Melanin-Concentrating Hormone, Neuropeptide E-I, and MCH Receptor 1

13

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Abstract

Melanin-concentrating hormone (MCH) and neuropeptide E-I (NEI) are neuropeptides produced from the pro-melanin-concentrating hormone gene, which are found in many vertebrates playing prominent roles in maintaining the homeostatic balance. While the hypothalamus is its primary site of synthesis, cells synthetize MCH in other areas of the brain and multiple peripheral tissues. Its receptor, MCH receptor 1 (MCHR1), is also found in the brain and peripheral tissues. In addition to neuromodulatory actions, MCH also plays substantial neuroendocrinological roles, including interactions with sex steroids, growth hormone, cortisol/corticosterone, thyroid hormones, prolactin, vasopressin, and oxytocin. These roles are mediated by direct innervation of hypophysiotropic neurons located in multiple brain areas, direct action of MCH and NEI in the adenohypophysis and release of MCH in the bloodstream through the

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neurohypophysis. In this chapter, a detailed description of the MCH peptidergic system is provided, focusing on the distribution of MCH synthesis, its differential processing during lactation, the peripheral tissues where MCH or MCHR1 are produced, and the principal hormonal axes that are influenced by MCH.

Keywords

Hypothalamus · Neuropeptides · Sex steroids · Growth hormone · Cortisol · Thyroid · Prolactin

Abbreviations

α-MSH	α-melanocyte-stimulating hormone
aa	Amino acid(s)
Acb	Nucleus *accumbens*
ACTH	Adrenocorticotropic hormone
Arc	Arcuate nucleus
CART	Cocaine- and amphetamine-regulated transcript
CORT	Cortisol/Corticosterone
CRF	Corticotropin-releasing factor
E2	Estrogen/Estradiol
EB	Estrogen benzoate
ERα	Estrogen receptor α
FSH	Follicle-stimulating hormone
GH	Growth hormone
GnRH	Gonadotropin-releasing hormone
GPCR	G protein-coupled receptor
HPA	hypothalamic-pituitary-adrenal axis
HPG	hypothalamic-pituitary-gonadal axis
HPS	hypophyseal portal system
HPT	hypothalamic-pituitary-thyroid axis
IHy	Incerto-hypothalamic area
LH	Luteinizing hormone
LHA	Lateral hypothalamic area
MCH	Melanin-concentrating hormone
MCHR1	Melanin-concentrating hormone receptor 1
MCHR2	Melanin-concentrating hormone receptor 2
ME	Median eminence
mfb	Medial forebrain bundle
MGOP	Melanin gene overprinted polypeptide
MPOA	Medial preoptic area
NEI	Neuropeptide E-I
NGE	Neuropeptide G-E
NH	Neurohypophysis

OT	Oxytocin
OTR	Oxytocin receptor
OVX	Ovariectomy/Ovariectomized
P4	Progesterone
PC	Prohormone convertase(s)
PMCH	Pre-pro-melanin-concentrating hormone
PPD	*Postpartum* day
PRL	Prolactin
PRLR	Prolactin receptor
PVH	Paraventricular hypothalamic nucleus
T3	Triiodothyronine
T4	Thyroxine
TH	Tyrosine hydroxylase
TIDA	Tuberoinfundibular dopaminergic
TM	Transmembrane
TRH	Thyrotropin-releasing hormone
TSH	Thyroid stimulating hormone
VP	Vasopressin
ZI	Zona incerta

13.1 Introduction

Melanin-concentrating hormone (MCH) and neuropeptide glutamic acid-isoleucine (neuropeptide E-I, NEI) are two neuropeptides found in tissues from multiple vertebrate species. Processed from a single precursor, these neuropeptides act in a large number of functions, ranging from the promotion of motivated behaviors to metabolic energy expenditure, autonomic control, and ventricular ciliary beating. Central to MCH and NEI functions are their interactions with multiple neuronal, neurohormonal, and hormonal systems. This feature has remained conserved across vertebrate evolution and is tied directly to the discovery of MCH and its related proteins.

Numerous species of fish and amphibians rely on skin color changes to better blend into their environment. These changes happen through the translocation of melanin and other pigments in special cells found in the skin of those animals. In 1931, Hogben and Slome hypothesized that a "pigmentary effector system" formed by two antagonistic factors—darkening (or *B* factor) and clearing (or *W* factor)—controls changes in skin pigmentation. These factors would be released in the circulatory system to perform their respective function at the skin level. In the 1950s, α-melanocyte-stimulating hormone (α-MSH) was identified as the *B* factor, as it promotes the darkening of fish scales, but the identity of the *W* factor would remain a mystery for another three decades.

In 1983, Kawauchi et al. isolated a heptadecapeptide from the chum salmon hypophysis that performs the role expected for the hypothetical *W* factor. When applied to melanophores, that peptide promoted the congregation of melanin

molecules within cells, resulting in visible pallor. Given its intracellular effect, the peptide was named *melanin-concentrating hormone*. While scale color alterations have limited translational potential to mammals, it was also established that teleost MCH could modulate the release of adrenocorticotropic hormone (ACTH), a neuroendocrine role of great scientific interest. The discovery that hypothalamic extracts of rats display activities similar to salmonid MCH led to the search of a mammalian homolog, culminating in 1989 on the discovery of the gene and protein (Nahon et al. 1989; Vaughan et al. 1989).

The identification of both the gene and protein unleashed over three decades of intense interrogation of mammalian MCH. Some of the remarkable advances include the mapping of MCH and NEI in the central nervous system of rats (Bittencourt et al. 1992), the discovery of its orexigenic properties (Qu et al. 1996), the identification of its first and second receptors (see recommended literature for references), the mapping of MCH receptor 1 (MCHR1) (Hervieu et al. 2000; Saito et al. 2001), the description of electrophysiological properties of MCH neurons and MCH (Gao and van den Pol 2001; van den Pol et al. 2004), the implication of MCH in sleep modulation (Verret et al. 2003), the identification of MCHR1 as a ciliary receptor (Berbari et al. 2008), the publication of a comprehensive transcriptome of MCH neurons (Mickelsen et al. 2017), and the discovery of volume transmission as part of MCH communication (Noble et al. 2018).

Among the roles played by MCH, we now know that it has a significant relationship with multiple neuroendocrine systems, including oxytocin (OT), vasopressin (VP), prolactin (PRL), sex steroids, cortisol/corticosterone (CORT), and thyroid hormones. This chapter will detail the neuroanatomy of MCH neurons and their relationship with endocrine systems, including the emerging perspective that MCH may constitute a hypophysiotropic and neurohypophyseal hormone in mammals.

13.2 Genes, Proteins, and Phylogenetic Origins

In most vertebrates, the MCH peptidergic family comprises a single gene (*Pmch*) that encodes a full-length peptide precursor (PMCH), which is proteolytically processed to generate three peptides—MCH, NEI, and neuropeptide glycine-glutamic acid (neuropeptide G-E, NGE). The mammalian *Pmch* gene is formed by three exons and is located downstream of multiple promoter motifs, including an AP-1 site, an interferon-ɣ responsive element, and a glucocorticoid response element. The sequence of MCH is encoded predominantly by Exon 3, the most conserved among different species (Fig. 13.1) (Breton et al. 1993; Viale et al. 1997).

The complete mammalian precursor is 165 amino acid (aa) residues long. At the N-terminal sits a 21 aa signal peptide that initiates the posttranslational processing of PMCH and is cleaved after translation. An 87 aa-long structural chain separates the bioactive component of PMCH from the signal peptide. Prohormone convertases (PCs) cleave at residue Lys^{109} to release the bioactive C-terminal, while PC2 cleaves the Lys^{129}-Lys^{130} dibasic pair to form NGE. The remaining NEI-MCH peptide is

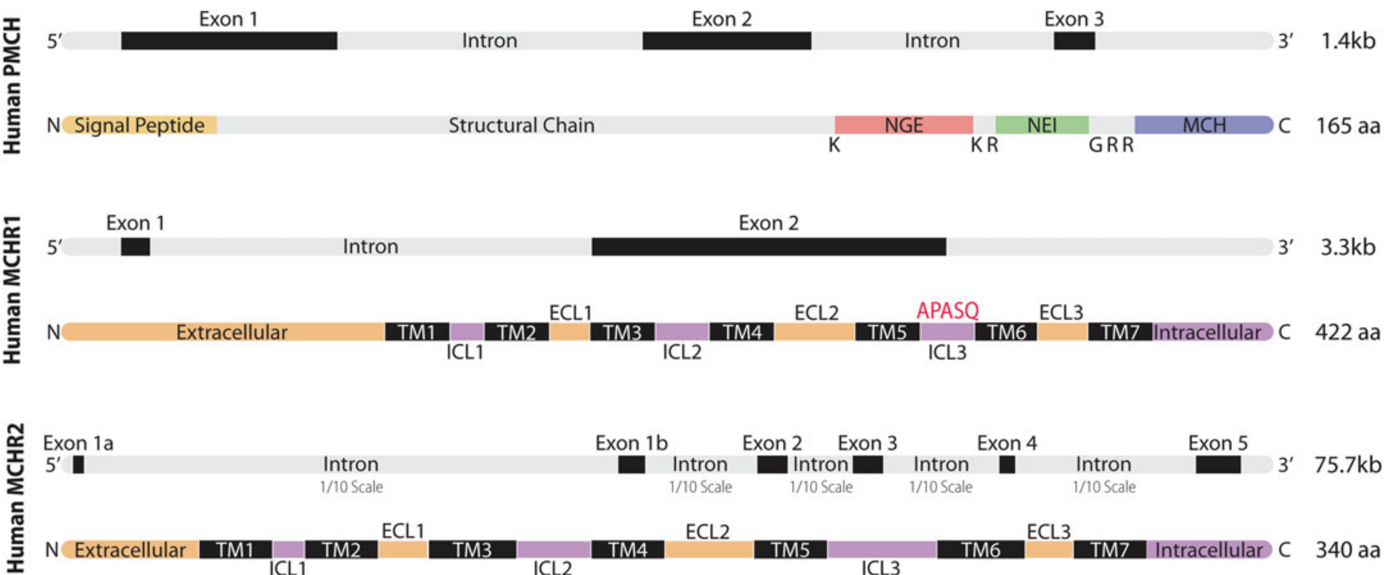

Fig. 13.1 Genome structure of the mammalian MCH peptidergic family. Human PMCH is formed by the expression of three exons, resulting in a 165-aa protein. Cleavage (K, R) and amidation sites (G) at which proteolytic processing occurs are indicated. Human MCHR1 is a 7-TM GPCR, with a long N-terminal extracellular tail, formed from the expression of two exons. The ciliary targeting sequence of MCHR1 is indicated by 3 letters within the third intracellular loop. Human MCHR2, on the other hand, is substantially larger, containing 5 exons with alternative splicing of the first exon. Introns in the MCHR2 gene are represented on a 1:10 scale due to their large size. Abbreviations: *ECL* extracellular loop, *ICL* intracellular loop, *MCH* melanin-concentrating hormone, *MCHR1* MCH receptor 1, *MCHR2* MCH receptor 2, *NEI* neuropeptide E-I, *NGE* neuropeptide G-E, *PMCH* pro-melanin-concentrating hormone, *TM* transmembrane

Fig. 13.2 Proteins of the mammalian MCH peptidergic family. Hexagons represent individual amino acid residues. Blue indicates negatively charged residues, and red indicates positive residues. Mammalian MCH is a nonadecapeptide with a cyclic structure due to a cysteine bridge between residues MCH^{7} and MCH^{16} (black dotted line). While positively charged residues are found in and near the ring portion, negative residues are found in the N-terminus. A chevron indicates residue MCH^{17}, which potentiates the binding of MCH to its receptors. Neuropeptide E-I and NGE are linear peptides with a predominant anionic character and have no known receptor or mechanism of action. Abbreviations: *MCH* melanin-concentrating hormone, *NEI* neuropeptide E-I, *NGE* neuropeptide G-E

cleaved at the Arg^{145}-Arg^{146} dibasic site by multiple PCs, including PC1/3, PC2, PACE4, PC5/6-A, PC5/6-B, and PC7. Amidation at the last residue of NEI produces mature NEI, a 13 aa-long linear peptide, while mature MCH is 19 aa residues-long, with a cyclic conformation resulting from a disulfide bridge formed between cysteine residues (Fig. 13.2) (Viale et al. 1999b).

Mature MCH exerts its activity through two known G protein-coupled receptors (GPCRs) called MCHR1 and MCHR2. The *Mchr1* gene encodes MCHR1 and comprises two exons: the first exon encodes a small portion of the N-terminus of the receptor (27 aa), while the second exon encodes the remaining 326 residues, for a final size of 353 residues (Fig. 13.1). The mature protein displays the characteristic seven transmembrane domains typical of GPCRs, three consensus sites for asparagine-linked glycosylation in the extracellular N-terminus, and two phosphorylation sites for protein kinase A, six for protein kinase C, and one for protein kinase CK2, allowing MCHR1 multiple levels of phosphorylation (Saito et al. 2013). In its final conformation, a central hydrophobic region separates hydrophilic pockets between transmembrane (TM) domains 3 and 7 and TM domains 4, 5, and 6 (Macdonald et al. 2000).

In addition to MCHR1, a second paralog, MCHR2, is found in most vertebrates (Tan et al. 2002). Its encoding gene, *Mchr2*, is substantially more complex than *Mchr1*, with six exons and five intronic sequences of variable length. The first two exons, E1a and E1b, are splice variants, with E1a expression generating a putatively truncated version of MCHR2 in the N-terminus. When E1b is expressed, MCHR2 is 340 residues in length, with typical GPCR features, including seven transmembrane domains, two N-linked glycosylation sites, a DRY motif located at the end of TM3, and a potential palmitoylation site in the C-terminal region (Fig. 13.1).

Both MCHR1 and MCHR2 bind MCH with high selectivity in the nanomolar range and do not respond to other MCH peptidergic family elements (NEI, NGE, Melanin Gene Overprinted Polypeptide—MGOP), natriuretic peptides, opioids, or

melanocortinergic peptides. Likewise, there is no binding to somatostatin or somatostatin-like peptides, despite MCH receptors sharing some sequence similarity with somatostatin receptors. The activation of MCHR1 has a predominantly inhibitory character, with multiple intracellular mechanisms engaged. These mechanisms include the inhibition of forskolin-mediated cAMP production and activation of mitogen-activated protein kinases, preferentially through Gi/Go, and an increase in intracellular Ca^{2+} mediated by Gq (for a review of MCH receptor functions, see Presse et al. 2014). Activation of MCHR1 also leads to its rapid internalization (Saito et al. 2004). On the other hand, activation of MCHR2 has a more limited intracellular effect, increasing intracellular levels of Ca^{2+} and IP_3 production through Gq.

An important aspect of the MCHR1 neurobiology is its translocation to the primary cilium (Berbari et al. 2008). Primary cilia are single non-motile microtubule-based organelles found in cells through the neuroaxis. The interior of primary cilia (axoneme) is gated from the rest of the cell, allowing the primary cilia to have different membrane and axoneme compositions compared to the cytoplasm and cellular membrane, in a mechanism that depends on specialized transport proteins. Primary cilia are mainly considered sensory structures, harboring multiple membrane receptors that can bind to neuroactive substances in the surrounding extracellular space.

A short consensus sequence within the third intracellular loop of MCHR1 is responsible for its targeting to the primary cilium in a transport process mediated by proteins of the BBSome complex (Berbari et al. 2008; Nagata et al. 2013). Ciliary MCHR1 is present in multiple cell culture models and is widespread in the brain of rats and mice. In the absence of primary cilia, as is the case with some cellular lineages, MCHR1 is found in the somatic membrane but displays attenuated action. Electrophysiological studies suggest MCHR1 is also located in the presynaptic membrane, but direct visualization of synaptic MCHR1 is still lacking.

Activation of ciliary MCHR1 by MCH leads to primary cilia shortening, in a process dependent on Gi/o but independent of cell cycle and receptor internalization. This process has been observed in multiple cellular models and, more recently, in hippocampal slices of rats and mice (Hamamoto et al. 2016; Kobayashi et al. 2020; Tomoshige et al. 2017). While the functional significance of ciliary shortening has not been established, it has been suggested to play a role in desensitizing primary cilia to external stimuli. Furthermore, altered ciliary length and morphology have been observed in a significant number of neurological diseases, warranting further investigation.

Present evidence suggests that the founder genes of *Pmch*, *Mchr1,* and *Mchr2* originated in phylostratum 11, at the time of vertebrate divergence (for a detailed review, see Diniz and Bittencourt 2019). This idea is supported by immunohistochemical studies using antibodies directed to salmon MCH that revealed MCH-like immunoreactivity in neurons of lampreys, and potential homologs of *Mchr1* and *Mchr2* that have been identified in the genome of *Petromyzon marinus*. In broad terms, the MCH gene family has remained well conserved throughout vertebrate evolution, with substantial conservation of sequence and structure for both MCH and

its receptors, contrasted only by a remarkably low similarity between MCHR1 and MCHR2. There are, however, notable exceptions for both MCH and its receptors.

At the time of the teleost divergence, a retroposition event likely led to the formation of two *Pmch* orthologs in this clade: *pmcha* and *pmchb* (the nomenclature used in this chapter follows that established in Diniz and Bittencourt (2019) and may not match that used in older works found in the literature). Teleost *pmcha* retained more similarities to the *Pmch* founder gene, although it was subject to a less strict selective pressure which resulted in substantial variability among teleost species. On the other hand, *pmchb* diverged into coding a shorter 17 residues-long MCH_B with residue substitutions in the N-terminal, bioactive ring and C-terminus, which is remarkably conserved between teleost species. In salmonids, a whole-genome duplication event led to the formation of a total of four *Pmch* paralogs (*pmcha1, pmcha2, pmchb1,* and *pmchb2*). The peptide isolated by Kawauchi et al. (1983) during the original discovery of MCH corresponds to MCH_B. The second exception concerns the MCH receptors in the Glires (Lagomorpha + Rodentia) clade, where a frameshift mutation in the *Mchr2* gene resulted in an inactive truncated protein, as observed in Lagomorpha species. The lack of functional MCHR2 ultimately led to the loss of *Mchr2* in rodents, including some of the most common laboratory models. This has severely impacted our understanding of MCH function in primates, where both functional receptors are found.

13.3 MCH/NEI Neurons

Melanin-concentrating hormone and NEI extensively colocalize within the rodent nervous system, a property believed to be shared by other species (Bittencourt et al. 1992; Bittencourt 2011; Diniz et al. 2019). There are two main exceptions to that rule: the preoptic cluster (described in detail in the next section), where MCH immunoreactivity is present in neuronal somas in the absence of NEI, and the interanterodorsal thalamic nucleus of rats, where MCH^+ fibers are found but only scattered NEI immunoreactivity has been reported. The importance of those differences is not known, in part due to a lack of understanding of NEI mechanisms of action. Even less is known about NGE, the third neuropeptide derived from PMCH, as its constitutive synthesis is yet to be demonstrated in mammals. Therefore, for the remainder of this chapter, the term *MCH neuron* will be used to describe neurons that synthesize both MCH and NEI, unless otherwise stated.

Within the neuron, MCH and NEI occupy different subcellular compartments (Fig. 13.3a–a'). Immunoreactivity to MCH is found predominantly within the Golgi apparatus's saccules, with a preference for its trans face, resulting in a honeycomb pattern when observed under high-resolution optical microscopy. Outside the soma, MCH immunoreactivity forms discrete puncta that extend into proximal branches, suggesting its packaging in vesicles for transport. On the other hand, NEI is a diffuse immunoreactive signal that fills the soma, proximal dendrites, and the axon, while sparing the nucleus, which led us to hypothesize that MCH and NEI may be released

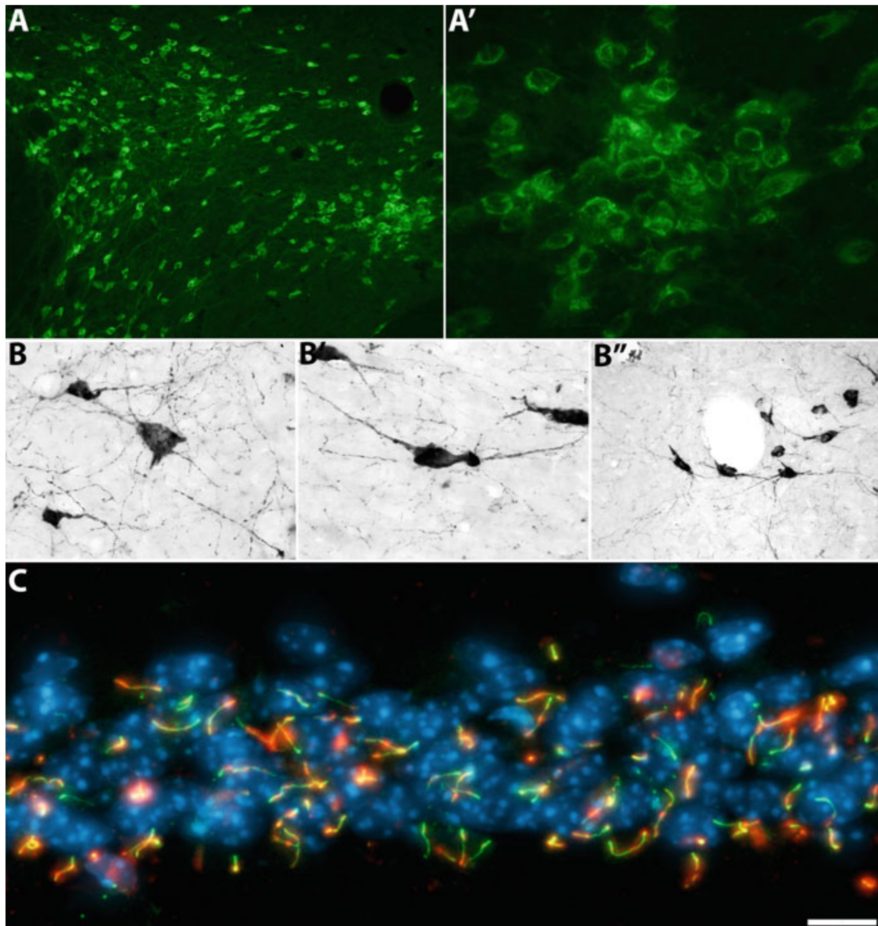

Fig. 13.3 Morphological properties of MCH and MCHR1. Mouse photomicrographs illustrating the morphological diversity of MCH and MCHR1 labeling in rodents. (**a**) Immunolabeled MCH neurons are found widespread throughout the lateral hypothalamus; (**a'**) Upon closer examination, it is possible to observe MCH labeling restricted to the soma surrounding, but not within, the nucleus. Neurons MCH^{+} can be categorized based on their morphology, including: (**b**) multipolar; **b**′) bipolar; and **b**″) crescent, when they are associated with blood vessels. (**c**) Immunolabeling of MCHR1 (red) is found in adenylate cyclase 3 (AC3—green)-positive cilia in multiple areas, including the pyramidal layer of CA1, illustrated here. Scale bar: **a**—120 μm; **a'**—30 μm; **b**, **b**′—20 μm; **b**″—40 μm; **c**—10 μm

through different intracellular mechanisms, based on the cellular compartment they occupy.

In addition to MCH and NEI, MCH neurons synthesize many other neuroactive substances, although some aspects of this are not fully understood. Melanin-concentrating hormone neurons express the mRNA for genes involved in the synthesis of GABA, including *Gad1* and *Gad2*, suggesting MCH neurons are

predominantly GABA inhibitory in nature. Recent studies, however, revealed that MCH neurons do not express the vesicular GABA transporter gene (*Slc32a1*), rendering them virtually unable to transport and release GABA vesicles through known mechanisms, despite having the machinery to synthesize it. On the other hand, MCH neurons express the vesicular glutamate transporters (*Slc17a6* and *Slc17a8*), which indicates MCH neurons are predominantly glutamate excitatory in nature, as previously demonstrated for a subgroup of septum-projecting neurons (Chee et al. 2015; Mickelsen et al. 2017). More studies are necessary to fully understand the neurotransmitter identity of MCH neurons.

In addition to neurotransmitter machinery, MCH neurons produce several other neuroactive substances. Integral colocalization has been reported between MCH and α-dystrobrevin, a protein associated with the structural integrity of muscle fibers also found in glial cells and, more rarely, in neurons. Nesfatin-1 is an 82-aa peptide with anorexigenic activity when injected centrally, and reports indicate that 90% of MCH neurons are Nesfatin-1 positive. The cocaine- and amphetamine-regulated transcript (CART) is a neuropeptide found in multiple brain areas that is upregulated following administration of some abuse substances, and it colocalizes with MCH in a region-dependent manner that ranges between 66–90% of tuberal MCH neurons. Other markers found in MCH neurons include the expression of mRNA for the synthesis of galanin (55%), a polypeptide found in several brain areas and implicated in multiple functions through its hyperpolarizing character; pronociceptin (35%), precursor of a 17-aa neuropeptide implicated in pain processing and fear; proenkephalin (30%), a precursor of peptides implicated in nociception; and thyrotropin-releasing hormone (TRH, $<10\%$), a hypophysiotropic hormone (Mickelsen et al. 2017). Other instances of co-expression include acetylcholinesterase in the absence of choline acetyltransferase, purposely for the modulation of cholinergic transmission, secretogranins, and the monocyte chemoattractant protein 1/chemokine ligand 2 (see recommended literature).

Contrasting to their substantial neurochemical heterogeneity, MCH neurons are remarkably uniform in terms of electrophysiological properties. In awake animals or slices, MCH neurons are predominantly quiescent. In these conditions, lateral hypothalamic area (LHA) neurons have low resting membrane potential (-61.3 ± 0.9 mV) and shallow spontaneous spike frequency (0.15 ± 0.1 Hz). These neurons also show spike frequency adaptation, with interspike interval increased by over 70% between the first and second halves of long current injections (van den Pol et al. 2004). Mature MCH neurons hyperpolarize in response to GABA and depolarize in response to glutamate, while immature MCH neurons can show depolarizing responses to GABA during the development period. These neurons undergo a progressive reduction in excitability in immature animals, reaching their most quiescent levels at four weeks of age in mice and seven weeks of age in rats (Li and van den Pol 2009; Linehan et al. 2018). While only occasional spikes are observed during slow-wave sleep, MCH neurons discharge at their maximum rate during paradoxical sleep showing a phasic firing pattern, and during the exploration of novel objects (Blanco-Centurion et al. 2019; Hassani et al. 2009).

A large number of post- and pre-synaptic mechanisms controls the electrophysiological behavior of MCH neurons (for a detailed review, please see Diniz and Bittencourt 2017). Postsynaptically, multiple neurochemical messengers mediate depolarization through transient receptor potential channels and sodium-calcium exchangers. Depolarizing agents include VP through the V1a receptor; OT through its receptor (OXTR), orexins through subtype 2 of their receptor; glucose, which gains entry to MCH neurons through the glucose transporter 3 and shunts ATP-sensitive K^+ channels; insulin, through the insulin receptor; and ATP, possibly through the purinergic receptor P2. Multiple mechanisms mediate postsynaptic hyperpolarization, including acetylcholine through the muscarinic cholinergic receptor; serotonin through a still unidentified receptor; norepinephrine and dopamine through the $\alpha 2_A$ adrenergic receptor; neuropeptide Y through its receptor; and nociceptin-1 mediated by the nociceptin/orphanin receptor, in an effect that is also mediated by dynorphin action on the K-opioid receptor. Hyperpolarization often occurs through G protein-coupled inwardly rectifying K^+ channels and voltage-dependent calcium channels. Most of the messengers that act postsynaptically on MCH neurons also act presynaptically to modulate both GABAergic and glutamatergic transmission into MCH neurons.

Morphologically, MCH neurons display a wide range of characteristics. These neurons can be classified into three main types depending on their morphology: multipolar, bipolar, and crescent, and that morphological classification has been well conserved, at least in mammals (Fig. 13.3b–b''). Multipolar neurons typically have 3–5 primary dendrites and no particular orientation, with medium and large cell bodies. Bipolar neurons have their longer axis preferentially oriented mediolaterally, with two or three primary dendrites and substantial branching in a short distance from the soma. Crescent neurons are a subtype of bipolar neurons found associated with the wall of blood vessels. The two primary dendrites of crescent neurons envelop blood vessel walls, and fibers from those neurons are often found within the wall of blood vessels. Ultrastructurally, MCH neurons display invaginated nuclei and well-developed Golgi apparatus and rough endoplasmic reticulum. No direct membrane apposition is found between MCH neurons and other MCH neurons or surrounding unlabeled cells in the LHA. The general appearance of MCH neurons has been described as similar to that of parvocellular hypophysiotropic neurosecretory neurons (Bittencourt et al. 1992; Diniz et al. 2019).

Axons from MCH neurons are thin (0.1–0.2 μm in diameter) and predominantly unmyelinated, containing small electron-translucent (30–60 nm in diameter) and large dense-core vesicles (80–150 nm). Immunoreactive material is found within the large dense-core vesicles but not in the small translucent ones. The primary contacts formed by MCH neurons are asymmetric and end on the dendritic shafts of unlabeled neurons, while axosomatic contacts are uncommon (Bittencourt et al. 1992).

13.4 Distribution

13.4.1 MCH in the Nervous System

In most early vertebrates, MCH neurons occupy a predominantly medial position within the diencephalon, often in the vicinity of the third ventricle (for a detailed description of MCH in multiple species, see Diniz and Bittencourt 2019). In these species, MCH neurons are found in the dorsomedial hypothalamic nucleus and the periventricular area, with neurons in lamprey reported to contact the interior of the third ventricle. In teleosts, MCH_B neurons drifted ventrally, occupying the lateral tuberal nucleus, a brain structure strongly associated with the hypophysis, reflecting the intimate association between MCH_B and neurosecretion, while MCH_A neurons retained their position adjacent to the ventricles. In sauropsids (reptiles and birds), there was a minor lateralization event, with some MCH neurons found in the lateral hypothalamus.

There is substantial variation in the distribution of MCH neurons between species, including phylogenetically close ones. In all mammalian species, the largest group of MCH neurons is found in the tuberal hypothalamus, predominantly in the lateral zone, intermingled with the crossing fiber groups of the medial forebrain bundle (*mfb*). These neurons form a shell that envelops orexin neurons, located in the core of the LHA. Interspecies variations include the mediolateral and the dorsoventral extensions of the MCH group of cells. In addition to the lateral zone, MCH neurons are also found in the dorsomedial area of the medial zone of the hypothalamus and, in some cases, in the periventricular zone. Neurons extend into the mammillary hypothalamus, often ending anterior to the medial mammillary nucleus. Rodents have been the most extensively examined species in MCH distribution and will be used in this section as the prototypical distribution.

Projections from MCH neurons are found widespread throughout the neuroaxis (Fig. 13.4). All major neuronal groups receive at least some MCH^+ fibers, except for some brainstem motor nuclei. A detailed description can be found elsewhere (Bittencourt et al. 1992; Bittencourt and Diniz 2018; Diniz et al. 2019), but some of the densest areas of innervation include the medial septal nucleus, the dorsal hippocampus, and all hypothalamic zones. Individual targets of innervation relevant to the neuroendocrine role of MCH include both internal and external layers of the median eminence (ME) and the neurohypophysis (NH) (Fig. 13.5), in addition to central projections that will be detailed in the appropriate sections. In the NH, MCH^+ axons processes and their swellings—predominantly Herring bodies—are identified in proximity to other axonal terminals and the basal membrane of fenestrated capillaries characteristic of this area, suggesting direct release of MCH in NH blood vessels to reach the general circulation. On the other hand, no immunoreactivity to MCH is found among specialized adenohypophyseal cells, as expected for a hypophysiotropic hormone released in the portal circulation (Fig. 13.6).

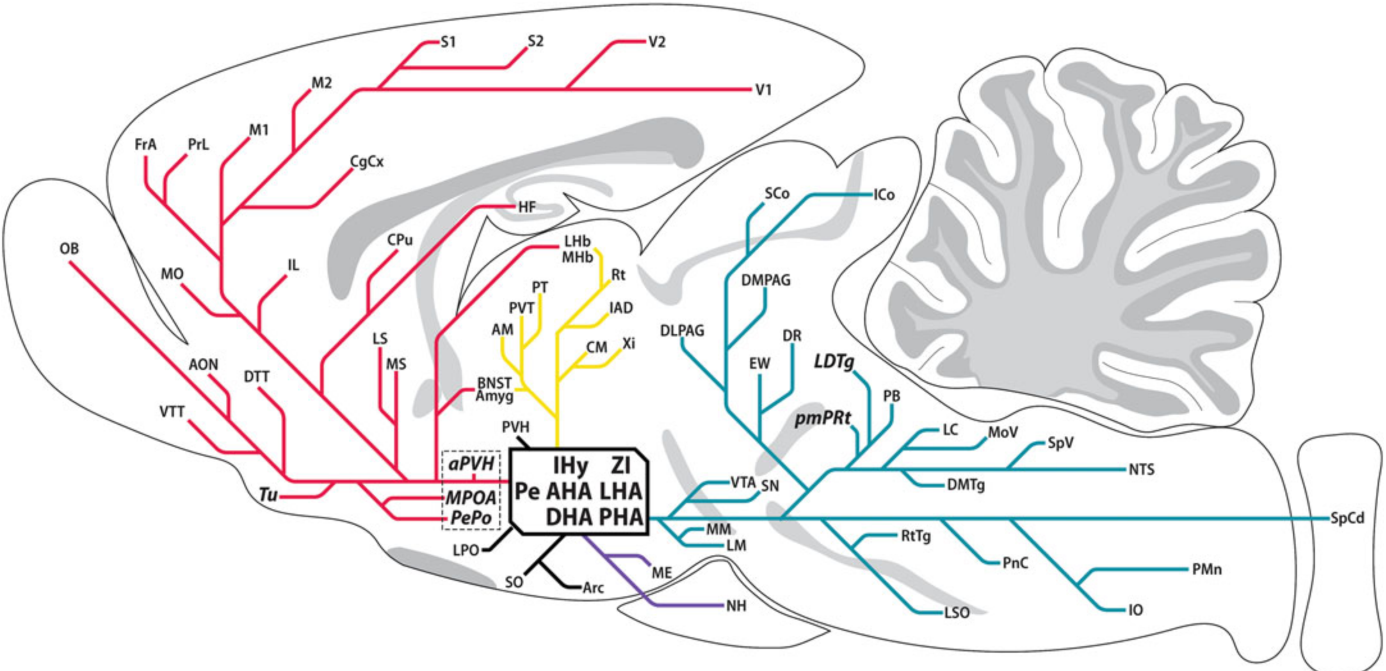

Fig. 13.4 Schematic representation of the main sites of innervation by MCH neurons in the rodent brain. Bold letters indicate structures where MCH neurons are found, a dotted box indicates the group of MCH neurons detectable only in lactating animals, and lines represent projections. There are four main projection routes (indicated by different colors), in addition to intra-hypothalamic connections (indicated in black). **Ascending pathway (red)**: through the ascending pathway, projections from MCH neurons reach the preoptic area, all structures of the basal telencephalon, the olfactory bulb and other olfaction-associated areas, the septal area, caudate-putamen, hippocampus, and multiple areas of the cortical mantle. **Periventricular pathway (yellow)**: the periventricular pathway turns dorsal and allows the innervation of multiple thalamic and epithalamic nuclei. **Hypophyseal pathway (purple)**: fibers in the hypophyseal pathway reach the

external and internal layers of the median eminence and the neurohypophysis. **Descending pathway (green)**: fibers in the descending pathway run throughout the rostrocaudal extent of the mesencephalon, pons, and medulla, ending within the spinal cord. Areas innervated by the descending pathway include the periaqueductal gray matter, the colliculi, raphe nuclei, and olivary areas. Abbreviations: *AHA* anterior hypothalamic area, *AM* anteromedial thalamic nucleus, *Amyg* amygdaloid complex, *AON* accessory olfactory nucleus, *aPVH* anterior part of the paraventricular hypothalamic nucleus, *Arc* arcuate nucleus, *BNST* bed nucleus of the stria terminalis, *CgCx* cingulate cortex, *CM* centromedial thalamic nucleus, *CPu* caudate-putamen, *DHA* dorsal hypothalamic area, *DLPAG* dorsolateral part of the periaqueductal gray matter, *DMPAG* dorsomedial part of the periaqueductal gray matter, *DMTg* dorsomedial tegmental nucleus, *DR* dorsal raphe nucleus, *DTT* dorsal tenia tecta, *EW* Edinger-Westphal nucleus, *FrA* frontal association cortex, *HF* hippocampal formation, *IAD* interanterodorsal thalamic nucleus, *ICo* inferior colliculus, *IHy* incerto-hypothalamic area, *IL* infralimbic cortex, *IO* inferior olivary complex, *LC* locus coeruleus, *LDTg* laterodorsal tegmental nucleus, *LHA* lateral hypothalamic area, *LHb* lateral habenular nucleus, *LM* lateral mammillary nucleus, *LPO* lateral preoptic area, *LS* lateral septal nucleus, *M1* primary motor cortex, *M2* secondary motor cortex, *ME* median eminence, *MHb* medial habenular nucleus, *MM* medial mammillary nucleus, *MO* medial orbital cortex, *MoV* motor nucleus of the trigeminal nerve, *MPOA* medial preoptic area, *MS* medial septal nucleus, *NH* neurohypophysis, *NTS* nucleus of the solitary tract, *OB* olfactory bulb, *PB* parabrachial nucleus, *Pe* preoptic nucleus, *PePo* periventricular preoptic nucleus, *PHA* posterior hypothalamic area, *PMn* paramedian reticular nucleus, *pmPRt* paramedian pontine reticular formation, *PNC* caudal pontine reticular nucleus, *PrL* prelimbic cortex, *PVH* paraventricular hypothalamic nucleus, *PVT* paraventricular thalamic nucleus, *PT* paratenial thalamic nucleus, *Rt* reticular thalamic nucleus, *RtTg* reticular tegmental nucleus, *S1* primary somatosensory cortex, *S2* secondary somatosensory cortex, *SCo* superior colliculus, *SN* substantia nigra, *SO* supraoptic nucleus, *SpCd* spinal cord, *SpV* spinal trigeminal nucleus, *Tu* olfactory tuberculum, *V1* primary visual cortex, *V2* secondary visual cortex, *VTA* ventral tegmental nucleus, *VTT* ventral tenia tecta, *Xi* xiphoid nucleus, *ZI* zona incerta

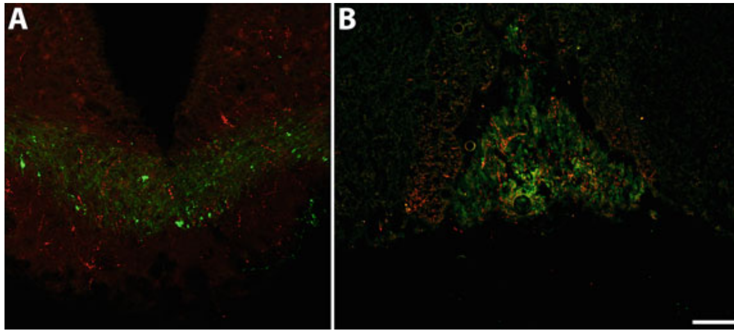

Fig. 13.5 Innervation of MCH in the median eminence and neurohypophysis. Rat photomicrographs illustrating the presence of MCH⁺ fibers (red) in neuroendocrine areas. (**a**) MCH⁺ axons can be found both in the internal layer of the median eminence, indicated in green by the presence of OT⁺ fibers, and in the adjacent external layer. (**b**) Both MCH⁺ and OT⁺ fibers are also found in the neurohypophysis, indicating MCH fibers course through the internal layer and into the neurohypophysis, where they contact blood vessels. Scale bar: 200 μm. The photomicrograph in B has been reproduced from Costa et al. (2019)

13.4.1.1 Lateral Zone of the Tuberal Hypothalamus

As previously mentioned, the LHA is the leading site of MCH synthesis. Neurons in the LHA are predominantly multipolar and intensely stained, and are found intermingled with the *mfb*, where multiple varicose MCH⁺ fibers are found. Dendrites from MCH neurons often protrude transversally to the *mfb*, allowing these neurons to tap into the most extensive fiber bundle crossing the anteroposterior extent of the neuroaxis. These neurons form a continuous mass of neurons, but they can be divided into three groups for didactic purposes. The first group, and the largest, is found in the area between the internal capsule and the fornix, with a large number of neurons forming a triangular shape between the internal capsule and the overlying zona incerta (ZI). The second group of neurons is found within the space between the optic tract and the ventral internal capsule. These neurons are large and strongly labeled, comprising the magnocellular group of MCH neurons. The third group is composed of neurons within or closely associated with the perifornical nucleus, displaying a characteristic centrifugal arrangement surrounding the fornix. Neurons in the perifornical group are often found in posterior levels of the tuberal hypothalamus compared to the other two groups. Some neurons of the perifornical area project to the ME and NH (Cvetkovic et al. 2003). The MCH neurons of the LHA are strongly associated with integrative functions.

13.4.1.2 Zona Incerta

Closely associated with the LHA is a narrow band of MCH neurons located in the ZI. These neurons are separated from the LHA by a narrow band of stain-free neuropil, and they form a compact layer of 3 to 4 cells, with their main axis oriented horizontally following the substantial mediolateral extent of the ZI. Little is known

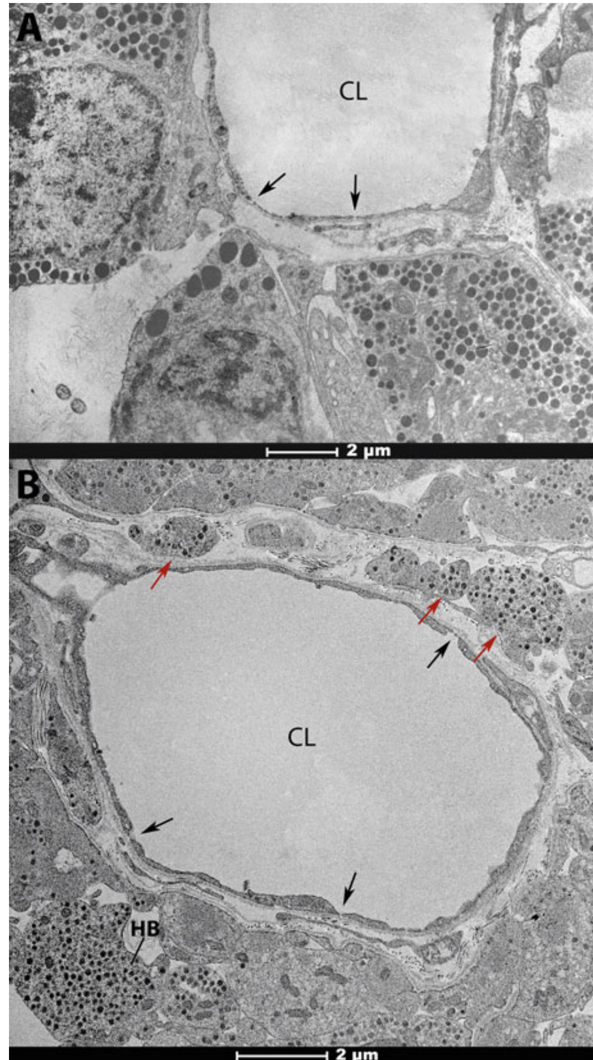

Fig. 13.6 Ultrastructural aspects of MCH immunolabeling in the hypophysis. Transmission electron microscopy of rat hypophyses subject to gold-conjugated immunolabeling for MCH. (**a**) Adenohypophysis. Although electrodense vesicles are abundantly found adjacent to capillary lumina (CL), no MCH immunolabeling is found; (**b**) Neurohypophysis. Secretion granules containing MCH immunoreactive material are observed in Herring Bodies (HB) and axonal processes (red arrows) in proximity to fenestrated capillaries. Note the endothelial pores (black arrows). Scale bars are indicated directly in the figure

about the specific functions of these neurons due to the difficulties associated with separating them from the adjacent LHA.

13.4.1.3 Medial Zone of the Tuberal Hypothalamus

MCH neurons in the medial zone of the hypothalamus are concentrated in the dorsal area, including the anterior hypothalamic area posterior to the paraventricular hypothalamic nucleus (PVH) and the dorsomedial hypothalamic area. These neurons are predominantly multipolar and are often continuous with LHA neurons, forming a single sheet of neurons that blankets the dorsal hypothalamus. No specific functions have been assigned to this specific group of neurons.

13.4.1.4 Incerto-hypothalamic Area

Dorsal to the dorsomedial hypothalamic group is a second, smaller group found in the incerto-hypothalamic area (IHy), a poorly differentiated zone located between the hypothalamus and the ZI (Sita et al. 2003, 2007). Neurons in the IHy can be differentiated from their ventral counterparts by their clear bipolar shape and mediolateral orientation. Neurochemically, the IHy can be identified by the presence of tyrosine hydroxylase (TH)-positive neurons of the dopaminergic group A13 that are found intermingled with MCH neurons, often forming extensive somatic contacts in the absence of colocalization. While the function of the dorsomedial hypothalamic area MCH neurons is poorly understood, the IHy MCH neurons are implicated in integrating energy status for the modulation of hormone secretion and sexual behavior, and projections of some of these neurons target the ME and NH (Cvetkovic et al. 2003).

13.4.1.5 Anterior Periventricular Nucleus

Some neurons in the dorsomedial hypothalamus extend towards the periventricular nucleus. This trend is particularly evident in rats, as MCH neurons are found clearly within its anatomical limits. In this species, a cluster of neurons is concentrated in the third ventricle's dorsal pole, merging with the adjoining dorsomedial area and IHy. These neurons extend projections into the lumen of the third ventricle, possibly contacting the cerebrospinal fluid.

13.4.1.6 Preoptic Cluster

Exclusively in lactating females, a small but well-defined group of MCH neurons is found in the medial preoptic area (MPOA), preoptic periventricular nucleus, and the anterior part of the PVH. These neurons have been described in multiple strains of rats and mice, although they are substantially more challenging to detect in mice due to lower levels of mRNA expression and protein synthesis and incongruence between Cre activation and gene expression (Alvisi et al. 2016; Beekly et al. 2020; Costa et al. 2019; Diniz et al. 2019; Knollema et al. 1992). These neurons express *Gad67* mRNA in rats, but there is no colocalization between MCH and the VGLUT2 or VGLUT3 genes in mice, with contradictory reports about Slc32a1 expression (Beekly et al. 2020; Teixeira et al. 2020), raising essential questions about their neurotransmitter profile. These neurons are also negative for kisspeptin, OT, and TH (Rondini et al. 2010; Teixeira et al. 2020).

While *Pmch* mRNA is not detected in the preoptic cluster in cycling virgin females and pregnant dams, expression starts at shallow levels on the fifth *postpartum* day (PPD) and increases as lactation progresses, reaching its maximum levels around the 15th–16th PPD, coinciding with the pups' eruption of the incisors and their transition into solid foods. Expression of *Pmch* and MCH immunoreactivity remain elevated through the late lactation period, either slowly decreasing towards the 26th PPD when pups are kept with the mothers or rapidly fading when pups are weaned on the 22nd PPD.

Preoptic cluster MCH neurons are intrinsically linked with the lactation process and the offspring, as the number of MCH neurons in this area is positively correlated

with *postpartum* litter size, and the number of cells in multiparous dams is lower compared to primiparous dams (what may be linked with maternal memory) (Ferreira et al. 2017; Teixeira et al. 2020). The mechanism that tethers preoptic MCH neurons and the offspring is not fully understood. While tactile stimulation of the nipples by the pups leads to ample synthesis of the early activation protein FOSB in the hypothalamus, including the preoptic area, this marker is not found colocalized with MCH neurons. A neurohormonal mechanism, however, seems more plausible, as preoptic MCH neurons contain both prolactin (PRL) and estrogen/estradiol (E2) receptors and respond to PRL through the expression of *Stat5* (Alvisi et al. 2016; Teixeira et al. 2020).

Preoptic MCH neurons have been implicated in maternal behavior. Intranuclear injections of MCH into the MPOA of rat dams in the fifth or sixth PPD decrease appetitive components of maternal behavior, including retrieval, licking, and nest building, while sparing consummatory aspects of behavior. Constitutive deletion of MCHR1 has a similar effect, decreasing nesting, maternal aggression, and pup retrieval (Alachkar et al. 2016; Benedetto et al. 2014). Preoptic MCH neurons may also be involved in the release of MCH in the bloodstream. Peripheral injection of a retrograde tracer in the blood leads to labeled MCH^+ neurons in all areas of the preoptic cluster, suggesting these neurons have open terminals in the NH (Costa et al. 2019).

13.4.1.7 Other Clusters

Smaller numbers of neurons are found in several brain areas, including the olfactory tubercle, the dorsomedial part of the tuberomammillary nucleus, the posterior hypothalamic area, the paramedian pontine reticular formation, and the laterodorsal tegmental nucleus (the latter found exclusively in female rats) (Bittencourt et al. 1992; Rondini et al. 2007). Little is known about these additional groups.

13.4.2 MCH in the Periphery

Expression of *Pmch* mRNA or MCH immunoreactivity has been detected in multiple peripheral tissues, but MCH and NEI are not fully processed outside of the brain, resulting in the production of a peptide containing both MCH and NEI epitopes (Viale et al. 1997). This allows the discrimination between MCH produced in the brain and NEI-MCH produced by peripheral tissues. Structures that include *Pmch*-expressing cells are the heart, lungs, stomach, intestine, pancreas, adrenal glands, testis, ovary, and immunological cells (Fig. 13.7). In the gastrointestinal tract, *lamina propria* cells of the mucosal plexus of the duodenum and the antral portion of the stomach express *Pmch* mRNA (Hervieu et al. 1996). In the testis, both *Pmch* expression and MCH immunoreactivity are found in Sertoli cells surrounding seminiferous tubules (Hervieu and Nahon 1995). In the pancreas, it is expressed in pancreatic islets (Pissios et al. 2007). Finally, expression of *Pmch* has been reported in splenocytes, thymocytes, lymphocytes, PBMCs, granulocytes, and $Th2^+$ human

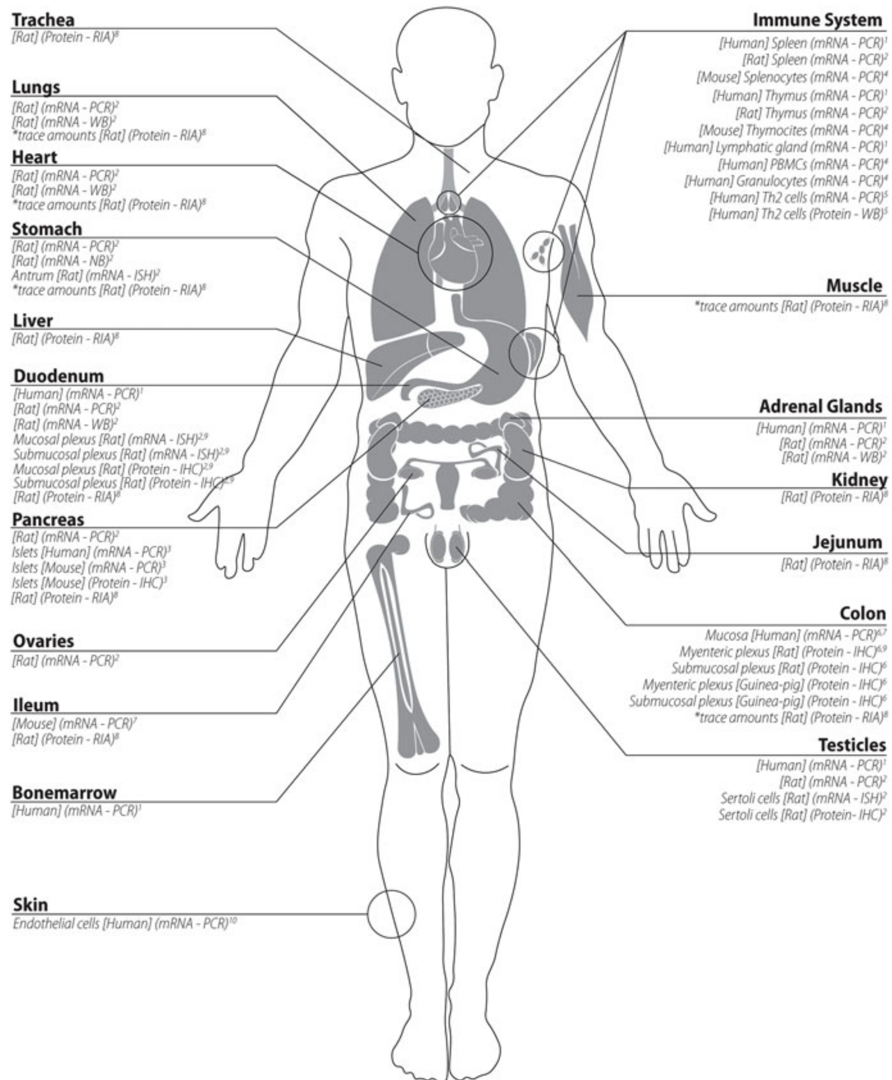

Fig. 13.7 Reports of *Pmch* expression and MCH synthesis in peripheral tissues of mammals. Reports are organized by organ/system, tissue, target, and technique. References: 1—Viale et al. (1997); 2—Hervieu and Nahon (1995); 3— Pissios et al. (2007); 4—Verlaet et al. (2002); 5—Sandig et al. (2007); 6 —Kokkotou et al. (2008); 7—Kokkotou et al. (2009); 8—Lelesz et al. (2016); 9—Hervieu et al. (1996). Abbreviations: *IHC* immunohistochemistry, *ISH* in situ hybridization, *PCR* polymerase chain reaction, *RIA* radioimmunoassay, *WB* western blotting

cells (Sandig et al. 2007; Verlaet et al. 2002). The role played by peripheral NEI-MCH is poorly understood.

13.4.3 MCH in the Circulation

Free mammalian MCH has been found in blood derivatives of rodents and humans. Detection has been performed with both enzymatic and radioimmunoassay methods using commercial and custom kits. Methodological differences make it challenging to directly compare different works, especially when considering that the MCH antibody used in commercial kits may react in a non-specific manner with proteins found in blood from *Pmch* knockout animals (Waters and Krause 2005). In this section, a brief description of each work will be provided.

Naufahu et al. (2017)—This work is possibly the most impactful publication regarding the presence of MCH in the bloodstream, considering that the authors developed an in-house radioimmunoassay and extensively characterized it, although they did not evaluate their test against a sample of *Pmch* KO animals (the gold standard of specificity validation). Perhaps even more important is the fact that this test only binds to NEI-MCH (the peripheral form of MCH) at supraphysiological levels, suggesting the values observed in this study correspond to brain-originated MCH released into the bloodstream. In this cross-sectional study, levels of MCH were determined in the plasma of over 230 adults and compared to body metabolic and morphological properties. Fasting plasma MCH levels were found in the range between 19.5 and 70.4 pg/ml (19.5 pg/ml is the lower limit of detection for the test). Complex relationships were found between circulating levels of MCH and body parameters. Males with BMI > 30 have higher average levels of MCH than males with BMI < 20. Males display a positive correlation between BMI and MCH, while females display the opposite correlation. In older individuals, there is an increase in MCH after eating, while younger individuals show a correlation between MCH and insulin area-under-the-curve. Leptin and MCH are positively correlated in lean males while negatively correlated in males with excess fat.

Schmidt et al. (2015)—In this work, sera from patients with major depressive disorder or controls were investigated using a commercial fluorescence immunoassay kit. While baseline levels of MCH do not differ between unmedicated patients and controls, female patients treated with mirtazapine showed a decrease in MCH levels during treatment.

Carnier et al. (2010)—In this work, sera from post-pubertal obese adolescents undergoing interdisciplinary treatment were investigated using a commercial radioimmunoassay kit. Baseline MCH was found to be 10.65 ng/ml, being upregulated to 12.25 ng/ml after short-term therapy and downregulated to 9.90 ng/ml after long-term therapy. After long-term therapy, MCH and leptin were found to be inversely correlated.

Gavrila et al. (2005)—In this work, sera from 108 healthy individuals were investigated using a commercial radioimmunoassay kit. Baseline serum levels of MCH were 97.8 ± 22.8 pg/ml, with average values significantly lower for men than

women. Levels of MCH were positively correlated with BMI, fat mass, and percentage of fat, while negatively correlated with lean mass. In this same study, fasting for two days increased serum MCH levels.

Sun et al. (2004)—In this work, sera from male and female Wistar rats were analyzed using a commercial competitive immunoassay from kit. Levels of MCH were non-significantly decreased in rats following lesions of the PVH or ventromedial hypothalamic nucleus, and non-significantly increased in lactating rats on the 12th PPD ($n = 5$; 17.6 ± 0.6 ng/ml) compared to nonlactating controls ($n = 5$; 13.7 ± 3.6 ng/ml).

Stricker-Krongrad et al. (2001)—In this work, plasmas from 20 lean and 20 obese Zucker rats were analyzed using a commercial competitive immunoassay kit. Plasma levels of MCH were 7.2 ± 0.8 ng/ml in lean animals and 12.5 ± 1.3 ng/ml in obese animals, a statistically significant difference.

Bradley et al. (2000)—In this work, plasmas from male Sprague-Dawley animals were analyzed using a commercial radioimmunoassay kit. Plasma levels of MCH were found to range between 54 and 397 pg/ml.

13.4.4 MCHR1 in the Brain

As is the case with MCH, most of the anatomical mapping of MCHR1 has been performed in rodents, with brief descriptions of other species available in the literature (Fig. 13.8) (Chee et al. 2013; Diniz et al. 2020; Hervieu et al. 2000; Saito et al. 2001). The presence of MCHR1$^+$ cilia in some relevant neuroendocrine populations is illustrated in Fig. 13.9.

13.4.4.1 Neocortex

There is ample expression of *Mchr1* mRNA and MCHR1 synthesis in the rat and the mouse cortical mantle, including layers II, III, IV, V, and VI. Primary cilia containing MCHR1 are abundantly found in all layers except for layer I, mimicking the pattern of gene expression. Synthesis of MCHR1 is found throughout the neocortex with minimal variation between areas.

13.4.4.2 Olfactory Areas

Olfactory areas display some of the densest concentrations of MCHR1$^+$ primary cilia in the mouse brain. Vast numbers of labeled cilia are found in the granular, internal plexiform, mitral, and glomerular cell layers. In the latter, labeled cilia are strongly associated with TH$^+$ glomerular cells and, to a lesser extent, with calretinin-positive cells. Additional sites include layer 2 of the piriform cortex, medial part of the anterior olfactory nucleus, dorsal and ventral *tenia tecta*, dorsal and intermediate endopiriform nucleus, and olfactory tubercle.

13.4.4.3 Hippocampal Formation

The hippocampal formation displays a very characteristic pattern of MCHR1 immunoreactivity, with dense ciliary labeling found in pyramidal cells of CA1, CA2, and,

Fig. 13.8 Schematic representation of the distribution and density of ciliary MCHR1 in the mouse brain. The size of red circles indicates the relative density of MCHR1$^+$ cilia in the brain. The area marked by a discontinuous line represents the amygdaloid complex. Abbreviations: *II* cortical layer II, *III* cortical layer III, *IV* cortical layer IV, *V* cortical layer V, *VI* cortical layer VI, *Acb* nucleus *accumbens*, *ACo* anterior cortical nucleus of the amygdala, *AHA* anterior hypothalamic area, *AON* anterior olfactory nucleus, *Arc* arcuate nucleus, *AVPV* anteroventral preoptic nucleus, *BL* basolateral nucleus of the amygdala, *BM* basomedial nucleus of the amygdala, *BNST* bed nucleus of the stria terminalis, *CA1Py* pyramidal layer of the CA1 field of the hippocampus, *CA2Py* pyramidal layer of the CA2 field of the hippocampus, *CC* central column of the spinal cord, *Ce* central nucleus of the amygdala, *CM* centromedial thalamic nucleus, *CPu* caudate putamen, *DMH* dorsomedial hypothalamic nucleus, *DR* dorsal raphe nucleus, *DTM* dorsal tuberomammillary nucleus, *GlO* glomerular layer of the olfactory

bulb, *GrA* granular layer of the accessory olfactory bulb, *GrO* granular layer of the olfactory bulb, *IAM* interanteromedial thalamic nucleus, *IG* induseum griseum, *IMD* intermediodorsal thalamic nucleus, *IP* interpeduncular nuclei, *LC* locus coeruleus, *LG* lateral geniculate nucleus, *IHy* incerto-hypothalamic area, *LHA* lateral hypothalamic area, *LHb* lateral habenular nucleus, *LSd* dorsal part of the lateral septal nucleus, *LSv* ventral part of the lateral septal nucleus, *MD* mediodorsal thalamic nucleus, *Me* medial nucleus of the amygdala, *MG* medial geniculate nucleus, *MHb* medial habenular nucleus, *MiO* mitral layer of the olfactory bulb, *MnPo* median preoptic nucleus, *MnR* median raphe nucleus, *MPON* medial preoptic nucleus, *MS* medial septal nucleus, *PAG* periaqueductal gray matter, *PePo* preoptic periventricular nucleus, *PHA* posterior hypothalamic area, *PMD* dorsal premammillary nucleus, *PMV* ventral premammillary nucleus, *PoC* posterior column of the spinal cord, *PSTh* parasubthalamic nucleus, *PVH* paraventricular hypothalamic nucleus, *PVT* paraventricular thalamic nucleus, *RCh* retrochiasmatic nucleus, *Re* nucleus reuniens, *SN* substantia nigra, *SPF* subparafascicular nucleus, *Sub* subiculum, *Tu* olfactory tubercle, *VM* ventromedial thalamic nucleus, *VN* trigeminal nuclei, *VTA* ventral tegmental area, *VTT* ventral tenia tecta, *Xi* xiphoid nucleus

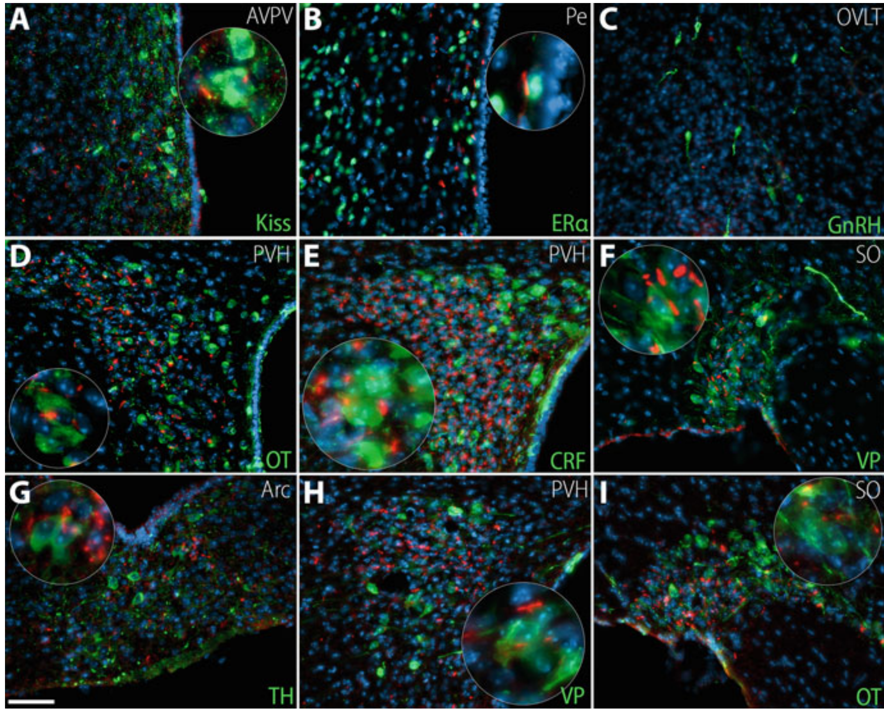

Fig. 13.9 Presence/absence of ciliary MCHR1 in neuroendocrine areas. Mouse photomicrographs showing the co-distribution between ciliary MCHR1 (red) and neuroendocrine populations (green) in the rodent brain. Highlights of the proximity between labeled cilia and neurons are provided in higher magnification circles, when applicable. Scale bar: 50 μm. Abbreviations: *Arc* arcuate nucleus, *AVPV* anteroventral preoptic area, *CRF* corticotropin-releasing factor, *ERa*—estrogen receptor α, *GnRH* gonadotropin-releasing hormone, *Kiss* kisspeptin 1, *OT* oxytocin, *OVLT organum vasculosum* of the *lamina terminalis*, *Pe* periventricular hypothalamic nucleus, *PVH* paraventricular hypothalamic nucleus, *SO* supraoptic nucleus, *TH* tyrosine hydroxylase, *VP* vasopressin. Adapted with permission from Diniz et al. (2020)

to a lesser extent, CA3. Primary cilia immunoreactive to MCHR1 in CA1 are substantially longer than those in CA3, and they undergo ciliary shortening in response to MCH while those in CA3 do not. Fasting in adult mice also reduces the length of CA1 MCHR1-positive cilia, while CA3 cilia remain unchanged (Kobayashi et al. 2020). Minimal numbers of MCHR1$^+$ cilia are found in the *oriens* and *radiatum* strata, and almost no immunoreactivity is found in the dentate gyrus, despite the vast numbers of primary cilia in that structure. The subgranular zone of the dentate gyrus is one of the main sites of differences between rats and mice, with rats displaying a small but well-delimited layer of MCHR1+ cilia that is absent in mice.

13.4.4.4 Subcortical Telencephalic Structures

MCHR1$^+$ cilia are abundantly found in subcortical structures. In both rats and mice, the nucleus *accumbens* (Acb) is among the densest areas of *Mchr1* mRNA expression and MCHR1$^+$ cilia. In the mouse caudate-putamen matrix, a mediolateral gradient is observed, with the highest density of positive cilia found closer to the wall of the lateral ventricles, while no labeling is found in the striasomes. No labeling is observed in the rat caudate-putamen, making this structure the second major dimorphic area between rats and mice. Moderate labeling is observed in the ventral pallidum and medial part of the globus *pallidus*, while sparse labeling is found in the lateral globus *pallidus* and the central part of the lateral septal nucleus. In the amygdaloid complex, only scattered MCHR1$^+$ cilia are found in the basolateral, basomedial, medial, and central nuclei.

13.4.4.5 Thalamus

Several thalamic areas display moderate numbers of MCHR1$^+$ cilia, including the *paratenial* nucleus, paraventricular thalamic nucleus, medial thalamic nuclei, and medial habenular nucleus. In the paraventricular nucleus, MCHR1$^+$ cilia are often found co-distributed with calretinin. While no immunoreactivity is detected in the ZI proper, many TH$^+$/MCHR1$^+$ neurons are found in the IHy.

13.4.4.6 Hypothalamus

In the hypothalamus, small but dense clusters of MCHR1$^+$ cilia are found in the preoptic periventricular nucleus, PVH, supraoptic nucleus, and arcuate nucleus (Arc). In the preoptic hypothalamus, MCHR1 positive cilia are associated with kisspeptin neurons and, to a lesser extent, estrogen receptor α (ERα)-positive cells, but not with gonadotropin-releasing hormone (GnRH) cells (Fig. 13.9). In the PVH, MCHR1$^+$ cilia are co-distributed with OT and corticotropin-releasing factor (CRF), while in the supraoptic nucleus MCHR1$^+$ cilia are often found adjacent to VP$^+$ neurons (Fig. 13.9). Positive cilia are associated with TH neurons in the Arc, but no exact co-distribution is found with αMSH or CART. Moderate numbers of labeled cilia are found in the MPOA, anterior hypothalamic area, and posterior hypothalamic area. The dorsomedial and ventromedial hypothalamic nuclei are mostly devoid of labeling.

13.4.4.7 Brainstem

Scattered labeled cilia are found in the dorsal midbrain, including the area surrounding the mesencephalic aqueduct and the superior colliculus, with a slight preference for the *stratum opticum* in the latter. Ventrally, MCHR1 is found in the ventral tegmental area, *paranigral* nucleus, parabrachial pigmented area, interpeduncular nucleus, and *pars compacta* of the *substantia nigra*. In the midbrain-pons transition area, labeled cilia are observed in the dorsal and medial raphe nuclei. In the posterior brainstem, small numbers of MCHR1 positive cilia are found in select sensory nuclei, including the ventral cochlear nucleus and the nucleus of the solitary tract.

13.4.4.8 Spinal Cord

In the spinal cord, MCHR1^{+} cilia are present in the dorsal grey column, comprehending the area of Rexed laminae II and III, while only scattered in the area surrounding the central canal.

13.4.5 MCHR1 in the Periphery

In addition to the brain, *Mchr1* expression and MCHR1 immunoreactivity have been found in multiple peripheral tissues, allowing MCH, either released in the circulation or produced locally at the periphery, to influence a large number of physiological systems (Fig. 13.10). These systems include: musculoskeletal—skeletal muscle, tongue, bones; cardiorespiratory—heart, lung, trachea; digestive—esophagus, stomach, small intestine, duodenum, colon, liver; urogenital—kidney, prostate, uterus; immune—splenocytes, lymphocytes, thymocytes, PBMCs, granulocytes; and endocrine—hypophysis, cortex and medulla of the adrenal gland and thyroid gland. The presence of *Mchr1* mRNA has also been reported in adipocytes, the skin, and the placenta (Bradley et al. 2000; Chung et al. 2012; Hill et al. 2001; Hoogduijn et al. 2002; Kokkotou et al. 2008, 2009; Saito et al. 1999; Segal-Lieberman et al. 2006; Takahashi et al. 2001; Verlaet et al. 2002). More recently, both *Mchr1* mRNA and MCHR1 immunoreactivity have been reported in the rat mammary gland. Positive signals are found in the skin covering the glands in both virgin and lactating females, but MCHR1 is found in the parenchyma exclusively in lactating animals, with maximal expression in samples collected on the 19th PPD. Immunoreactivity was found to be associated with alveolar secretory cells, suggesting an active role of circulating MCH in milk ejection, which may explain why MCHR1 ablation or inactivation mid-lactation results in decreased milk production (Alachkar et al. 2016; Battagello et al. 2020).

13.5 Interactions Between MCH and Hypophysiotropic Hormonal Systems

13.5.1 Sex Steroids

There is a complex relationship between MCH, NEI, and luteinizing hormone (LH) that is highly dependent on the hormonal *milieu* of the animal (for an in-depth discussion, see Naufahu et al. 2013). These interactions include both direct and indirect MCH and NEI actions over the release of GnRH and direct and indirect actions of sex steroids over MCH neurons. The high complexity of these interactions has led to a large body of studies in the literature that is difficult to interpret and, at times, contradictory. A schematic representation of MCH in the hypothalamic-pituitary-gonadal (HPG) axis is provided in Fig. 13.11.

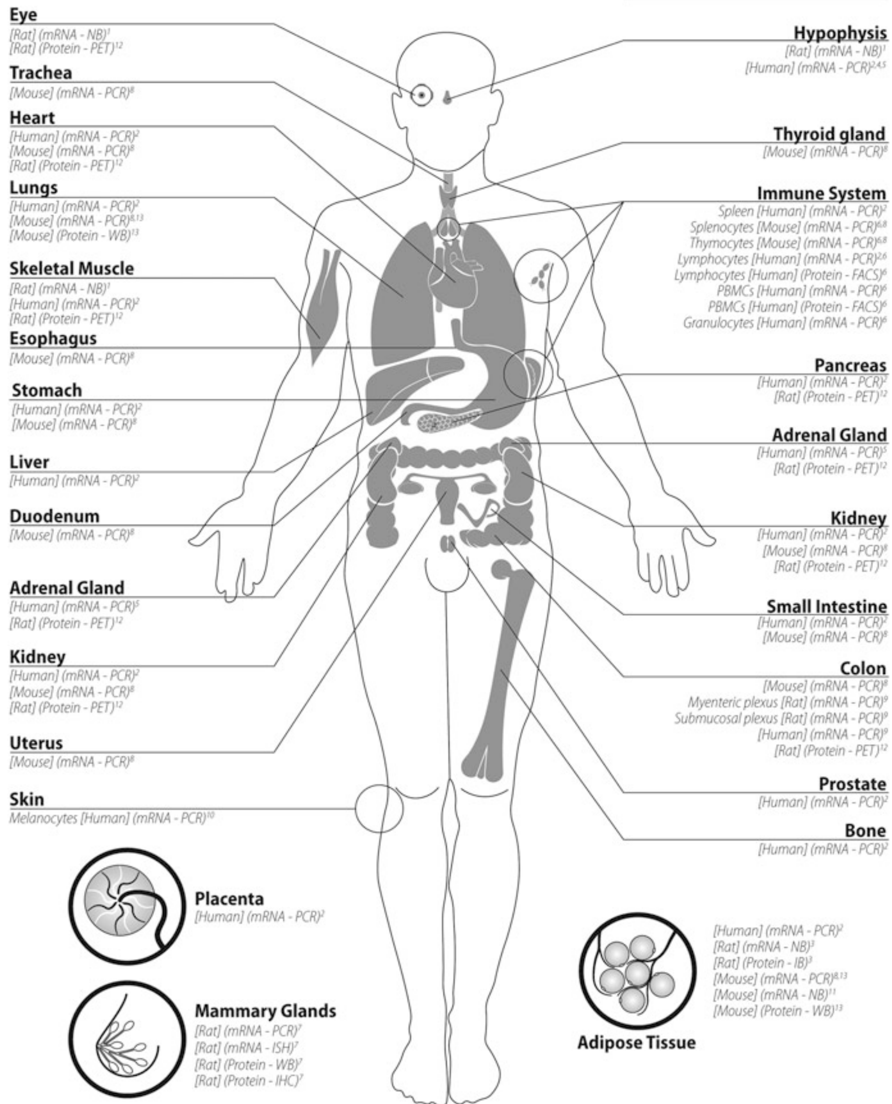

Fig. 13.10 Reports of *Mchr1* expression and MCHR1 synthesis in peripheral tissues of mammals. Reports are organized by organ/system, tissue, target, and technique. References: 1—Saito et al. (1999); 2—Hill et al. (2001); 3—Bradley et al. (2000); 4—Segal-Lieberman et al. (2006); 5—Takahashi et al. (2001); 6—Verlaet et al. (2002); 7—Battagello et al. (2020); 8—Chung et al. (2012); 9—Kokkotou et al. (2008); 10—Hoogduijn et al. (2002); 11—Bradley et al. (2002); 12—Philippe et al. (2016); 13—Balber et al. (2019). Abbreviations: *FACS* fluorescence assisted cell sorting, *IB* immunoblotting, *IHC* immunohistochemistry, *ISH* in situ hybridization, *NB* northern blotting, *PCR* polymerase chain reaction, *PET* positron emission tomography, *RIA* radioimmunoassay, *WB* western blotting

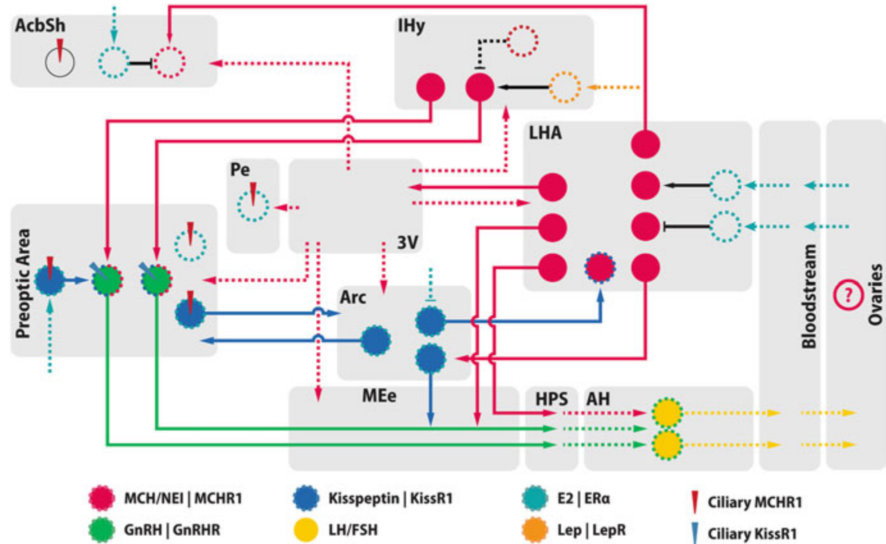

Fig. 13.11 The role of MCH in the hypothalamic-pituitary-gonadal (HPG) axis. Schematic representation of the main components of the HPG axis and their relationship with MCH neurons. For clarity, not all elements of the HPG system are represented . Circles represent cells and their neuromodulator/hormonal content, broken circles represent receptors in the surface of the cells or of unknown subcellular location, continuous lines indicate wired projections, broken lines represent humoral communication or unknown pathways, thin wedges represent ciliary receptors, and black drawings represent cells or relationships that have not been determined. Elements drawn out of gray boxes may originate from multiple or undetermined areas. A question mark is used in cases where MCH or MCHR1 has been reported in peripheral structures but there were no dedicated confirmatory studies. Abbreviations: *3V* third ventricle, *AcbSh* shell of the nucleus *accumbens*, *AH* adenohypophysis, *Arc* arcuate nucleus, *E2* estrogen/estradiol, *ERα* estrogen receptor α, *FSH* follicle-stimulating hormone, *GnRH* gonadotropin-releasing hormone, *GnRHR* GnRH receptor, *HPS* hypophyseal portal system, *IHy* incerto-hypothalamic area, *KissR1* kisspeptin receptor 1, *Lep* leptin, *LepR* leptin receptor, *LH* luteinizing hormone, *LHA* lateral hypothalamic area, *MCH* melanin-concentrating hormone, *MCHR1* MCH receptor 1, *MEe* median eminence, external layer, *NEI* neuropeptide E-I, *Pe* periventricular hypothalamic nucleus

13.5.1.1 The Actions of MCH/NEI on LH Release

Available evidence suggests NEI has a positive effect on the release of LH. When injected intraventricularly in rats, NEI leads to a transient increase in LH concentration in the blood as soon as 10 min and persisting up to 90 min (study endpoint), both in males and in ovariectomized (OVX) females treated with estrogen benzoate (EB) and progesterone (P4) (Attademo et al. 2004). The actions of MCH, on the other hand, are less straightforward. Injection of MCH into the MPOA and the ME leads to an increase in LH secretion in OVX females treated with high levels of EB. Accordingly, immunoneutralization of MCH in the MPOA leads to a decrease in LH secretion in OVX females in the absence of hormonal supplementation. The addition of P4, however, abolishes the inducing effect of MCH over LH release in the MPOA and leads to an impaired surge in LH when MCH is injected into the IHy

(Gonzalez et al. 1997; Murray et al. 2000a, 2006). Intraventricular injections of MCH in OVX females treated with low levels of EB lead to decreased LH secretion (Tsukamura et al. 2000). These results indicate that MCH action over LH secretion depends on the site of action and the hormonal status of the animals, including circulating leptin. The immunoneutralization of MCH in the MPOA blocks the increase in LH secretion caused by leptin injection in the IHy (Murray et al. 2000b).

13.5.1.2 GnRH Neurons as Mediators of MCH Action Over LH Release

GnRH neurons in the medial septal nucleus, *organum vasculosum* of the *lamina terminalis* and preoptic area receive extensive contacts (60%–90%) from NEI^+ and MCH^+ fibers both in rats and mice of both sexes. Contacts between MCH^+ fibers and $GnRH^+$ neurons have also been observed in the human infundibular nucleus, although these contacts appear to be less extensive ($17.7 \pm 3.3\%$). The existence of MCH synapses onto GnRH neurons has been confirmed through electron microscopy (Skrapits et al. 2015; Ward et al. 2009; Williamson-Hughes et al. 2005; Wu et al. 2009). Expression of *Mchr1* mRNA has been reported in approximately half of the GnRH population, although no $MCHR1^+$ cilia are found in those cells (Diniz et al. 2020; Williamson-Hughes et al. 2005). A subset of kisspeptin-sensitive GnRH neurons of the medial septal nucleus and diagonal band of Broca respond postsynaptically to MCH, suggesting MCH and kisspeptin signals converge onto those neurons (Wu et al. 2009).

Given that we detected ciliary MCHR1 closely associated with kisspeptin neurons, MCH may act synaptically on GnRH neurons and through volume transmission over kisspeptin neurons to perform its complex regulation of LH secretion. In addition to action over GnRH somas in the basal forebrain and preoptic hypothalamus, NEI^+ fibers have been identified in close apposition to $GnRH^+$ fibers coursing through the external layer of the ME, raising the possibility that MCH and NEI also modulate LH secretion by altering the release of GnRH into the hypophyseal portal system (HPS) through axo-axonal contacts. This would explain why incubation of ME of proestrus female rats with 10^{-10} or 10^{-9} M of MCH leads to increased GnRH in the media after 30 min (Attademo et al. 2006; Chiocchio et al. 2001; De Paul et al. 2009; Ward et al. 2009; Williamson-Hughes et al. 2005).

13.5.1.3 The Direct Action of MCH and NEI on Gonadotropes

Both MCH^+ and NEI^+ varicose fibers are found in the external lamina of the ME, with dense fiber plexuses found near blood vessels, leading to the suggestion that MCH and NEI are released in the HPS. The addition of NEI to isolated hypophyses increases LH release in the culture media after 1 hour, remaining high for up to 5 hours (study endpoint). Likewise, the incubation of isolated hypophyses obtained from proestrus females with MCH leads to increased LH and follicle-stimulating hormone (FSH) in the culture media. These secretory changes are accompanied by the development of the rough endoplasmic reticulum and Golgi apparatus, accompanied by a reduction in secretory granules in the presence of vesicle exocytosis. These results strongly suggest that both MCH and NEI act directly on gonadotropes to promote LH release and possibly FSH (Chiocchio et al. 2001; De Paul et al. 2009).

13.5.1.4 Sex Steroids Actions on MCH Neurons

The orexigenic (but not locomotor) effect of intraventricular MCH in OVX females is suppressed in EB-supplemented animals compared to controls, suggesting EB influences orexigenic circuits of MCH in a specific manner (Messina et al. 2006; Santollo and Eckel 2008). This effect is at least partially mediated by the action of MCH in the Acb. While activation of MCHR1 in the Acb leads to an increase in feeding in males, only OVX females without treatment displayed a similar response, while supplementation with EB abolished that effect. Given that *Mchr1* mRNA and estrogen receptor 1 (*Esr1*) mRNA are co-distributed in the shell of the Acb, it is likely that both MCH and E2 signaling converge in the Acb to modulate the orexigenic effect of MCH (Terrill et al. 2020).

Hyperestrogenemia has temporally sensitive effects on the expression of *Pmch* mRNA in the LHA. Implantation of E2 pellets in male mice upregulates *Pmch* expression after 48 hours but downregulates it after 22 days and prevents the increase in *Pmch* transcription secondary to caloric restriction (Morton et al. 2004; Mystkowski et al. 2000; Tritos et al. 2004). Physiological single injections of EB or an ERα agonist promote a decrease in the total number of MCH neurons in the LHA 9 and 6 hours after injection, respectively (Santollo and Eckel 2013). In OVX cynomolgus monkeys, acute injections of EB promote a rise in MCH and NEI in the hypothalamus within 72 hours post-administration (Viale et al. 1999a). This effect seems to be mediated through a polysynaptic circuit since MCH neurons of the LHA lack ERα, despite the presence of both markers in the lateral hypothalamus (Muschamp and Hull 2007; Santollo and Eckel 2013). The exception to this is the preoptic cluster, where 70% of MCH neurons colocalize with ERα, allowing E2 to act directly on those neurons (Teixeira et al. 2020).

13.5.2 Growth Hormone

Evidence indicates that both MCH and NEI act as pro-growth hormone (GH) hypophysiotropic hormones (Fig. 13.12). The addition of MCH and NEI in nanomolar concentrations leads to an increase (62% and 124%, respectively) in GH secretion when applied to human fetal hypophyseal cells and mouse hypophyses. A similar phenomenon has been reported in the teleost *Cichlasoma dimerus*, suggesting a pro-GH action of MCH has been conserved in vertebrate evolution (Pérez-Sirkin et al. 2012; Segal-Lieberman et al. 2006).

13.5.3 Cortisol

As is the case with sex steroids, there is a complicated relationship between MCH and the physiological machinery involved in modulating CORT release, with conflicting reports in the literature as the precise role of MCH (Fig. 13.13). Most works point to a pro-secretion role for MCH in the basal release of CORT, while it suppresses release in stress conditions.

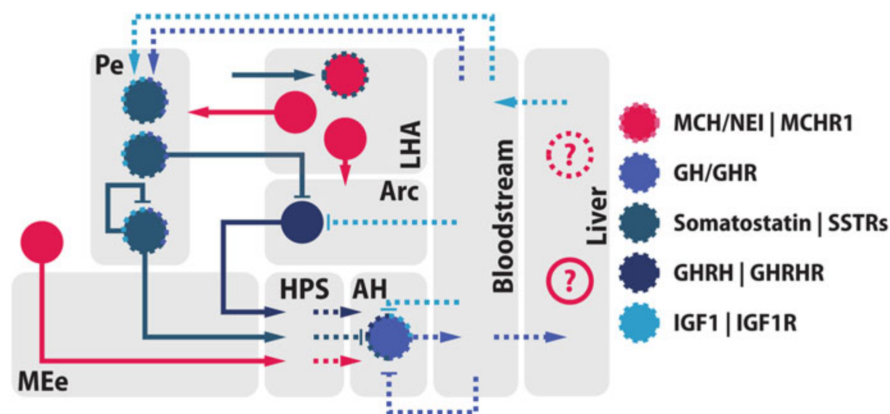

Fig. 13.12 The role of MCH in the hypothalamic-pituitary-somatotropic axis. Schematic representation of the main components of the axis and their relationship with MCH neurons. For clarity, not all elements of the system are represented. Circles represent cells and their neuromodulator/hormonal content, broken circles represent receptors in the surface of the cells or of unknown subcellular location, continuous lines indicate wired projections, broken lines represent humoral communication or unknown pathways. Elements drawn out of gray boxes may originate from multiple or undetermined areas. A question mark is used in cases where MCH or MCHR1 has been reported in peripheral structures but there were no dedicated confirmatory studies. Abbreviations: *AH* adenohypophysis, *Arc* arcuate nucleus, *GH* growth hormone, *GHR* GH receptor, *GHRH* growth hormone-releasing hormone, *GHRHR* growth hormone-releasing hormone receptor, *HPS* hypophyseal portal system, *IGF1* insulin-like growth factor 1, *IGF1R* insulin-like growth factor 1 receptor, *LHA* lateral hypothalamic area, *MCH* melanin-concentrating hormone, *MCHR1* MCH receptor 1, *MEe* median eminence, external layer, *NEI* neuropeptide E-I, *Pe* periventricular hypothalamic nucleus, *SSTR* somatostatin receptor

13.5.3.1 The Effect of MCH/NEI on CRF Neurons

The addition of MCH or NEI to hypothalamic explants raises the level of CRF in the medium (56% over baseline and 134% over baseline, respectively), suggesting MCH promotes the release of CRF (Jezová et al. 1992). This effect is likely mediated by the substantial population of CRF neurons in the PVH associated with $MCHR1^{+}$ primary cilia. However, it should be noted that a different study found no increase in CRF release after incubation of rat hypothalamic explants with synthetic rat MCH (Navarra et al. 1990).

13.5.3.2 The Effect of MCH/NEI on ACTH Release

Intraventricular injection of nanomolar concentrations of MCH in male Wistar rats was reported to result in a transient rise in plasma ACTH (approximately 44% over baseline) 10 min after injection that disappears after 20 min. Intranuclear injection of MCH into the PVH has a more intense effect, increasing plasma ACTH by 133% over baseline after 10 min. Intraventricular injection of 3 μg of MCH 2 hours after the beginning of the light phase elevates plasma ACTH within 15 min of injection, but not at 30 min. Intraventricular injection of 50 μg of MCH in rats leads to an increase in plasma ACTH levels (200–300% of baseline) as soon as 5 min after

Fig. 13.13 The role of MCH in the hypothalamic-pituitary-adrenal (HPA) axis. Schematic representation of the main components of the HPA axis and their relationship with MCH neurons. For clarity, not all elements of the HPA system are represented. Circles represent cells and their neuromodulator/hormonal content, broken circles represent receptors in the surface of the cells or of unknown subcellular location, continuous lines indicate wired projections, broken lines represent humoral communication or unknown pathways, and thin wedges represent ciliary receptors. A question mark is used in cases where MCH or MCHR1 has been reported in peripheral structures but there were no dedicated confirmatory studies, or when the exact role of MCH neurons over other neurons has not been determined. Abbreviations: *3V* third ventricle, *ACTH* adrenocorticotropic hormone, *AH* adenohypophysis, *CORT* cortisol/corticosterone, *CRF* corticotropin-releasing factor receptor, *CRFR* CRF receptor, *GR* glucocorticoid receptor, *HPS* hypophyseal portal system, *LHA* lateral hypothalamic area, *MC2R* melanocortin receptor 2, *MCH* melanin-concentrating hormone, *MCHR1* MCH receptor 1, *MEe* median eminence, external layer, *NEI* neuropeptide E-I, *PVH* paraventricular hypothalamic nucleus, *V1R* vasopressin receptor 1, *VP* vasopressin

injection and remained elevated up to 17 min (experiment endpoint) (Kennedy et al. 2003; Smith et al. 2006, 2009). It should be noted, however, that other researchers found a decrease in plasma ACTH levels following intraventricular injections of nanomolar concentrations of MCH in male Wistar rats during the light phase or after stress in the dark phase, and a decrease in plasma ACTH levels following handling stress both 45 and 90 min after stimulation (Bluet-Pajot et al. 1995; Ludwig et al. 1998), or no alteration whatsoever after intracerebroventricular infusion of MCH or NEI at 10 µg/h for 24 h in adult OVX ewes (Parkes 1996).

13.5.3.3 The Effect of MCH/NEI on CORT Release

Intranuclear injections of picomolar concentrations of MCH into the PVH of male rats increase circulating CORT (66% over baseline) after 10 min (Kennedy et al. 2003). Accordingly, intraventricular injections of 3 µg of MCH 2 hours after the beginning of the light period substantially elevates circulating levels of CORT after 15 min, remaining elevated up to 60 min (study endpoint) (Kennedy et al. 2003; Smith et al. 2006). On the other hand, no alterations in circulating CORT were found following continuous injection of MCH or NEI intraventricularly in OVX ewes, and intraventricular injections of MCH in rats were found to decrease levels of circulating CORT following handling stress (Ludwig et al. 1998; Parkes 1996).

13.5.3.4 Interactions Between MCH and CORT in Teleosts

Synthetic salmon MCH at picomolar concentrations reduces ACTH release by isolated hypophyses of stressed trout and inhibits the induced secretion of ACTH by CRF, and removed hypophyses from stressed trout chronically injected with salmon MCH release less ACTH *in vitro* (Baker et al. 1985, 1986). Immunoneutralization of MCH in isolated trout hypothalamus also indicates a depressive role for MCH over ACTH release (Baker et al. 1985, 1986; Green et al. 1991). Injection of MCH in trout reduces the stress-induced rise in plasma CORT during the first hour, but not the total rise over the experiment (Gilchriest et al. 2001). As a side note, the tilapia homolog of mammalian NGE was reported to stimulate ACTH release from hypophyses *in vitro* (Gröneveld et al. 1996).

13.5.4 Thyroid Hormones

13.5.4.1 The Actions of MCH on Thyroid Hormones

Evidence in the literature points to MCH having an effect on the release of thyroid hormones, both centrally and peripherally (Fig. 13.14). Administration of 1 μM MCH or NEI reduces the basal production of TRH (73% and 40%, respectively). Accordingly, intraventricular injection of nanomolar concentrations of MCH in male rats depresses plasma thyroid-stimulating hormone (TSH) at 10 (29% reduction from

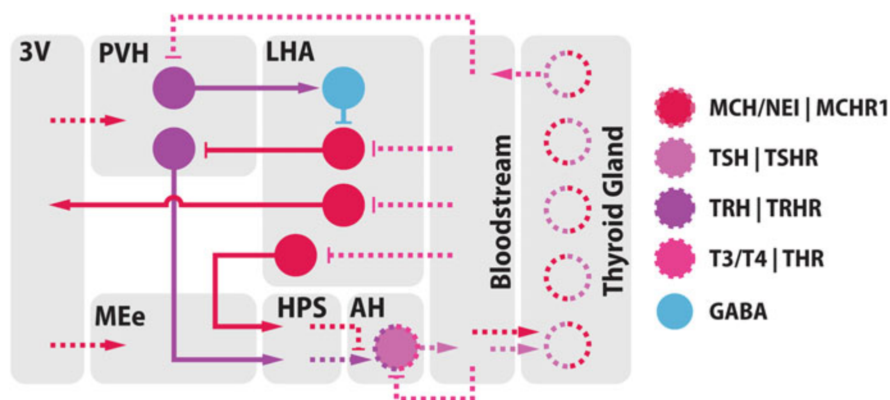

Fig. 13.14 The role of MCH in the hypothalamic-pituitary-thyroid (HPT) axis. Schematic representation of the main components of the HPT axis and their relationship with MCH neurons. For clarity, not all elements of the HPT system are represented. Circles represent cells and their neuromodulator/hormonal content, broken circles represent receptors on the surface of the cells or of unknown subcellular location, continuous lines indicate wired projections, and broken lines represent humoral communication or unknown pathways. Abbreviations: *3V* third ventricle, *AH* adenohypophysis, *HPS* hypophyseal portal system, *LH* lateral hypothalamic area, *MCH* melanin-concentrating hormone, *MCHR1* MCH receptor 1, *MEe* median eminence, external layer, *PVH* paraventricular hypothalamic nucleus, *T3* triiodothyronine, *T4* thyroxine, *THR* thyroid hormone receptor, *TRH* thyrotropin-releasing hormone, *TRHR* thyrotropin-releasing hormone receptor, *TSH* thyroid-stimulating hormone, *TSHR* thyroid-stimulating hormone receptor

baseline) and 60 (53% reduction from baseline) minutes. At the level of the adenohypophysis, MCH counteracts the TRH stimulation of TSH release without inducing a reduction in basal release (Kennedy et al. 2001). There is also evidence for a peripheral action of MCH, as *Mchr1* mRNA is found in the thyroid gland, with expression levels that are substantially higher than that of other peripheral tissues. In *Mchr1* KO animals, T4 secretion in response to TSH is reduced compared to WT mice, and circulating levels of thyroxine (T4), triiodothyronine (T3), and rT3 are all significantly depressed (Chung et al. 2012).

13.5.4.2 The Effect of Thyroid Hormones on NEI Synthesis and Release

There is an intricate pattern of interaction between NEI content, time of day, and circulating thyroid levels. While LHA levels are unaltered, NEI content in the perifornical nucleus decreases after 24 days of altered thyroid hormone levels, regardless of the time of the day when the measurement has taken place. Other areas with altered NEI levels include the *organum vasculosum* of the *lamina terminalis*, anteroventral periventricular nucleus, preoptic hypothalamus, PVH, and ME (Ayala et al. 2011, 2013). There is also an effect of TRH on MCH cells that occurs indirectly, through the excitation by TRH of GABA neurons presynaptic to MCH neurons in the LHA (Zhang and van den Pol 2012).

13.5.5 Prolactin

13.5.5.1 The Action of MCH on PRL Release

When injected into the ventricle, 1 μg of MCH leads to a decrease in the levels of 3,4-dihydroxyphenylacetic acid in the ME and an increase in serum PRL, as expected from an inhibitory action over tuberoinfundibular dopamine neurons (TIDA) in the Arc (Yang and Shieh 2005). This effect is likely to be the result of a direct action of MCH on TIDA neurons, as there is extensive presence of ciliary MCHR1 in TH^+ neurons of the arcuate nucleus (Fig. 13.15). Considering that primary cilia are specialized in detecting signals in the surrounding space, and the permeability of the Arc to bloodstream originated signals, it is possible that MCH originated in both the hypothalamus and the bloodstream may inhibit TIDA neurons to disinhibit the secretory activity of lactotropes in the adenohypophysis. It should be noted that some authors did not detect changes in basal plasma levels of PRL in male rats following intraventricular injection of nanomolar concentrations of MCH (Bluet-Pajot et al. 1995).

13.5.5.2 The Action of PRL on MCH Neurons

There is an ambivalent response of MCH to PRL depending on the brain area. Hyperprolactinemia leads to decreased *Pmch* expression (approx. 33%) in the lateral hypothalamus compared to sham-operated controls (Garcia et al. 2003). The opposite is observed in the preoptic cluster, however, as suppression of PRL secretion through bromocriptine decreases the number of MCH neurons in the lactating MPOA, and *Stat5* knockout animals also display fewer MCH neurons in that area

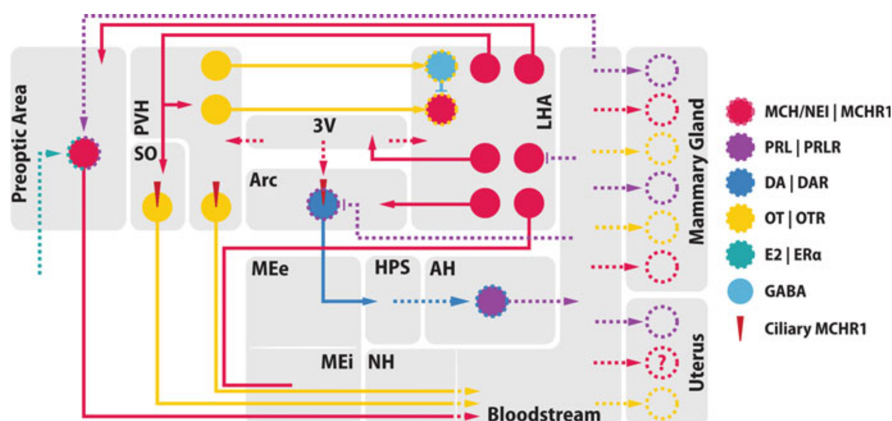

Fig. 13.15 Interactions between MCH, prolactin and oxytocin during lactation. Schematic representation of the main components involved in the hormonal control of lactation processes. For clarity, not all elements are represented. Circles represent cells and their neuromodulator/hormonal content, broken circles represent receptors on the surface of the cells or of unknown subcellular location, continuous lines indicate wired projections, broken lines represent humoral communication or unknown pathways, and thin wedges represent ciliary receptors. A question mark is used in cases where MCH or MCHR1 has been reported in peripheral structures but there were no dedicated confirmatory studies. Abbreviations: *3V* third ventricle, *AH* adenohypophysis, *Arc* arcuate nucleus, *DA* dopamine, *DAR* dopamine receptor, *E2* estrogen/estradiol, *ERα* estrogen receptor α, *HPS* hypophyseal portal system, *LHA* lateral hypothalamic area, *MCH* melanin-concentrating hormone, *MCHR1* MCH receptor 1, *MEe* median eminence, external layer, *MEi* median eminence, internal layer, *NEI* neuropeptide E-I, *NH* neurohypophysis, *OT* oxytocin, *OTR* OT receptor, *PRL* prolactin, *PRLR* PRL receptor, *PVH* paraventricular hypothalamic nucleus, *SO* supraoptic

(Kokay et al. 2020; Teixeira et al. 2020). The action of PRL on preoptic MCH neurons likely results from a direct mechanism: approximately 60% of the *Pmch*-expressing neurons in the MPOA and periventricular nucleus also express the PRL receptor gene (*Prlr*), and more than 90% of neurons in the area colocalize with pSTAT5 after an acute PRL injection. Furthermore, treatment of animals with PRL resulted in a robust synthesis of pSTAT5 in MCH neurons (Kokay et al. 2020; Teixeira et al. 2020).

13.6 Interactions Between MCH and Neurohypophysial Hormonal Systems

13.6.1 Vasopressin

13.6.1.1 Neuroanatomical Substrate

Mouse VP neurons of the PVH synthesize a gene reporter linked to *Mchr1* expression and are co-distributed with MCHR1^{+} primary cilia, albeit to a less extent to VP neurons in the supraoptic nucleus (Fig. 13.13) (Chee et al. 2013; Diniz et al. 2020). This indicates that VP neurons, or at least a subpopulation of those neurons, can

respond to extracellular MCH. Conversely, MCH neurons have V1a receptors. Through these receptors, VP causes a postsynaptic excitatory effect on MCH neurons, depolarizing them and increasing spike frequency under current clamp. Vasopressin also enhances both excitatory and inhibitory synaptic transmission into MCH neurons (Yao et al. 2012).

13.6.1.2 Physiological Actions of MCH

Despite the anatomical substrate, there is no evidence for physiological interactions between MCH and VP. Administration of 1 μM MCH to hypothalamic explants of adult male Wistar rats does not affect VP release, and continuous intraventricular injection of human MCH in OVX ewes does not change circulating levels of VP (Kennedy et al. 2003; Parkes 1996). There is also no evidence of MCH action at the NH level since the addition of nanomolar or micromolar concentrations of MCH to dissociated rat hypophyses results in no change in VP release (Parkes and Vale 1993). The physiological relevance of MCH-VP interactions therefore remains unclear. It is noteworthy that there is some degree of functional overlap between the two populations. Intraventricular injection of microgram concentrations of MCH in male Long-Evans rats significantly increases water intake in the absence of food within 2 hours of intervention. While central MCH promotes water ingestion, administration of an MCHR1 antagonist alone does not influence water intake, suggesting MCH is not constitutively part of the water intake circuitry (Clegg et al. 2003; Morens et al. 2005). There may also be interactions between MCH and VP in the CORT secretion actions of VP, but further experiments are necessary.

13.6.1.3 Physiological Actions of NEI

Administration of NEI to dissociated hypophyses of adult rats reduces VP secretion after 1 ($57 \pm 10\%$ of the baseline) and 3 hours ($68 \pm 11\%$), suggesting a direct role for NEI in modulating the release of VP by terminals in the NH (Parkes and Vale 1993).

13.6.2 Oxytocin

13.6.2.1 Neuroanatomical Substrate

Mouse oxytocinergic neurons of the PVH synthesize a gene reporter linked to *Mchr1* expression and are substantially co-distributed with $MCHR1^+$ primary cilia (Chee et al. 2013; Diniz et al. 2020). This indicates that OT neurons, or at least a subpopulation of those neurons, can respond to extracellular MCH. Fibers immunoreactive to both MCH and NEI have been found in moderate densities in areas rich in OT and VP neurons, such as the PVH and the SO, and close to OT fibers coursing through the internal layer of the ME towards the NH (Costa et al. 2019). These internal layer MCH fibers form axon terminals, with large dense- cores vesicles immunoreactive to MCH found apposed to other local axons. These terminals are also found in the NH, where OT^+ fibers release their contents into the bloodstream.

Finally, the presence of MCH neurons in the anterior PVH during lactation could allow for the paracrine-like release of MCH to act in nearby OT neurons (Fig. 13.15).

Conversely, approximately 60% of MCH neurons have been reported to express the gene for the OT receptor (*Oxtr*), through which application of OT to current-clamped MCH neurons leads to depolarization and a substantial increase in firing rate. Oxytocin also enhances inhibitory synaptic communication of MCH neurons, but unlike VP, it does not change excitatory activity. This effect seems to be mediated by a very small population (0.7 ± 0.4% of the total OT population) of OT neurons in the PVH (Sanathara et al. 2018; Yao et al. 2012).

13.6.2.2 Physiological Actions of MCH and NEI

Addition of nanomolar and micromolar concentrations of MCH to dissociated rat hypophyses leads to an increase in OT secretion after 3 hours (188 ± 29%), suggesting MCH facilitates the release of OT by terminals in the NH. The addition of NEI in a similar experimental design has an even more intense pre-release effect, increasing the release of OT after 1 (245 ± 89%) and 3 (209 ± 64%) hours (Parkes and Vale 1993). Melanin-concentrating hormone neurons have also been implicated as mediators of some of the central actions of OT (Phan et al. 2020; Sanathara et al. 2018, 2020).

13.7 Perspectives

While the role of MCH as a hormone in teleosts is unquestionable, its status as a central neuromodulator in mammals has been favored in the literature, fueled mainly by the discovery that its role in adaptive color change is not a universal feature in early vertebrates but an acquisition in the teleost lineage (Baker and Bird 2002), and the attention given to the orexigenic actions of MCH, which has attracted much of the research into this neuropeptide. However, based on past and recent developments in the field and an overarching view of the literature, it is our opinion that the roles of hypophysiotropic hormone, neurohypophysial hormone, and neuromodulator for MCH should be treated with equal importance.

Hormones are a large class of chemical messengers with diverse compositions, functions, and origins. The term "hormone" was coined by Ernest Starling in a series of lectures to the Royal College of Physicians in 1905 (Starling, 1905 *apud* Hirst 2004) following his discovery of secretin in 1902 (Bayliss and Starling 1902). *Per* the original definition, hormones are messengers that "have to be carried from the organ they are produced to the organ which they affect by means of the bloodstream" (Starling, 1905 *apud* Hirst 2004), drawing from the concept of a chemical reflex, happening independent of the nervous system. This definition still holds, but modern concepts have expanded endocrine communication to include other modalities, including paracrine, autocrine, and intracrine communication.

To account for some particularities, hormones produced by neurons (and some specialized cells) are categorized as neurohormones. Within the nervous system, two classes of neurohormones are found: hypophysiotropic neurons and hypophyseal

hormones. Hypophysiotropic hormones consist of a highly specialized class of hormones produced by neurons in the hypothalamus, with axonal projections that reach the external layer of the ME to release these hormones in the HPS. From there, hypophysiotropic hormones reach the adenohypophysis (endocrine in origin) to modulate the activity of somatotropes, lactotropes, gonadotropes, corticotropes, and thyrotropes. On the other hand, neurohypophyseal hormones are not released in the HPS but directly into the general circulation. These hormones are produced by a specialized class of neurons, magnocellular secretory neurons of the PVH and supraoptic nucleus. Axons from these neurons travel through the internal layer of the ME and the hypophyseal stalk to reach the NH, where the neurohypophyseal hormones are released in blood vessels.

Melanin-concentrating hormone fits the criteria of both a hypophysiotropic and a neurohypophyseal hormone, while NEI can be classified as a hypophysiotropic hormone based on current data. Immunoreactive fibers for MCH and NEI are found in the external layer of the ME forming varicose buttons in the neighborhood of blood vessels. This allows for the release of MCH and NEI in the HPS. In the adenohypophysis, MCH promotes the release of LH and FSH, while NEI promotes LH release by gonadotropes; MCH and NEI promote the release of GH by somatotropes; and MCH counteracts the TRH-dependent increase in TSH release by thyrotropes. Since MCH also acts on hypophysiotropic neurons that will ultimately modulate the action of adenohypophyseal cells, one may ask why a direct hypophysiotropic effect is beneficial. While speculative, we believe a direct role over adenohypophyseal cells allows MCH and NEI to bypass possible antagonistic signals being integrated by hypophysiotropic neurons.

Several lines of evidence also support the neurohypophyseal role of MCH: (1) MCH neurons send axons through the internal layer of the ME and into the NH; (2) MCH neurons are labeled following injection of a retrograde tracer in the bloodstream; (3) MCH is found in the plasma and serum, and its mature form matches that produced in the brain, but not the NEI-MCH form produced in the periphery; (4) MCHR1 receptors are widely found in peripheral tissues, allowing for action away from the organ where MCH is produced; (5) MCHR1 is found in several targets under strong endocrine regulation, including the mammary glands. Taken together, these data indicate the existence of a complete MCH endocrine axis that can affect multiple systems.

Melanin-concentrating hormone has been implicated in over 12 families of functions, including feeding and water consumption, energy balance, sleep, learning, mood, sexual behavior, maternal behavior, sensory integration, ciliary beating, immunological function, and cardiorespiratory function. Understanding this neuropeptidergic system is essential for understanding vertebrate physiology, both in natural and in pathological conditions. The central roles of MCH as a neuromodulator, however, have overshadowed the hormonal aspect of MCH and NEI, and much remains to be elucidated about these hormones' role in the periphery. In part, the challenges associated with understanding the hormonal aspects of MCH and NEI derive from the often contradictory or hard to replicate results available in the literature. The development of new techniques and increased transparency in the

reporting of materials and methods should ease some of those challenges and accelerate profoundly necessary discoveries. Several outstanding questions remain unanswered, including:

- Neuropeptide E-I plays multiple roles in modulating neuroendocrine functions, including potent actions at the level of the adenohypophysis that, in some cases, surpass MCH actions. However, there is no known NEI receptor or mechanism of action.
- The tilapia homolog of NGE has been reported to promote the release of ACTH. It is still unknown whether NGE is constitutively produced in mammals and if it has any actions.
- Synaptic roles for MCH have been extensively described, but direct visualization of MCHR1 in the synapse has proven challenging.
- MCHR1 has been identified in multiple peripheric tissues where no role for it has been described.
- Both MCH and MCHR1 have been identified in reproductive organs, but their exact role in reproductive physiology through these organs is poorly understood.
- MCH neurons in the brain respond to the lactation period and project to the NH, and MCHR1 is found in the mammary gland, but there is no known mechanism of MCH action on milk secretion.
- MCH plays a role in modulating sex steroids, but this modulation depends on the hormonal milieu of the animal and appears contradictory in some studies. The exact role of MCH in the presence of sex steroids needs further investigation.
- Opposing actions of MCH have been reported in the release of CORT, and no single model of action has been produced.
- Vasopressin neurons of the PVH express *Mchr1* and contain ciliary MCHR1, but no direct interaction between MCH and VP has been demonstrated.
- While progress has been made in understanding the role of MCH in central actions of OT, there are substantial gaps in our understanding of how these two neurohormones interact peripherally.

Key References

Kawauchi et al. (1983)—The first paper to isolate MCH material from hypophyses.

Bittencourt et al. (1992)— Prototypical distribution of MCH and NEI sites of synthesis and projections.

Qu et al. (1996)—Initial description of the orexigenic role of MCH.

Saito et al. (1999)—One of multiple papers describing MCHR1 as the specific MCH receptor.

Verret et al. (2003)—Discovery of an MCH role in sleep architecture.

Van den Pol et al. (2004)—Description of neurophysiological properties of MCH neurons.

Berbari et al. (2008)—Identification of MCHR1 as a ciliary receptor.

Mickelsen et al. (2017)—Transcriptome of MCH neurons, revealing major neurochemical properties.

Noble et al. (2018)—Empirical evidence of volume transmission in the MCH peptidergic system.

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