Physiology & Behavior 174 (2017) 42–48

Contents lists available at ScienceDirect

Physiology & Behavior

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

The effect of orexin 1 and orexin 2 receptors antagonisms in the basolateral amygdala on memory processing in a passive avoidance task

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HIGHLIGHTS

- Role of orexin receptors 1/2 (OR1X/OR2X) on memory in the basolateral amygdala (BLA)
- Post-training inhibition of OR1X in BLA impaired passive avoidance consolidation.
- Pre-retrieval inhibition of OR1X in BLA impaired passive avoidance retrieval.
- Post-training inhibition of OR2X did not impair passive avoidance consolidation.
- Pre-retrieval inhibition of OR2X in BLA impaired passive avoidance learning task.

ARTICLE INFO

Article history:
Received 29 December 2016
Received in revised form 4 February 2017
Accepted 3 March 2017
Available online 06 March 2017

Keywords:
Orexin receptors
Learning
Memory
Passive avoidance learning
Basolateral amygdala

ABSTRACT

There is an extensive evidence concerning basolateral amygdala (BLA) function to hippocampal memory processing. However, few researches have addressed the orexinergic system roles in this modulation. Then, the present study aims at investigating the action of orexin 1 and 2 receptors in BLA on passive avoidance (PA) learning. Wistar male rats (n = 79) were trained to avoid foot shock in one type of PA task. The rats were injected bilaterally into BLA, a selective orexin 1 receptor antagonist, SB-334867-A (3, 6, 12 μg/0.5 μl), and an orexin 2 receptor antagonist, TCS-OX2–29 (2.5, 5, 10 μg/0.5 μl), after training or before retrieval of the inhibitory avoidance task. Control rats received dimethyl sulfoxide at the same volume. The amount of learning was assessed 24 h later. The time of the first entrance to the dark compartment and the total time spending in the light compartment were measured as criteria for the avoidance memory. The results showed that consolidation and retrieval were significantly impaired by SB-334867-A administration into BLA in 3, 6 and 12 μg/0.5 μl. However, TCS-OX2-29 in 2.5 μg/0.5 μl could not influence neither consolidation nor retrieval. The TCS-OX2-29 administration into BLA impaired memory retrieval in 5 and 10 μg/0/5 μl but not the consolidation. It gives the impression that orexinergic system of the BLA plays an important role in regulation of memory processing and learning in the rats.

1. Introduction

Memories are not equal to all events and are highly influenced by emotional state [1]. There is evidence that emotionally arousing experiences activate the amygdala. In the basolateral amygdala (BLA), this activation results in modulation of memory-related processes in the other brain regions [2]. Previous studies have demonstrated that drugs affecting neuromodulatory systems within the BLA could modulate the memory of many emotionally arousing training tasks, including inhibitory avoidance [3]. Moreover, the emotionally modulating effects of several neurotransmitter systems on memory formation depend on the different neuronal circuits interactions within the BLA [4]. The power of memorization is modulated by a range of neurotransmitters, including norepinephrine, acetylcholine [5,6], GABA [7], and opioids [8]. Orexinergic system may also have a role in aversive and appetitive learning depending on the situation and the context of the stimulus in that orexin signaling can facilitate attentional behavior and some types of learning and memory [9]. Orexinergic neurons are confined to the lateral hypothalamus and neighboring areas [10,11], and extensively project to the entire central nervous system. Particularly plentiful

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http://dx.doi.org/10.1016/j.physbeh.2017.03.004
0031-9384/

projections are observed in the amygdala, hippocampus, [12,13,14], cerebral cortex, and bed nucleus of the stria terminalis [12]. The hypothalamic neuropeptides orexin A and orexin B interact with the orexin 1 and orexin 2 receptors (OX1R and OX2R), both of which are orphan G-protein-coupled receptors (GPCRs). Orexin A has been shown to have 10-fold higher potency at OX1R than at OX2R, whereas orexin B binds with similar affinity to both receptors [12,13,15]. OX1R and OX2R are located throughout the central nervous system. Such receptors are plentifully expressed, particularly in brain regions responsible for arousal regulation (e.g. the locus coeruleus, dorsal raphe, and tuberomammilary nucleus), feeding (e.g. the lateral, arcuate, and paraventricular nuclei), and memory processing (e.g. the septum, hippocampus, amygdala, and locus coeruleus) [12,13,15,16].

Telegdy and Adamik have reported that intracerebroventricular (i.c.v) orexin A infusion was able to enhance consolidation and retrieval processes in passive avoidance (PA) learning [17]. Palotai and Telegdy have shown that central administration of orexin B could augment acquisition, consolidation of memory, and recalling [18]. They also found that EMPA, a selective antagonist of the OX2R, completely reversed the effect of orexin B on memory consolidation [18]. Still, the distinct role of each receptor in various memory processes needs to be fully clarified. Region-specific pharmacological manipulations are an effective tool in elucidating these various effects. A selective OX1R antagonist, SB-334867-A (SB), with nano-molar affinity at the OX1R, retains at least 50-fold selectivity over various GCRs (e.g. OX2R). SB-334867-A (SB) is used as a tool to evaluate the physiological and behavioral importance of the endogenous orexinergic system [12]. However, this antagonist cannot help in studying the function of OX2R, because orexin A has affinity to OX1R and OX2R [12].

In this study, animals were infused with the selective OX2R antagonist TCS-OX2-29 (TCS) in order to test whether OX2R contributes to PA learning. The molecular weight of this antagonist is 433.97 Da, the IC50 is 40 nM on OX2R, and it is 250 times more selective for OX2R than OX1R [19]. Even though the presence of orexin terminals and receptors has been described in the amygdala [13,15], there is little knowledge concerning activity of the orexinergic system in the stress-related and PA learning. Therefore, here remains some crucial questions about orexin's role in learning and memory, for example, in what types of learning is orexin involved? Evidence for this is limited and somehow combined and little more is known about orexin roles in emotional and stress-related learning and behavior [9]. Previous studies demonstrated that orexin neurons and signaling represent an interesting new goal for clinical interventions in various disorders. Orexinergic drugs could also have a considerable ability to treat deficits in learning, and other cognitive disorders [9]. Thus the aim of the present study was to clarify the physiological function of the orexinergic system of the BLA on memory modulation. For this purpose, we centrally infused an OX1R antagonist (SB) and an OX2R antagonist (TCS) into the BLA region. Then, the effects of OX1R and OX2R inactivation on consolidation and recall were evaluated in a PA learning task.

2. Materials and methods

2.1. Animals

One hundred and twelve adult male wistar rats with body weights ranging from 200 to 250 g were sourced from the Laboratory Animal Institute of Mazandaran University of Medical Science. Three to 4 animals were housed in each cage with free access to food and water. Rats were maintained on a standard 12 h light/12 h dark cycle, at a constant temperature (25 ± 2 °C). There were 8 rats in each group; however, data from thirty three animals was excluded because the cannula was not in the right place. All behavioral procedures were conducted between 8:00 AM and 12:00 PM. The experiments were conducted according to internationally established principles for the use of experimental animals. Also, this study has been approved by Ethics Committee of Mazandaran Medical Science University.

2.2. Surgical procedure and microinjection

After anesthetization by intraperitoneal (i.p) injection of ketamine and xylazine (dose: 100 and 2.5 mg/kg, respectively), the animals were placed in stereotaxic instrument and then two guide cannulas were fixed bilaterally exactly into the BLA region (AP: −2.7 mm from bregma; ML: ±4.8 mm from midline; DV: 7.7 mm from the surface of skull). This coordinate was according to the Paxinos and Watson atlas [20]. To fix the cannulas into the skull, we used a small bolt and dental acrylic. After 1-week of recovery, a behavioral test was conducted. Treatment microinjections were administered through the 21-gauge guide cannula (21 gauge) with injection needles (27 gauge) joined at one end to the polyethylene tubes and the other end to 5 μl Hamilton microsyringes. The injection needles were 1 mm longer than the guide cannulas. Once the injection needles were properly placed, 0.5 μl of the vehicle, 3, 6, or 12 μg/0.5 μl of SB, or 2.5, 5, or 10 μg/0.5 μl of TCS were delivered into each side of the BLA. The infusion time was 3–4 min and the needles were kept in the cannulas for at least 1 min after infusion. Dimethyl sulfoxide (DMSO) was used as the vehicle. DMSO does not have any significant influence on PA learning and memory [21,22]. The doses of both antagonists were based on previous literature [12,23,24].

2.3. PA apparatus

A PA apparatus was used to test learning and memory. This apparatus was made of light and dark chambers of identical size (20 cm × 20 cm × 40 cm) with a rectangular door in the middle (8 cm × 8 cm) connecting the two chambers. Stainless steel rods (2 mm diameter) were positioned on the floor of the apparatus spaced 1 cm apart. An electrical stimulator was attached to the floor in the dark partition. The behavioral test was done in standard conditions in an acoustically insulated room. The light compartment was illuminated by a 100 W lamp positioned 40 cm over the compartment [12].

2.4. Behavioral assessing

2.4.1. PA training

First, all the rats were habituated to the apparatus in two trials. In these sessions, 10 s after placing the rats in a light chamber (facing against the door) the door was elevated. Rats have an instinctive inclination to the dark chamber. Immediately after the rats entered the dark chamber, the door was shut and 30 s later they were taken out of the dark partition and returned to their home cage. After 30 min, the habituation trial was repeated and the first acquisition trial was conducted at an equal time interval. The time it took for the animal to place all its four paws inside the dark compartment, i.e. the acquisition step-through latency (STLa), was recorded. As soon as the animal had unequivocally entered the dark partition, the rectangular door was released and an electric shock (50-Hz, 1 mA for 1.5 s) was given. After 20 s, the rats were returned to their home cage. After 2 min, the procedure was repeated. Each rat experienced a foot-shock every time it reentered dark chamber. Acquisition training was considered complete whenever the rat remained in the light partition for 120 consecutive seconds [12,25].

2.4.2. Retention assessment

Twenty-four hours after the last training trial, a retention test was carried out. In this trial, each rat had to stay in the light compartment for 10 s, then the door was opened. The time latency of the rat entering into the dark compartment for the first time, i.e. the retention step-through latency (STLr), and the total time it spent in the light compartment, i.e. the total time in the light compartment (TLC), over 300 s were