF antibody (Jezova et al. 1992). Thus, stimulation of MCH1R seems to cause activation of the hypothalamus-pituitary-adrenal (HPA) axis through increases in CRF excretion. On the other hand, several contrasting studies have been reported. Briefly, icv, intra-amygdaline or intra-hippocampal MCH administration was reported to exert dose-response anxiolytic effects (Monzon et al. 2001) consistent with experiments showing anti-anxiety properties for MCH in a test called Vogel's punished drinking test (Kela et al. 2003). However, another study reported that exogenous MCH induced a moderate decrease in ACTH secretion under resting conditions when injected during the light phase (Bluet-Pajet et al. 1995). These divergent results regarding the role of MCH may be attributed to its wide circadian variation or negative feedback in the basal HPA axis. It is also likely that the different routes of MCH administration and/or the various rodent models used to score the behavior may produce such differences in the function of MCH in the regulation of anxiety.

The most recent studies using genetic and pharmacological approaches have provided support for the anxiogenic effects of the MCH-MCH1R system (Fig. 2). For example, chronic administration of the MCH1R antagonist SNAP7941 showed efficacy for reducing anxiety, and mimicked antidepressant effects in modified forced swim tests (Borowsly et al. 2002). Other non-peptide MCH1R antagonists, ATC0065 and ATC0175, were synthesized and their oral administration produced anxiolytic and antidepressant activities in a series of behavioral models (Chaki et al. 2005). Similarly, the MCH1R antagonist GW3430 produced anxiolytic-like effects in animal models of anxiety (Smith et al. 2006). Furthermore, direct delivery of the MCH1R peptide antagonist compound B to the nucleus accumbens shell blocked feeding and further produced an antidepressant-like effect in forced swim tests, while

injection of MCH into the nucleus accumbens shell increased depressive behavior as described above (Georgescu et al. 2005). Given these reports, the MCH-MCH1R system is involved in not only regulation of feeding and energy balance but also regulation of mood and emotion via the hypothalamic-nucleus accumbens neural association. Characterization of the phenotypes of MCH1R-deficient mice revealed anxiolytic-like behavior when tested by a number of behavioral paradigms commonly used to assess fear and anxiety responses in rodents (Smith et al. 2006; Roy et al. 2006), and further revealed antidepressant-like behavior in female mice, but not male mice (Roy et al. 2007). It is noteworthy that MCH1R-selective antagonists had anxiolytic-like effects in wild-type mice, but not in MCH1R-deficient mice (Smith et al. 2006).

Overall, although a consistent link between the MCH-MCH1R system and mood has not yet been established, the effects of MCH1R antagonists in animal models suggest that these compounds deserve further investigation as potential etiologic treatments for affective disorders.

5
Peripheral Roles of the MCH–MCH Receptor System

MCH was initially isolated as a pituitary peptide in teleost fish in which the activity of the hormone decreased skin pigmentation (Kawauchi et al. 1982). Although the expressions of both MCH and MCH1R have been identified in human melanocytes and melanoma cell lines (Saito et al. 2001b; Hoogduijin et al. 2002), their physiological roles in the skin have not yet been fully elucidated. Pathologically, MCH1R on melanocytes was reported to be one of the targets of autoantibody responses in vitiligo, which is a common depigmentation disorder resulting from the loss of melanocytes in the skin (Kemp et al. 2002).

In the process of evolution from fish to mammals, it is likely that MCH and its counteracting hormone MSH have changed their primary functions from melanocyte regulation to energy metabolism. In the periphery, MCH or MCH1R expression in some of the digestive systems or adipose tissues is associated with energy and lipid metabolism. Hervieu and coworkers detected MCH immunoreactivity and mRNA expression in the lamina propria of the duodenum and colon in both humans and rats (Hervieu et al. 1996). The end product of the MCH gene in the digestive tract is an immature form of the MCH precursor, NEI-MCH, which consists of the 17-amino acid NEI attached to the N-terminal of MCH (Fig. 1B). Feeding was more potently boosted by icv administration of NEI-MCH than MCH, and this effect may arise via reduced susceptibility to proteases (Maulon-Feraille et al. 2002). The authors of the latter study also suggested the possibility that NEI-MCH may act as superagonist in vivo.

Since both white and brown adipose tissues express MCH1R, it is also possible that adipose tissues are directly regulated by MCH in the circulation. In mouse 3T3-L1 adipocytes, MCH induced rapid and transient increases in ERK1/2 and pp70 S6 kinase, which activated the transcriptional activity of leptin (Bradley et al. 2002). MCH may not directly regulate triglyceride metabolism in adipocytes, since it had no effect on lipogenesis or lipolysis in 3T3-L1 adipocytes and primary cultures of murine white adipose tissue (Bradley et al. 2002). Combined with the fact that central administration of MCH increased fat mass (Ito et al. 2003), it is anticipated that humoral or neuronal factors mediate MCH-expressing neurons in the CNS and adipose tissues.

In contrast to the possible endocrine actions in adipose tissues, autocrine cells expressing both MCH and MCH1R were recently found in vagus nerve system (Burdyga et al. 2006) and pancreatic islet (Pissios et al. 2007), both associate with energy metabolism. In the nodose ganglion of vagus neurons, which transmit chemical and physical inputs from digestive systems to the CNS, 10% of the neural soma coexpressed MCH and MCH1R. MCH and MCH1R are simultaneously increased by fasting, and then both decreased upon refeeding. Interestingly, cholecystokinin (CCK) is responsible for the suppression of the MCH system in MCH-expressing autocrine neurons through CCK1R. A further important fact is that the anorectic CART peptide is colocalized with MCH in MCH-expressing neurons. CCK reciprocally regulates the expressions of CART and MCH, and the orexigenic ghrelin counteracted CCK (Lartige et al. 2007). These findings suggest that modulation of gut-brain signaling is involved in the control of food intake.

The autocrine system of MCH and MCH1R is also found in beta cells in both human and mouse pancreatic islets. Genetic interventions of the MCH gene modify the size of the pancreatic islets, since mice overexpressing MCH exhibited islet hyperplasia (Shimada et al. 1998; Ludwig et al. 2001), while MCH-knockout mice had a significantly reduced beta cell mass (Pissios et al. 2007). MCH also increased insulin secretion, and altered the expressions of islet-enriched genes, such as glucagon, forkhead homeobox A2, hepatocyte nuclear factor (HNF) 4 and HNF1. These data illustrate that the autocrine system of MCH partially regulates beta-cell mass dynamics and islet secretory functions.

MCH immunoreactivity is also measurable in the human circulation. Plasma MCH levels were positively correlated with fat mass and increased by 25% after fasting (Gavrilla et al. 2004), consistent with the orexigenic and fat-increasing nature of MCH. However, many issues regarding circulating MCH, including its processing, source and dynamics, remain unsolved.

Taken together, the MCH system may play significant roles in peripheral tissues and be involved in energy metabolism. Studies of the MCH system in peripheral tissues will provide important findings for lipid and glucose metabolism that may provide direct links to the clinical implications of drugs targeting MCH receptors.

6 Conclusions

In recent years, obesity therapy has become a major focus of pharmaceutical research. The worldwide market for obesity therapeutics has increased dramatically over the past decade, and obesity has been linked with numerous risk factors. Combined with the knowledge that the hypothalamic area is one of the critical sites for the control of energy expenditure, the discovery of the different roles of MCH has attracted the interest of many research groups. In conjunction with energy balance, recent progress has suggested that the MCH-MCH1R system is involved in the regulation of certain types of complex behavior, such as stress, anxiety, and depression. Since many research groups have MCH receptor antagonist programs, it is likely that several compounds will succeed in advancing highly selective antagonists with pharmacokinetic properties into the clinical setting for obesity and mood disorders. Questions still largely remain as to how the signals from MCH are integrated into intracellular mechanisms that change neuronal activity, and how MCH neurons interact with other neuronal populations and finally control satiety, mood and emotion. For this purpose, identification of live MCH neuron by a viral approach has a substantial advantage (van den Pol et al. 2004). Full understanding of such complex brain circuitry will lead to deep insights into the clinical associations between anxiety, depression and eating disorders.

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