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Review

Effects of changes in energy homeostasis and exposure of noxious insults on the expression of orexin (hypocretin) and its receptors in the brain

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ABSTRACT

This review summarizes data regarding the brain expression of the orexin (hypocretin) system including: prepro-orexin (PPO), orexin A (OxA), orexin B (OxB) and the two orexin receptors 1 and 2 (OxR1, OxR2). Clinical data is limited to OxA and OxB in cerebral spinal fluid and serum/plasma, thus necessitating the development of animal models to undertake mechanistic studies. We focus on changes in animal models that were either exposed to a regime of altered sleep, metabolic energy homeostasis, exposed to drugs and noxious insults. Many more expressional studies are available for PPO, OxA and OxB levels, compared to studies of the receptors. Interestingly, the direction and pattern of change for PPO, OxA and OxB is inconsistent amongst studies, whereas for the receptors, there tends to be increased expression for both OxR1 and OxR2 after alterations in energy homeostasis, and an increased expression after noxious insults or exposure to some drugs. The clinical implications of these results from animal models are discussed in light of the findings from human studies, and future research directions are suggested to fill knowledge gaps with regard to the orexin system, particularly during early brain development.

Abbreviations: ARC, Arcuate Nucleus; BMI, Body Mass Index; CO_2, Carbon dioxide; CPAP, Continuous positive airways pressure; CSF, Cerebral Spinal Fluid; DMH, Dorsal Medial Hypothalamus; DMN, Dorsal Medial Nucleus; DR, Dorsal Ralphe Nucleus; FR, Food restriction; HFD, High Fat Diet; Icv, Intracerebroventricular; IHC, Immunohistochemistry; ISH, In situ hybridization; i.p., Interperitoneal; LHA, Lateral Hypothalamic Area; LC, Locus Coeruleus; MHN, Medial hypothalamic nucleus; mRNA, Messenger ribonucleic acid; Nac, Nucleus accumbuns; non-REM, Non-Rapid Eye Movement; OSA, Obstructive Sleep Apnea; OxA, Orexin A; OxB, Orexin B; OxR1, Orexin receptor 1; OxR2, Orexin receptor 2; PCR, Polymerase chain reaction; PD, Postnatal day; PPO, Prepro-orexin; PVN, Paraventricular Nucleus; PPT, Pedunculopontine Tegmental Nucleus; PFA, Perifornical Area; REM, Rapid Eye Movement; RIA, Radioimmunoassay; s.c., Subcutaneous; SON, Supraoptic Nucleus; SONr, Supraoptic nucleus, retrochiasmatic part; TMN, Tuberal Mammillary Nucleus; VMH, Ventral medial hypothalamus; VTA, Ventral tegmental area; WB, Western blot

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1. Introduction

Since the discovery of orexin (also known as hypocretin) in 1998 (de Lecea et al., 1998; Sakurai et al., 1998) much has been studied regarding the function of the orexinergic system and its role in clinical diseases/disorders; particularly eating behavior and sleep related disorders. Initially, orexin was identified as having a prominent role in feeding behavior based on its pharmacological activity, and its localization to the lateral hypothalamus (also known as the feeding center), with the name orexin produced from the Greek word *orexis* meaning appetite (Sakurai et al., 1998). Subsequently, orexin neuronal activity was shown to regulate wakefulness, based on the finding that sufferers of the sleep disorder narcolepsy had deficient orexin signaling (Crocker et al., 2005; Thannickal et al., 2000, 2003). Many reviews have been written even just recently (Progress in Brain Res 2012 (V198), Vitamin and Hormones 2012 (V89)). However, none of these reviews has yet summarized the data available regarding effects of common experimental paradigms (changes in energy homeostasis or noxious insults) on the *expression* of the orexinergic system including prepro-orexin (PPO), orexin A (OxA), orexin B (OxB), and the receptors 1 and 2 (OxR1, OxR2).

This review will thus provide a comprehensive summary of studies aimed at determining how changes to normal homeostasis and exposure to various insults alters expression of the orexin system. These studies evaluate expression of PPO and the peptides, as well as how the receptors change in response to drugs of abuse such as nicotine, ethanol and cocaine, and other noxious insults (encompassing hypoxia and/or hypercapnia). Given the various roles of the orexinergic system, we have grouped the review into four thematic sections: (1) Metabolic, (2) Sleep, (3) Drugs, and (4) Noxious insults. In humans, these frequently overlap so it can be very difficult to delineate which parameter links to the changes in the orexin system. Animal models utilizing specific paradigms often help resolve this issue, although the heterogeneous nature of the experimental models can then add different problems. By reviewing the animal paradigms available and highlighting homogeneity or differences across studies, we will provide a description of their implications, including proposed mechanisms for the changes.

2. The orexin system

The orexin system consists of three genes, the precursor PPO gene that encodes two neuropeptides, OxA and OxB, and a separate gene for each of the receptors OxR1 and OxR2. The human PPO gene is 131 amino acids and located on chromosome 17q21 (Sakurai et al., 1999). The sequences are 83% equivalent in humans and rats; between rats and mice the polypeptide is 95% identical (Sakurai et al., 1999). The gene consists of two exons and an intervening intron. The PPO sequence, including the OxA and OxB sequences are encoded on the second exon. Separating OxA and OxB in the PPO polypeptide sequence, is a Lys-Arg basic pair that is thought to be a recognition site for a prohormone convertase. OxB is encoded by amino acids 69 to 96, making it a 28 amino acid linear peptide (Sakurai et al., 1999).

OxR1 and OxR2 are class A, G-protein coupled receptors (GPCR) of 425 and 444 amino acid sequences, respectively; with 64% identical sequencing (Sakurai et al., 1998). OxA has a ten times higher affinity for OxR1 than OxB, while both bind to OxR2 with equal affinity (de Lecea et al., 1998; Sakurai et al., 1998). Studies have shown that other neuropeptides do not bind to orexin receptors (Holmqvist et al., 2001; Smart et al., 2001) as the orexin receptors have a low (30%) homology with other GPCRs (Kukkonen et al., 2002). This suggests that the binding electrochemical and stereochemistry of the orexin neuropeptides is specific to the epitopes expressed on orexin receptors. Generally, OxR1 signals via G_q coupled proteins while OxR2 signals via G_q or $G_{i/o}$ (Karteris et al., 2001; Zhu et al., 2003). However, this is still disputed (Kukkonen, 2013).

3. Brain regions involved in orexin innervation

A summary of the main areas of OxR1 and OxR2 expression, projection and physiological roles attributed to the brain regions, is provided in Fig. 1. The number of orexin containing neurons is relatively small compared to neurotransmitter systems in the brain (Deurveilher et al., 2006). However, they receive major inputs from serotonergic, glutamatergic, muscarinic, adenosine, GABAergic and catecholaminergic receptors (Sakurai, 2007) as well as glucose, leptin and neuropeptide Y