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Research report

Opposite effects of sleep rebound on orexin OX_1 and OX_2 receptor expression in rat brain

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Abstract

Orexins (hypocretins) have been implicated in the regulation of the normal sleep–wake cycle, in sensorimotor programming, and in other homeostatic and neuroregulatory processes. The present study examined the effects of sleep deprivation (SD) and sleep recovery on the expression of orexin 1 receptors (OX_1R) and orexin 2 receptors (OX_2R) throughout the brain. Rats were sacrificed either immediately after 96 h of sleep deprivation (SD group) or after SD followed by 24 h of sleep recovery (Rebound group). Prepro-orexin mRNA showed a non-significant increase in the SD group relative to controls, but a pronounced and significant increase in the Rebound group (+88%, $P < 0.007$). Similarly, sleep deprivation produced no effect on OX_1R or OX_2R mRNA levels. However, in the Rebound group, OX_1R mRNA levels increased significantly, compared to either control or SD groups, in 37 of 92 brain regions analyzed, with particularly strong effects in the amygdala and hypothalamus. Changes in OX_2R mRNA levels were also seen only in the sleep Rebound group, but they were fewer in number (10 out of 86 regions), were in the direction of decreased rather than increased expression, and were predominantly confined to cerebral cortical areas. These observations indicate that some factor associated with sleep recovery, possibly the compensatory increase in REM sleep, has strong effects on the orexin system at the mRNA level. They further indicate that OX_1 and OX_2 receptors are affected in opposite way and that the former are more vulnerable to these effects than the latter.

Theme: Neurotransmitters, modulators, transporters, and receptors
Topic: Peptides: anatomy and physiology

Keywords: Sleep deprivation; Hypocretin; In situ hybridization; Gene expression; Hypersomnia; REM sleep

1. Introduction

Orexin A and B, also referred to as hypocretin 1 and 2, are hypothalamic neuropeptides derived by a proteolytic process from the same 130-amino acid precursor prepro-orexin [22]. Orexins A and B were first identified by de Lecea et al. [4] in mRNAs from rat hypothalamus and were initially associated with feeding behavior [4,22], although subsequent evidence has been inconsistent in this respect [25,34]. The prepro-orexin gene is expressed in a very restricted, largely perifornical area of the hypothalamus [15,18]. Two G protein-coupled receptors for orexins have been identified, the orexin receptor type 1 (OX_1R) and orexin receptor type 2 (OX_2R) [22]. Orexin A binds equally to both receptor subtypes, while orexin B has a preferential affinity for OX_2R [9,22]. The large number of projections of orexin-producing cells throughout the central nervous system [18] and the widespread distribution of the two receptors in the brain [8,13,32] suggest that these neuropeptides may have broad physiological functions.

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Orexins and their receptors are linked to canine narcolepsy [11] and have been found to be substantially depleted in post-mortem hypothalamus [19,30] as well as in cerebrospinal fluid (CSF) of human narcoleptic patients [33]. Targeted disruption of the prepro-orexin gene in mice results in a phenotype similar to human narcolepsy [1]. While orexin involvement in the sleep–waking cycle remains clear [9,20], more recent evidence has emphasized a broader role for orexins in other regulatory and somatomotor functions [10,25,31,36].

One approach to investigate the relationship between orexins and the regulation of sleep and wakefulness is to examine the effects of sleep deprivation on the expression of the peptide and its receptors. Prepro-orexin mRNA levels in mouse and rat hypothalamus have been reported to be either unchanged after short-term (6 h) sleep deprivation [6,28] or only slightly elevated [21]. On the other hand, studies measuring the orexin A peptide in CSF have reported increases after longer periods of SD [17,36]. The present study examined the possibility that longer periods of sleep deprivation may be necessary to significantly affect prepro-orexin mRNA levels. We also examined, for the first time, the effects of sleep deprivation and sleep recovery on the expression of orexin 1 and orexin 2 receptors in the same brains.

2. Materials and methods

2.1. Sleep deprivation

Male Wistar rats (300–400 g) from our own colony were housed in pairs with food and water available at all times. Lights were on between 7 AM and 7 PM. Rats were deprived of sleep using the classical platform method. Sixteen animals were individually placed on round (6.5 cm diameter) platforms in 23 × 23 × 35 cm containers filled with water up to 1 cm below the platform surface. In this procedure, the animal is aroused from sleep when loss of muscle tone makes it fall off the platform. In our laboratory, this procedure results in a complete abolition of REM sleep, with some loss (37%) of slow wave sleep (SWS) also occurring [12]. After 96 h of sleep deprivation, one group of 8 animals was sacrificed by decapitation (Sleep Deprived group); a second group was returned to the home cage and allowed to sleep for 24 h before being sacrificed (Rebound group). Another group of 8 animals remained in their home cages throughout the procedure (Control group), in the same room where deprivation took place. All groups had food and water available ad libitum during sleep deprivation and recovery periods. All animal procedures were in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publications No. 80-23, 1996).

2.2. In situ hybridization

Rats were sacrificed by decapitation between 9 and 11 AM. Brains were rapidly removed, frozen over dry ice, and stored at −80 °C. Twenty-micron coronal sections were cut on a Leica cryostat at 0.3 mm intervals and mounted on Fisher Superfrost slides; several sets of slides were prepared covering the entire longitudinal extent of the brain. In situ hybridization (ISH) for prepro-orexin was performed using a 48 base oligonucleotide complementary to bases 258–305 (Genbank AF041241), tagged at the 3′ end with [^{35}S]dATP. OX_1R and OX_2R hybridization was performed using [^{35}S]UTP labeled riboprobes prepared by in vitro transcription with PCR fragments primed with the following sequences: for OX_1R (Genbank NM_013064.1), 918–937 and 1368–1349 and for OX_2R (Genbank NM_013074.1), 580–599 and 1028–1005. Probe specific activity was approximately 2×10^9 CPM/μg. Slide-mounted sections were first fixed in 4% paraformaldehyde, rinsed in PBS, acetylated in 0.1 M triethanolamine HCl containing 0.25% acetic anhydride, rinsed in 2× SSC, dehydrated in graded ethanol and 100% chloroform, re-hydrated and dried, and then hybridized with the respective probes. The ^{35}S-labeled probes were diluted in hybridization solution containing 50% formamide, 35% denhardts, 10% dextran sulfate, 0.1× SSC, salmon sperm DNA (300 μg/mL), yeast tRNA (100 μg/ml), and DTT (40 μM). Following overnight incubation at 60 °C (riboprobes) or 42 °C (oligo probe), the sections were rinsed in 4× SSC at 60 °C, treated in RNase A (20μg/mL) at 45 °C for 40 min, washed with agitation in decreasing concentrations of SSC containing 25 g/ml sodium thiosulfate, dipped in water, dehydrated in 70% ethanol, dried, then exposed to Kodak BioMax film at 4 °C for 2 days in the presence of calibrated standards.

Densitometric analyses were performed with an MCID AIS/C system (Imaging Research, St. Catharines, Ontario, Canada). Prepro-orexin analyses took into account optical density as well as area of signals along the anterior–posterior axis of the hypothalamus. The OX_1R signal was quantified in 92 brain regions and the OX_2R signal in 87 regions, defined according to the atlas of Paxinos and Watson [16]. Densitometric analyses were performed without awareness of group membership. For any subject, the final density value for any given brain region represented an average of multiple readings on 3–6 separate brain sections. Data were analyzed by one-way ANOVAs using Systat 5.0 Software (Evanston, IL) followed by *t* comparisons for brain regions where significant *F* values (*P* at least <0.05) were obtained.

3. Results

3.1. Prepro-orexin expression

Prepro-orexin ISH signals were entirely confined to the hypothalamus but formed a continuum across different nuclei. As shown in Table 1, the mean density of ISH signals averaged over the entire hypothalamus increased by 12% in the Sleep Deprived group (not statistically signifi-