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Research report

The action of orexin B on passive avoidance learning. Involvement of neurotransmitters

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HIGHLIGHTS

- Diffuse expression of orexins and their receptors in the brain.
- Orexins are involved in several physiological brain functions, including memory.
- Orexin B improves learning, consolidation and retrieval processes in rats.
- GABA-ergic, opiate, nitrergic, α- and β-adrenergic neurotransmissions are involved.

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ABSTRACT

The extensive projection of orexigenic neurons and the diffuse expression of orexin receptors suggest that endogenous orexins are involved in several physiological functions of the central nervous system, including learning and memory. Our previous study demonstrated that orexin A improves learning, consolidation and retrieval processes, which involves α- and β-adrenergic, cholinergic, dopaminergic, GABA-A-ergic, opiate and nitrergic neurotransmissions. However, we have little evidence about the action of orexin B on memory processes and the underlying neuromodulation. Therefore, the aim of the present study was to investigate the action of orexin B on passive avoidance learning and the involvement of neurotransmitters in this action in rats. Accordingly, rats were pretreated with the selective orexin 2 receptor (OX2R) antagonist, EMPA; the γ-aminobutyric acid subunit A (GABA-A) receptor antagonist, the bicuculline; a D2, D3, D4 dopamine receptor antagonist, haloperidol; the nonselective opioid receptor antagonist, naloxone; the non-specific nitric oxide synthase (NOS) inhibitor, nitro-L-arginine; the nonselective α-adrenergic receptor antagonist, phenoxybenzamine and the β-adrenergic receptor antagonist, propranolol. Our results demonstrate that orexin B can improve learning, consolidation of memory and retrieval. EMPA reversed completely the action of orexin B on memory consolidation. Bicuculline blocked fully; naloxone, nitro-L-arginine, phenoxybenzamine and propranolol attenuated the orexin B-induced memory consolidation, whereas haloperidol was ineffective. These data suggest that orexin B improves memory functions through OX2R and GABA-ergic, opiate, nitrergic, α- and β-adrenergic neurotransmissions are also involved in this action.

1. Introduction

The 33-amino acid orexin A (also known as hypocretin-1) and the 28-amino acid orexin B (also known as hypocretin-2) were described in the hypothalamus in 1998. Both neuropeptides are derived from the common precursor peptide (preproorexin) [1,2]. Orexin-expressing neurons are predominantly localized in the lateral hypothalamus area (LHA) and posterior hypothalamus and extensively project to the entire neuroaxis, including the cerebral cortex, olfactory bulb, thalamus, anterior and posterior hypothalamus, diagonal band of Broca, amygdala, hippocampus, bed nucleus of the stria terminalis, septum, brainstem and spinal cord [1–6].

Orexins activate at least two distinct G-protein coupled receptors, the orexin 1 receptor (OX1R) and the orexin 2 receptor (OX2R). OX1R has a 10 fold greater affinity for orexin A than orexin B, whereas OX2R has nearly equal affinity for both neuropeptides [2,7]. OX1R and OX2R are located on both pre- and post-synaptic processes, as well as on cell bodies [8,9]. Prominent expression of OX1R mRNA was found in the locus coeruleus (LC) and to a lesser extent in the dorsal raphe nucleus, hippocampal formation and

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tenia tecta. On the other hand, OX2R mRNA was identified mainly in the cerebral cortex, nucleus accumbens, paraventicular thalamic and subthalamic nuclei [10,11].

The extensive projection of orexigenic neurons and the diffuse expression of orexin receptors suggest that endogenous orexins may be involved in several physiological functions of the central nervous system (CNS), such as regulation of blood pressure [12,13], body temperature [14], drinking behavior [15], food intake [2], nociception [16], sleep–waking cycle [17,18], sleep disorder [19], reward and drug addiction [20], stress and arousal [21,22], LH secretion [23,24], prolactin secretion [25], and thyroid function [26]. The presence of numerous orexigenic terminals in the memory related brain regions, such as the hippocampus and amygdala suggests the involvement of orexins in learning and memory.

In our previous study, we have demonstrated that orexin A improves learning, consolidation and retrieval processes [27]. Several other studies provided further evidence about the memory enhancing action of orexin A [28,29] and about the role of OX1R in memory [30–32]. In contrast, the effect of orexin B on memory processes has not been elucidated. Additionally, there are indications that the orexins might act as neurotransmitters per se or in concert with other monoaminergic structures [33,34]. In our previous study, we demonstrated that a number of transmitters could be involved in the action of orexin A on memory consolidation [27]. However, we have little evidence about the underlying neuromodulation of the presumable effect of orexin B on learning and memory. Therefore, the first aim of the present study was to investigate the action of orexin B on passive avoidance learning and memory formation in rats. The second aim of our study was to investigate the involvement of neurotransmitters in mediating the action of orexin B on memory consolidation, thus the animals were pretreated with transmitter receptor antagonists prior to peptide administration.

2. Methods and materials

2.1. Experimental animals and ethics statement

Male Wistar rats, weighing 150–250 g were used. The animals were maintained and treated during the experiments in accordance with the instructions of the Ethical Committee for the Protection of Animals in Research of the University of Szeged (Szeged, Hungary), which specifically approved this study. The rats were kept in their home cages at a constant temperature (23 °C) on a standard illumination schedule with 12-h light and 12-h dark periods (lights on from 6:00 AM). Commercial food and tap water were available ad libitum. To minimize the effects of nonspecific stress, the rats were handled daily. All surgery was performed under anesthesia, and all efforts were made to minimize suffering.

2.2. Surgery

For intracerebroventricular (icv) administration, the rats were implanted with a stainless steel Luer canulla (10 mm long) aimed at the right lateral cerebral ventricle under Nembutal (35 mg/kg, intraperitoneally, ip) anesthesia. The stereotaxic coordinates were 0.2 mm posterior; 1.7 mm lateral to the bregma; 3.7 mm deep from the dural surface, according to the atlas of Pellegrino et al. [35]. Cannulas were secured to the skull with dental cement and acrylate. The rats were used after a recovery period of 5 days.

2.3. Treatments

Orexin B and the selective OX2R antagonist EMPA (*N*-ethyl-2-[(6-methoxy-pyridin-3-yl)-(toluene-2-sulphonyl)-amino]-*N*-pyridin-3-ylmethyl-acetamide) were purchased from Bachem Inc., Switzerland. Orexin B was applied via the icv cannula in a dose of 0.5 or 1.0 μg/animal. For combined treatment, only 1.0 μg orexin B was used. Orexin B was administered 30 min before the learning trial (learning), after the learning trial (consolidation) and 30 min before the 24 h test (retrieval). EMPA was injected icv in a dose of 2.0, 4.0 or 8.0 μg/animal.

EMPA and the receptor blockers were applied immediately after the learning trial, followed 30 min later by orexin B administration.

The following receptor blockers were used: bicuculline methiodide (Sigma, St Louis, USA), 1 mg/kg ip; haloperidol (G. Richter Budapest, Hungary) 10 μg/kg ip; naloxone hydrochloride (Endo Lab., New York, USA), 0.3 mg/kg ip; nitro-L-arginine (*N*-*w*-nitro-L-arginine, *N*-NA, Sigma-Aldrich, Budapest, Hungary), 10 μg/2 μl icv; phenoxybenzamine hydrochloride (Smith, Klein and French, Herts, UK), 2 mg/kg ip and propranolol hydrochloride (ICI, Macclesfield, UK), 10 mg/kg ip. The doses of the receptor blockers were selected on the basis of our earlier experience as being effective when administered with other neuropeptides, but not affecting the paradigm per se [27,36].

2.4. Behavioral testing

2.4.1. Passive avoidance test

One-trial learning, step-through passive avoidance behavior was measured according to Ader et al. [37]. The apparatus consists of two separate chambers connected through a guillotine door. One of the chambers was illuminated, while the other was dark. Rats were placed on the illuminated platform and allowed to enter the dark compartment. Since rats prefer dark to light, they normally entered within 5 s. Two additional trials were delivered on the following day. After the second trial, unavoidable mild electric footshocks (0.75 mA, 2 s) were delivered through the grid floor. The guillotine door was closed immediately after the rat entered the dark chamber and the animals could not escape the footshock. After this single trial, the rats were immediately removed from the apparatus and were treated. The consolidation of passive avoidance behavior was tested 24 h later. When the action of the peptide was tested on acquisition processes, the animals were treated before the single trial. When the action of the peptide was tested on retrieval processes the animals were treated with the peptide 30 min before the 24 h testing. For consolidation, the animals were treated with the peptide following the learning trial or first with the receptor antagonist and 30 min later with the peptide for combined treatment. In the 24 h testing each rat was placed on the platform and the latency to enter the dark compartment was measured up to a maximum of 300 s.

2.5. Statistical analysis

Statistical analysis of the behavioral testing was performed by two-way analysis of variance (two-way ANOVA), which was followed by Tukey's post hoc comparison test. Only the mean percentages were plotted and the standard error of the mean (SEM) is given in the figure captions. The differences between groups were examined by Tukey's post hoc comparison test, and a probability level of 0.05 or less was accepted as indicating a statistically significant difference.

3. Results

The action of orexin B on learning was studied when the peptide was administered 30 min prior to the learning trial. Orexin B lengthened significantly the latency of the avoidance response in the dose of 1 μg, whereas the 0.5 μg dose was ineffective [$F(2, 53) = 4.87$]; $p < 0.05$ (Fig. 1). Thus orexin B facilitated the learning and consolidation in a dose-dependent manner.