Behavioural Brain Research 316 (2017) 47–53

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Role of the orexin (hypocretin) system in contextual fear conditioning in rats

Huiying Wang[a], Sa Li[a], Gilbert J. Kirouac[a,b,*]

[a] Department of Oral Biology, College of Dentistry, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada
[b] Department of Psychiatry, College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada

HIGHLIGHTS

- Upregulation of ppOX mRNA in shocked rats.
- Upregulation of the orexin-1 receptor in shocked rats.
- Orexin-1 antagonist decreases contextual fear.

ARTICLE INFO

Article history:
Received 8 July 2016
Received in revised form 23 August 2016
Accepted 25 August 2016
Available online 26 August 2016

Keywords:
Fear
Hypocretin
Orexin
Posterior hypothalamus
Pharmacology

ABSTRACT

Orexin (hypocretin) neurons located in the posterior hypothalamus send projections to multiple areas of the brain involved in arousal and experimental evidence indicates that these neurons play a role in the physiological and behavioral responses to stress. This study was done to determine if the orexin system was involved in mediating the fear associated with shock context (5 × 2 s of 1.5 mA). First, real-time RT-PCR was used to examine changes in the mRNA levels for prepro-orexin (ppOX), the orexin-1 receptor (OX1R) and the orexin-2 receptor (OX2R) at two weeks post-shock. We found that the mRNA levels for ppOX and OX1R were increased in the posterior hypothalamus of shocked rats. In contrast, no significant difference was found in the midline thalamus or the locus coeruleus/parabrachial region. Second, the study examined if systemic injections of antagonists for orexin receptors attenuated the freezing related to contextual fear. The OX1R antagonist SB334867 (20 or 30 mg/kg; i.p.) decreased freezing while the same doses of the OX2R antagonist TCSOX229 had no effect. The dual orexin antagonist TCS1102 (20 mg/kg; i.p.) also decreased the freezing to the shock context. The results of the present study show upregulation of orexin activity and of the OX1R in the hypothalamus following exposure of rats to footshocks and highlight a specific role of OX1R in contextual fear.

1. Introduction

A group of neurons scattered in the perifornical region of the posterior hypothalamus that contain the peptide orexin (hypocretin) [1,2] provide widespread innervation of regions of the brain associated with the regulation of arousal [3]. The bioactive orexin peptides orexin-A and orexin-B are produced by the cleaving of prepro-orexin polypeptide (ppOX) [2]. Orexins act at G protein-coupled receptors called the orexin-1 receptor (OX1R), which is selective for orexin-A, and the orexin-2 receptor (OX2R), which is non-selective for both orexin-A and orexin-B [1,2]. Experimental evidence indicates that orexins stabilize arousal during periods of wakefulness [4–7] and that loss of orexin signaling causes a difficulty in maintaining wakefulness [6,7]. Other research shows that orexin neurons are activated in response to stressful conditions [8–12] and play a role in the physiological and behavioral responses to stress [9,13,14].

Previous research demonstrated that the level of ppOX mRNA was elevated at 6 days after rats that had received a brief episode of footshocks and remained elevated for up to 14 days [15,16]. More importantly, the elevated level of ppOX mRNA was positively correlated with freezing in rats exposed to the shock context. The post-shock increase in orexin activity suggested that the shock experience may lead to an increase in the baseline synthesis and release of orexins contributing to fear [16]. In support of this idea, oral administration of a dual orexin receptor antagonist called

* Corresponding author at: Department of Oral Biology, College of Dentistry, Rady Faculty of Health Sciences, 780 Bannatyne Avenue, Winnipeg, Manitoba, R3E 0W2, Canada.
E-mail address: Gilbert.Kirouac@umanitoba.ca (G.J. Kirouac).

http://dx.doi.org/10.1016/j.bbr.2016.08.052
0166-4328/

almorexant reduced fear-induced startle [17] and attenuated the increases in heart rate and blood pressure produced by exposing rats to the chamber in which they had previously experienced footshocks [9]. In addition, systemic injection of a dual orexin receptor antagonist TCS1102 reduced contextual fear following footshocks [16]. While the receptor subtype mediating these effects has not been examined at the pharmacological level, evidence from knockout mice indicates a role for the OX1R in both cued and contextual fear conditioning paradigms and a role for the OX2R in contextual fear conditioning paradigm [18].

This study was done to determine the role of the orexin system in the contextual fear conditioning. The first experiment examined if the mRNA levels for ppOX, OX1R and OX2R are enhanced in rats exposed to footshocks. The second experiment was done to examine if systemic injection of a dual orexin receptor antagonist (TCS1102) or selective antagonists for the OX1R (SB334867) or the OX2R (TCSOX229) attenuates the fear expressed in shocked rats when they are exposed to the shock context.

2. Methods

2.1. Animals and housing

Male Sprague-Dawley rats weighing 130–160 g (University of Manitoba, Winnipeg, Manitoba, Canada) were pair-housed in plastic cages in a colony room where they had free access to food and water. Animals were maintained on a 12-h light-dark cycle (lights on 6 AM) with controlled temperature (20–24 °C) and humidity (40–70%). All experimental procedures were approved by Research Ethics Review Board of University of Manitoba and in compliance with the Canadian Council on Animal Care.

2.2. Experimental approach and footshock procedure

Two separate groups of rats were exposed to footshocks. The first group (n = 25) was used to examine the mRNA levels for ppOX, OX1R and OX2R (Experiment 1). The second group (n = 124) was used to verify the effects of the dual and single orexin receptor antagonists on contextual fear (Experiment 2).

The rats were adapted to the experimenters prior to the shock procedure. The rats were transferred one at a time to a room (400–500 lx) which was exclusively dedicated for footshock delivery. The rats received footshocks over a 3 min period (5 × 2 s of 1.5 mA shocks presented randomly with an intershock periods of 10–50 s) following a 2 min acclimation period (MED Associates, Vermont, USA). Then the rats were kept in the chamber for another 60 s before they were returned to their home cages. Nonshocked rats were placed in the chamber for the same amount of time but no shocks were delivered. The shock chamber was cleaned with 10% alcohol after exposure of each rat.

2.3. Biochemical experiments

The rats were anaesthetized with chloral hydrate (600 mg/kg, i.p.) and perfused with ice-cold 0.1 M phosphate buffered saline (PBS) at 11 days post-shock. Brains were removed and placed in a matrix with 1 mm divisions to cut sections of the brain which included the target areas. Then coronal sections of the brain were placed on a plastic petri dish, and areas of interest (Fig. 1) were dissected using a stereo microscope according to a stereotaxic atlas [19]. All procedures were done on ice and all surfaces in contact with the brain tissue were wiped with RNaseZap (Life Technologies Inc., Ontario, Canada). The hypothalamus, midline thalamus and locus ceoruleus/parabrachial regions were collected in RNase-free microtubes containing RNAlater solution (Life Technologies Inc., Ontario, Canada) and stored at −80 °C for later analysis using

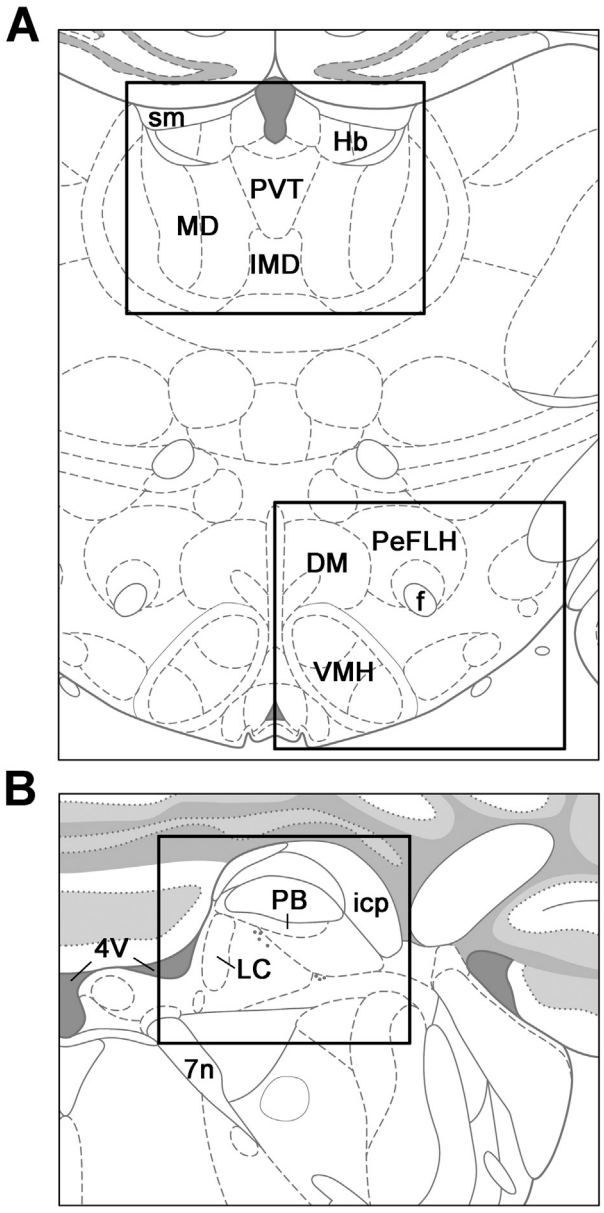

Fig. 1. Diagrammatic representation of the regions of the brain dissected for analysis of mRNA levels for prepro-orexin and orexin receptors using real time-PCR, adapted from [19]. Coronal section A was blocked between −1.0 ~ −4.0 mm from bregma while coronal section B was blocked between −8.5 ~ −10.5 mm from bregma. Abbreviations (A) DM: dorsomedial hypothalamic nucleus; f: fornix; Hb: habenular; IMD: intermediodorsal nucleus; MD: medial dorsal nucleus; PeFLH: perifornical lateral hypothalamus; PVT: paraventricular nucleus of the thalamus; sm: stria medullaris; VMH: ventromedial hypothalamus; (B) 4 V: 4th ventricle; 7n: facial nerve; icp: inferior cerebellar peduncle; LC: locus coeruleus; PB: parabrachial.

PCR. Real-time reverse-transcript PCR was done using methods as previously described [16].

Total RNA was extracted and purified from individual samples using RNeasy Mini Kit (Qiagen, Ontario, Canada) and first-strand cDNA synthesis was performed using qScript™ cDNA SuperMix (Quanta BioSciences, Maryland, USA). Quantitative PCR was performed on the genes of interest in triplicate on an Eco™ Real-Time PCR system (Illumina, California, USA). All PCR reactions were carried out in a PCR MasterMix consisting of 5.6 μl of RNase-free water, 0.4 μl of each primer mix (FWD + REV; 20 μM), 2.0 μl of cDNA, and 2.0 μl of MBI EVOlution EvaGreen® qPCR master mixes (MBI Lab Equipment, Quebec, Canada). A standard ramp speed was used in which denaturation at 95 °C for 15 min was followed by 40