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Orexins (hypocretins) and energy balance: More than feeding

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ABSTRACT

Initially implicated in the regulation of feeding, orexins/hypocretins are now acknowledged to play a major role in the control of a wide variety of biological processes, such as sleep, energy expenditure, pain, cardiovascular function and neuroendocrine regulation, a feature that makes them one of the most pleiotropic families of hypothalamic neuropeptides. While the orexigenic effect of orexins is well described, their central effects on energy expenditure and particularly on brown adipose tissue (BAT) thermogenesis are not totally unraveled. Better understanding of these actions and their possible interrelationship with other hypothalamic systems controlling thermogenesis, such as AMP-activated protein kinase (AMPK) and endoplasmic reticulum (ER) stress, will help to clarify the exact role and pathophysiological relevance of these neuropeptides have on energy balance.

1. Introduction

The role of the hypothalamus in the regulation of endocrine and autonomic functions has been known for more than 70 years (Everitt and Hokfelt, 1990; Bernardis and Bellinger, 1993, 1998; López et al., 2007c, 2009, 2010a; Schneeberger et al., 2014; Saper and Lowell, 2014). Proper co-ordination of these functions is of relevance for several basic physiological processes, such as feeding, drinking, neuroendocrine regulation, reproduction, lactation, cardiovascular function, metabolic control, thermoregulation and sleep-wake cycle. All these mechanisms are mediated by neurotransmitters and neuromodulators, often with redundant and overlapping roles, that provide a robust system to control body energy homeostasis (López et al., 2007c; Schneeberger et al., 2014; Saper and Lowell, 2014).

The lateral hypothalamic area (LHA) has classically been implicated in all the functions described above (Everitt and Hokfelt, 1990; Bernardis and Bellinger, 1993, 1998; López et al., 2007c, 2009, 2010a; Schneeberger et al., 2014; Saper and Lowell, 2014). However, the exact molecular mediators of those actions remained unknown until the latter part of last 20th century. The melanin-concentrating hormone (MCH) (Skofitsch et al., 1985; Bittencourt et al., 1992; Qu et al., 1996) was the first orexigenic peptide discovered that was located exclusively in LHA. Later, other feeding neuropeptides have found to be expressed at this level: galanin (GAL) (Hakansson et al., 1998), dynorphin (DYN) (Chou et al., 2001) and cocaine- and amphetamine-regulated transcript peptides (CARTps) (Koylu et al., 1998). However, the finding with most impact in terms of explaining the molecular underpinnings of the physiological relevance for LHA came in 1998, when two different laboratories independently discovered a new family of neuropeptides: the *orexins/hypocretins*. Using the directional tag polymerase chain reaction subtraction method *Sutcliffe* and colleagues found a novel mRNA, with expression restricted to the LHA. This mRNA encoded a 130-residue secretory protein, with a proteolytic site that gave rise to two C-terminally amidated peptides. One of these peptides shared an important homology with the gut secretin

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peptide family. For this reason, and considering also their hypothalamic origin, they were called *hypocretins* (Hcrt1 and Hcrt2) (de Lecea et al., 1998). At the same time *Yanagisawa* and colleagues used an intracellular calcium influx assay with cells expressing multiple orphan G-protein-coupled receptors to isolate two novel neuropeptides. Both neuropeptides were originated by the proteolytic processing from a common precursor expressed in the LHA. This location strongly suggested a potential effect on feeding behavior. In keeping with this hypothesis, intracerebroventricular (ICV) administration of these peptides in non-fasted rats stimulated food intake in a dose- and time-dependent fashion. Thus, due to their orexigenic activity, these novel neuropeptides were called *orexins* (OX-A and OX-B) (Sakurai et al., 1998). Seventeen years later orexins are now acknowledged to play a major role in the control of a wide variety of biological processes, such as sleep, energy expenditure, pain, cardiovascular function and neuroendocrine regulation, a feature that make them one of the most pleiotropic families of hypothalamic neuropeptides (Willie et al., 2001; Tsujino and Sakurai, 2009; López et al., 2009, 2010a; Herzig and Purhonen, 2010; Sakurai, 2014; Gao and Horvath, 2014).

2. Structure of the orexins/hypocretins

Although hypocretins and orexins were initially considered as different proteins (Flier and Maratos-Flier, 1998), it was soon demonstrated that they were identical molecules. Prepro-hypocretin gene is identical to prepro-OX gene, and both of them encode the 130-residue (rodent), or 131-residue (human, dog, pig) polypeptide, with a secretory signal and a proteolytic site. This precursor presents an important homology between different species (83% human-mouse; 95% mouse-rat) suggesting a relevant and conserved role in evolution. The processing of this precursor protein results in two peptides: OX-A/Hcrt1 and OX-B/Hcrt2. OX-A is a 33-amino acid peptide (3562 Da) with a N-terminal pyroglutamil residue and C-terminal amidation. The mature peptide shows two disulphide bounds in the N-terminal region (Cys-6-Cys-12; Cys-7-Cys-14). This feature is fundamental to its biological activity (Okumura et al., 2001). This structure is widely conserved in mammalians (human, rat, mouse, dog and pig) (Kilduff and Peyron, 2000; Hungs et al., 2001) and other vertebrate species, such as the genus *Xenopus* (Shibahara et al., 1999). Hcrt1 peptide shows an identical sequence to OX-A, but it was initially deduced with five more amino acid residues in the N-terminal region and a C-terminal glycine (de Lecea et al., 1998; Sakurai et al., 1998). OX-B, identical to Hcrt2, is a linear C-terminal amidated peptide of 28 amino acid residues (2937 Da), but with an N-terminal glycine (de Lecea et al., 1998; Sakurai et al., 1998). Mouse and rat OX-B show an identical sequence. Human OX-B exhibits two amino acid substitutions as compared to the rodent sequence. C-terminal amidation of both orexins seems to be essential for their biological activity (Sakurai et al., 1998). Evolutionary analysis has related orexins with the incretin family. In this sense it has been proposed that the prepro-OX gene arose 650 million years ago, in the early chordate lineage, as a result of a process of circular permutation of incretin gene (Alvarez and Sutcliffe, 2002).

3. Anatomy of the orexin/hypocretin system

The number of orexin neurons in the LHA is limited, with an estimation of about 70,000–90,000 immunoreactive cell bodies in this area in humans and about 4000 in the rat (de Lecea et al., 1998; Sutcliffe and de Lecea, 2000; Thannickal et al., 2000). These neurons are localized specifically in the region of the hypothalamus within the perifornical and dorsomedial hypothalamic (DMH) nuclei and LHA (de Lecea et al., 1998; Sakurai et al., 1998; Broberger et al., 1998; Elias et al., 1998; Date et al., 1999; Horvath et al., 1999). Low levels of prepro-OX mRNA expression have also been detected in the ependymal cell layer of the lateral-, the third- and the fourth ventricles (Kummer et al., 2001). The localization of the orexin cells in the LHA overlaps with the distribution of MCH, but the peptides are not co-expressed within the same neurons (Broberger et al., 1998; Elias et al., 1998). Hypothalamic orexin neurons also express mRNAs of DYN (Chou et al., 2001), GAL (Hakansson et al., 1999), secretogranin II (Risold et al., 1999) and Narp, a secreted neuronal pentraxin (Reti et al., 2002). In the rat, prepro-OX mRNA is detected in the LHA from birth onwards. The levels are low up to day 15, and then increases between days 15 and 20, when expression levels achieve values similar to those found during adulthood (Yamamoto et al., 2000a). In adults, there are sexual dimorphic differences in prepro-OX mRNA expression, with higher levels in females, thus suggesting gender-specific functions of orexins (Johren et al., 2002).

In contrast to the limited localization of orexin cell bodies in the LHA, orexin fibres show a widespread distribution in the central nervous system. In the hypothalamus orexin projections are mainly found in the paraventricular (PVH), ventromedial (VMH) and arcuate (ARC) nuclei (Horvath et al., 1999). Outside the hypothalamus orexin neurons project to the olfactory bulb, cerebral cortex, thalamus, the brainstem and all levels of the spinal cord (Broberger et al., 1998; Elias et al., 1998; Peyron et al., 1998; Date et al., 1999; Nambu et al., 1999). The projections with the highest density are found at the level of the locus coeruleus (LC) within the brainstem, where catecholaminergic neurons are innervated by orexin containing fibers, which suggested a potential role of these peptides in the regulation of the sleep–wake cycle (Peyron et al., 1998). In the spinal cord, long descending orexins axons from cervical to sacral segments are found in mouse, rat and human (van den Pol, 1999). In the spinal cord there are also dense immunoreactive orexin fibers in the marginal zone (lamina I) of the dorsal horn and moderately abundant in the area X, surrounding the central canal, and in the intermediolateral column. This location suggests a potential role of orexin neuropeptides in the modulation of sensory information, pain and in the regulation of the sympathetic (SNS) and parasympathetic (PSNS) nervous system (van den Pol, 1999; Bingham et al., 2001; Yamamoto et al., 2002).

Outside the central nervous system, orexin peptides are expressed in testis (Sakurai et al., 1998; López et al., 1999), where OX-A immunoreactivity has been documented along postnatal maturation, with strong peptide signal in Leydig cells and spermatocytes at specific stages of meiosis (Barreiro et al., 2005), as well as in both the myenteric and the submucosal plexus of the gut in several species (rat, mouse, guinea pig and human) (Kirchgessner and Liu, 1999; de Miguel and Burrell, 2002). In addition, orexin immunoreactivity was found in endocrine cells of the gastric and intestinal mucosa and also in the β cells of the pancreas (Kirchgessner and Liu, 1999; Heinonen et al., 2008). In the rat adrenal gland no expression of prepro-OX has been detected (López et al., 1999), however immunoreactivity for prepro-OX and OX-A has been described in human adrenal (Randeva et al., 2001). Finally, it is interesting to note the existence of OX-A in human plasma (Arihara et al., 2001; Dalal et al., 2001).

4. Orexin/hypocretin receptors

Two different orexin/hypocretin receptors have been cloned, called orexin/hypocretin 1 receptor (OX1R/Hcrtr1), with highest affinity for OX-A, and orexin/hypocretin 2 receptor (OX2R/Hcrtr2), which binds with high affinity to both OX-A and OX-B (Sakurai et al., 1998). These G-coupled proteins differ in the sense that OX1R is exclusively coupled to the Gq subclass of heterotrimeric G