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Review

Anatomical organization of the melanin-concentrating hormone peptide family in the mammalian brain

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ABSTRACT

More than 20 years ago, melanin-concentrating hormone (MCH) and its peptide family members – neuropeptide EI (NEI) and neuropeptide GE (NGE) – were described in various species, including mammals (rodents, humans, and non-human primates). Since then, most studies have focused on the role of MCH as an orexigenic peptide, as well as on its participation in learning, spatial memory, neuroendocrine control, and sleep. It has been shown that MCH mRNA or the neuropeptide MCH are present in neurons of the prosencephalon, hypothalamus and brainstem. However, most of the neurons containing MCH/NEI are within the incerto-hypothalamic and lateral hypothalamic areas. In addition, the terminals of those neurons are distributed widely throughout the central nervous system. In this review, we will discuss the relationship between those territories and the roles played by MCH/NEI, as well as the importance of MCH receptor 1 in the respective terminal fields. Certain neurochemical features of MCH- and NEI-immunoreactive (MCH-ir and NEI-ir) neurons will also be discussed. The overarching theme is the anatomical organization of an inhibitory neuropeptide colocalized with an inhibitory neurotransmitter in integrative territories of the central nervous system, such as the IHy and LHA. Although these territories have connections to few brain regions, the regions to which they are connected are relevant, being responsible for the organization of motivated behaviors. All available information on this peptidergic system (anatomical, neurochemical, hodological, physiological, pharmacological and behavioral data) suggests that MCH is intimately involved in arousal and the initiation of motivated behaviors.

1. Introduction

From its initial discovery in teleost fish [52,83], and subsequently in mammals in 1989 [72,119], to the present day, the melanin-concentrating hormone (MCH)[1] peptide family has been studied thoroughly at many different levels of analysis. This family comprises MCH itself and two additional neuropeptides, neuropeptide EI (NEI) and neuropeptide GE (NGE), processed from the MCH precursor [72,119]. Bittencourt et al. [9] reported the mean proportion of MCH-ir cells that colocalize with NEI in corresponding diencephalic neurons to be 96%. It is known that NEI can induce increases in grooming behavior and motor activity [93,94]. Sanchez et al. [95] also showed that high doses of NEI increase the maximum density and apparent affinity of a dopamine D_1 receptor antagonist, suggesting an interaction between this peptidergic system and the D_1 receptor in the striatum. It has also been shown that NEI and MCH exhibit antagonistic effects on the stress-induced release of adrenocorticotropic hormone (ACTH) in rats [12]. In addition Attademo et al. [3] demonstrated that NEI can induce the secretion and release of luteinizing hormone through various mechanisms. Furthermore, Kis-

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[1] *Abbreviations used:* α-MSH, alpha-melanocyte stimulating hormone; AcbSh, nucleus accumbens shell; ACTH, adrenocorticotropic hormone; ADA, adenosine deaminase; CART, cocaine- and amphetamine-regulated transcript; CNS, central nervous system; CTb, cholera toxin B subunit; DBB, diagonal band of Broca; DMH, dorsomedial nucleus of the hypothalamus; DY, Diamidino Yellow; FB, Fast Blue; FG, Fluoro-Gold; GABA, gammaaminobutyric acid; GAD67, glutamic acid decarboxylase-67; HF, hippocampal formation; IHy, incerto-hypothalamic area; -ir, immunoreactive; LDT, laterodorsal tegmental nucleus; LHA, lateral hypothalamic area; LHAa, anterior region of the lateral hypothalamic area; LHAp, posterior region of the lateral hypothalamic area; LHAt, tuberal region of the lateral hypothalamic area; LM, lateral mammillary nucleus; LS, lateral septal nucleus; MCH, melaninconcentrating hormone; MCHR1, MCH receptor 1; MCx, motor cortex; ME, median eminence; MFB, medial forebrain bundle; ML, *pars lateralis* of the medial mammillary nucleus; MS, medial septal nucleus; NEI, neuropeptide EI; NGE, neuropeptide GE; PAG, periaqueductal gray matter; PHA-L, *Phaseolus vulgaris* leucoagglutinin; PMCHL, pro-MCH-like; POMC, proopiomelanocortin; PP, posterior pituitary (gland); ppMCH, prepro-MCH; PPT, pedunculopontine tegmental nucleus; PVH, paraventricular nucleus of the hypothalamus; SFO, subfornical organ; TB, True Blue; VMH, ventromedial nucleus of the hypothalamus.

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tler-Heer et al. [60] showed that, although low doses of alpha-melanocyte stimulating hormone (α-MSH) and NEI increase the growth of neurofilament protein, higher doses of NGE are required in order to achieve the same effect. Hintermann et al. [48] presented an interesting body of data on the interactions among MCH, NEI, NGE, and α-MSH (and their respective receptors) at low doses of MCH, as well as the interactions among melanocortin receptors only at high doses of MCH. The MCH and proopiomelanocortin (POMC) systems might work agonistically or antagonistically in the central nervous system (CNS), as they do in the periphery through paracrine/autocrine mechanisms.

The structure of MCH has remained highly conserved over millions of years of evolution [20,25] and, despite the diversity of species in which it is found, its cellular expression has remained sequestered in discrete regions of the hypothalamus. However, in contrast to its neurohormonal role in regulating adaptive pigmentation in fish [58], MCH has been shown to have orexigenic effects in mammals [82]. This finding was made only 2 years after the cloning of the *ob* gene and its protein product, leptin [130], an adipocyte hormone that informs hypothalamic systems in terms of the levels of peripheral energy (fat) stores [97]. Both discoveries (of MCH as an orexigenic peptide and of leptin) came at a time when obesity and metabolic syndrome were becoming recognized as having grown to epidemic proportions in America and worldwide. Consequently, there was an explosion of interest in MCH and the regulation of energy balance, given that research into an orexigenic peptide expressed in a hypothalamic area historically implicated in appetitive control could foster key insights into the mechanisms of obesity. As a consequence, there was a tremendous increase in the number of published articles on food intake, feeding behavior, energy balance control and related themes. Between 1990 and 2004 there was a glut of published papers searchable in PubMed under the terms "food intake" and "MCH" (http://www.ncbi.nlm.nih.gov). Since that time, the number of novel "MCH vs. Feeding" studies has declined, indicating that work focused solely on MCH, its receptors, agonists, and antagonists is insufficient to answer fundamental and complex questions as to how normal feeding is regulated or to identify the mechanisms by which dysregulated feeding can lead to obesity. Nevertheless, there is a large body of evidence indicating that MCH is involved in feeding behavior: MCH mRNA is abundant in obese (*ob/ob*) mice [82]; MCH administration in the cerebral ventricles or to specific hypothalamic nuclei that express the MCH receptor (MCHR1) induces hyperphagia and increases body weight [1,40,64,81,87,89]; MCH "knock-out" mice display decreased body weight due to hypophagia and increased energy expenditure [102]; MCHR1-deficient mice are lean despite consuming excess food [23]; and administration of MCHR1-specific antagonists decrease body weight [13,101,117].

The distribution of MCH has been mapped in the rat [9], mouse [82], and cat [118], as well as in nonhuman primates, in humans [8,31], and, most recently, in birds [21]. In each of those species, mRNA expression of MCH and MCH immunoreactivity are localized principally to the lateral hypothalamic area (LHA), and to a lesser degree to the adjoining incerto-hypothalamic area (IHy). It seems that, regardless of species, the LHA has maintained its place and role.

The LHA is one of the most complex regions of the CNS, harboring at least 35 different cell types [37] distributed throughout 22 different subdivisions. It has been referred to as the "bed nucleus of the medial forebrain bundle" (MFB), and is arguably the major north–south fiber system of the brain [38,75,120]. The cells scattered among the LHA were once called "path neurons" [69], and the arrangement of those cells along the MFB resemble "unison notes on a musical score" [66]. Consequently, the LHA has been associated with a broad array of functions, ranging from sensory information processing to somatomotor responses and the regulation of motivated and goal-directed behaviors [41,97,114]. Nevertheless, the LHA has been strongly implicated in the control of hunger and thirst, although those data have been contested [2,111,125].

The second largest population of MCH-expressing neurons is found in the IHy, a region that is poorly understood in all of its aspects, including cytoarchitecture, neurochemical makeup and connectivity. Only recently have the projections of this region been described in detail [104]. Outputs from this region suggest that it participates in neuroendocrine functions related to motivated behaviors [104].

The projections of MCH-expressing hypothalamic neurons are exceptionally widespread, with major targets including the median eminence (ME), hippocampal formation (HF), prefrontal cortex (PfCx), periaqueductal gray matter (PAG), pars lateralis of the medial mammillary nucleus (ML), nucleus accumbens shell (AcbSh) and medial septal nucleus (MS). Therefore, it would seem appropriate to consider the LHA and IHy to be integrative centers, positioned to influence a broad array of systems and functions, and not as direct modulators of motor and effector functions.

Here we will review the organization of the MCH system in species ranging from rodents to man. Similarities and differences in terms of the sites of cellular expression and fiber distribution will be considered, with a view to clarifying the functional associations within this fascinating and important peptide system.

2. Cellular localization of prepro-MCH mRNA and MCH immunoreactivity

2.1. Rodents

2.1.1. Rat

After the discovery of MCH in fish [52], antisera raised against salmon MCH were used in a rat model in order to obtain maps of the anatomical sites producing the MCH, which were found to be located exclusively in the diencephalon – mainly within the LHA and IHy [34,96,107,129]. However, the use of those antibodies led to the conclusion that the perikarya in these regions also synthesize α-MSH and rat corticotropin-releasing factor (rCRF), and immunoreactivity to human growth hormone-releasing factor has been observed [33–35,43,51,73,79]. Likewise, the same group of cells in the LHA, identified by the use of an antibody against α-MSH, received the nickname of alpha-2 neurons, as alpha-1 cells are localized in the arcuate nucleus (Arc) and the nucleus of solitary tract (NTS) [57,96,123,124]. The Arc and NTS are the only two sites that truly express POMC mRNA and immunoreactivity for the α-MSH peptide [80].

The first map created using species-specific reagents was published in 1992 [9]. The authors found the combination of prepro-MCH (ppMCH) mRNA and MCH-ir in two diencephalic regions: the hypothalamus; and a region that at the time was referred to as the rostromedial zona incerta. However, the results of the hodological studies conducted by Sita et al. [104], indicated that this region belongs to the medial hypothalamic system. Because this region harbors the second largest population of ppMCH and MCH/NEI-ir neurons, a proposal was made to change its name to IHy [6,104] (Fig. 2). At least seven different diencephalic regions harbor MCH-containing cells: the IHy (Fig. 2); the anterior, tuberal, and posterior subdivisions of the LHA (LHA_a, LHA_t, and LHA_p) (Fig. 2); the anterior periventricular nucleus (Fig. 3); the dorsolateral part of the zona incerta (Fig. 2); and the dorsomedial part of the tuberomammillary complex (Fig. 3). Nevertheless, when we look more closely at the anatomical distribution of ppMCH mRNA, MCH-ir, and NEI-ir in the CNS of the normal rat, a moderate number of labeled cells appear in the medial hypothalamus, the caudal part of the anterior hypothalamic nucleus. These cells are localized