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Behavioral effects of orexin-A in the bed nucleus of stria terminalis of rat

Olga Hangodi [a,b,*], Barbara Urbán [c], Péter Inkő [c], Szilvia Tálos [c], Kristóf László [c], Éva E. Bagi [c], Éva M. Fekete [c], Balázs Lukáts [a,c], László Lénárd [b,c], Yutaka Oomura [d], Shuji Aou [a]

[a] *Department of Brain Science and Engineering, Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, Wakamatsu, Kitakyushu 808–0196, Japan*
[b] *Neurophysiology Research Group of the Hungarian Academy of Sciences, Pécs University, Medical School, Pécs, Hungary*
[c] *Institute of Physiology, Pécs University, Medical School, Pécs, Hungary*
[d] *Department of Integrative Physiology, Kyushu University, Faculty of Medicine, Fukuoka, Japan*

Abstract. Lateral hypothalamic orexin expressing neurons send fibers to various brain areas such as the brainstem, hypothalamus, cortex and limbic system, including the amygdala and the bed nucleus of stria terminalis (BST). The role of orexins in reward seeking, response to stress, learning and memory have recently been investigated. BST is known to be involved in the regulation of hormonal and behavioral responses to stress, anxiety and sexual behavior. The present study aimed to elucidate the involvement of orexin-A (OXA) in learning, anxiety and reinforcement processes in the BST. Bilateral OXA microinjections in the doses of 250 ng (70 pmol) and 500 ng (140 pmol) into the BST of male Wistar rats were performed 30 min prior to open field (OPF), elevated plus maze (EPM), conditioned place preference (CPP), or passive avoidance (PAV) tests. In the CPP, 250 ng OXA increased the number of entering into the treatment quadrant. The 500 ng OXA had significant anxiolytic effects on EPM. There was a mild increase in the locomotor activity in the OPF as well as in the EPM. In the PAV, OXA dose-dependently reduced the retention time to enter the dark room, indicating its suppressive effect on avoidance learning. Our findings reveal that OXA shows functional heterogeneity in learning and memory processes having a specific role in the modulation of PAV learning. The inhibition on avoidance learning is supposed to be due to the anxiolytic effect of OXA.

Keywords: Orexin; Bed nucleus of stria terminalis; Anxiety; Learning; Rat

* Corresponding author. Tel./fax: +81 936 956 115.
E-mail address: olga@brain.kyutech.ac.jp (O. Hangodi).

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1. Introduction

Neuropeptide orexins can be localized in the gut, pancreas and the spinal cord. In the brain they are expressed by a small group of neurons in the lateral hypothalamic and perifornical area [1,2]. These cells send fibers to various brain areas such as the brainstem, hypothalamus, cortex and limbic system, including the amygdala and the bed nucleus of stria terminalis (BST) [3]. The two types of orexins, orexin-A (OXA) and orexin-B, are derived from a common precursor, preproorexin. The orexinergic system coordinates metabolic, motivational, motor and arousal processes necessary to elicit environmentally appropriate behaviors. The role of orexins in reward seeking, response to stress, learning and memory are also being investigated. BST is known to be involved in the regulation of hormonal and behavioral responses to stress, anxiety and sexual behavior [4]. In our previous experiments, microinjections of OXA enhanced liquid food and water intake in the BST in a dose-dependent manner and these effects were blocked by a selective orexin-1 receptor antagonist [5]. The aim of the present study was to elucidate the involvement of orexin-A in learning, anxiety and reinforcement processes in the BST.

2. Materials and methods

Seventy-six male Wistar rats (weight: 250–350 g) were used in this series of experiments. Under anesthesia, bilateral stainless steel guide cannulae (22 Gauge) were implanted 1.5 mm dorsal to the BST (stereotaxic coordinates: AP: −2.8 mm, ML: ±4.0 mm, DV: 5.6 mm) [6]. After recovery, bilateral microinjections of OXA (Sigma, 0–6012) in the doses of 250 ng (70 pmol) or 500 ng (140 pmol), or saline were performed (as described previously) [5] 30 min prior to open field (OPF), elevated plus maze (EPM), conditioned place preference (CPP), or passive avoidance (PAV) tests recorded by Noldus Ethovision software.

OPF was performed under dim red light conditions in a square-shaped (50 × 50 cm) apparatus in order to examine the general motor activity (total distance moved). The EPM consisted of two enclosed and two open arms (50 × 50 cm each) extending from a central area. It was used for determining the rats' unconditioned response to a potentially dangerous environment and anxiety-related behaviors after OXA treatment, recording the time spent on open arms and on the end of the open arms as well as the total distance moved.

PAV was examined in a classical two-compartment box to study learning for stress stimuli. The apparatus consisted of light (L) and dark (D) compartments separated by a guillotine door. One day after habituation, 30 min after OXA or vehicle treatment, rats were placed in L and the door was opened. When a rat stepped with all four paws into D, a 0.8 mA/2 s unavoidable footshock was delivered through the grid floor. After 24 h, animals were tested for passive avoidance response retrieval. Latency of entering into the D (retention time) was recorded. The retrieval session ended when the animal entered D or remained in L for 1500 s.

Circular CPP (diameter: 83 cm) was applied for evaluation of the subjective effects of OXA. On day 1, rats were allowed to move freely in the apparatus for 15 min, and the time spent in each quadrant was recorded. During conditioning (days 2, 3) the apparatus was divided into four walled quadrants. OXA or saline microinjection was paired always with one cue-specific quadrant (treatment quadrant) for 15 min. On the test day (day 4), animals were tested for CPP as on day 1.