Physiology & Behavior 185 (2018) 79–86

Contents lists available at ScienceDirect

Physiology & Behavior

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

# Role of dorsal hippocampal orexin-1 receptors in modulation of antinociception induced by chemical stimulation of the lateral hypothalamus

Pooya Pourreza[a], Vahab Babapour[b], Abbas Haghparast[c,*]

[a] Department of Basic Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad university, Tehran, Iran
[b] Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Iran
[c] Neuroscience Research Center, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ARTICLE INFO

*Keywords:*
Inflammatory pain
Orexin-1 receptors
Lateral hypothalamus
CA1 region of hippocampus
Rat

ABSTRACT

The Hippocampus has a role not only in nociception but also in modulation of pain perception. In addition, orexinergic neurons present in the lateral hypothalamus (LH) have a recognized role in pain modulation. The presence of orexinergic projections from the lateral hypothalamus (LH) to the dorsal hippocampal Cornu Ammonis 1 (CA1) region raises the question of whether pain modulatory role of LH is mediated through the CA1. To elucidate the interactions between the LH and neural substrates involved in modulation of formalin-induced nociception, the study aimed to test the pain modulatory role of CA1 orexin receptors in the formalin test. Seventy-one male Wistar rats were unilaterally implanted with two cannulae above the LH and CA1. In the treatment groups, intra-CA1 administration of SB-334867, as an orexin-1 receptor antagonist, was performed 5 min before intra-LH microinjection of carbachol, as a cholinergic receptor agonist. In dimethyl sulfoxide (DMSO)-control group, DMSO and saline as well as in carbachol-control group, DMSO and carbachol were microinjected into the CA1 and LH, respectively. In all rats, the procedure was followed by subcutaneous injection of formalin after 5-min time interval. Carbachol reduced both phases of formalin-induced nociception. Intra-CA1 administration of SB-334867 antagonized the LH-induced analgesia during both phases in a dose-dependent manner. It seems that the blockade of orexin-1 receptors has more effects on reduction of antinociception during the late phase compared to the early phase. Pain modulatory role of orexinergic system in the formalin test through a neural pathway from the LH to CA1 provides the evidence that orexins can be useful therapeutic agents for chronic pain treatment.

## 1. Introduction

Orexin-A and -B are two neuropeptides made from a common precursor, prepro-orexin. They are also known as hypocretin-1 and -2 and two G protein-coupled receptors, i.e. orexin-1 and -2 receptors are differentially recruited by orexins [39]. A few number of neurons within the perifornical area, lateral and posterior hypothalamus produces orexin-A and -B [45]. The orexinergic system is involved in the regulation of feeding behavior, sleep/wakefulness, thermogenesis, homeostasis of energy as well as reward processing [19,45].

It has been demonstrated that electrical and chemical stimulation of the lateral hypothalamus (LH) for instance by glutamate or morphine relieves nociceptive responses in different pain models [1,6,11]. In this respect, administration of carbachol, a cholinergic receptor agonist, into the LH reduces nociceptive-related behaviors in acute pain models like foot withdrawal, tail-flick tests [22,37,38] and formalin-induced pain [16].

Carbachol is a potent muscarinic cholinergic receptor agonist, which activates LH orexin neurons [40]. Numerous supra-spinal and spinal sites integrated in pain modulation are innervated by orexinergic projections [28,31,33,46]. Previous studies have shown that modulation of the nociceptive responses through carbachol administration into the LH which is at least partially mediated through the orexin receptors into the ventral tegmental area (VTA) [17], nucleus accumbens (NAc) [51], periaqueductal gray (PAG) [14,15] and pons [21].

A body of evidence suggests that hippocampal formation is involved in nociception [26,42], and some neural populations in the dorsal hippocampal Cornu Ammonis 1 (CA1) region respond to persistent noxious stimuli [24]; so that, partial hippocampectomy has been used as a treatment for chronic pain [18]. Furthermore, the role of

* Corresponding author at: Neuroscience Research Center, School of Medicine, Shahid Beheshti University of Medical Sciences, P.O.Box: 19615-1178, Tehran, Iran.
*E-mail address:* Haghparast@sbmu.sc.ir (A. Haghparast).

https://doi.org/10.1016/j.physbeh.2017.12.036
Received 11 July 2017; Received in revised form 28 December 2017; Accepted 29 December 2017
Available online 30 December 2017
0031-9384/ 

hippocampus in the control of cognitive and emotional aspects of pain has been established [20,27]. Orexinergic projections are also found in the hippocampal formation including CA1 and Dentate gyrus [25,33]. In the current study, to elucidate the interactions between the LH and neural substrates involved in modulation of formalin-induced nociception, efforts were made to examine the pain modulatory role of the LH through the CA1 orexin-1 receptors in the formalin test.

## 2. Materials and methods

### 2.1. Animals

Adult male albino Wistar rats (n = 71) weighing 220–250 g were used in this project (Pasteur Institute, Tehran, Iran). During the whole period of experiments, animals were provided with free access to food and water. Animals were kept on a 12:12 h light:dark cycle (lights off at 7 PM).

### 2.2. Drug and drug administration

In this study, carbachol (Carbamylcholine chloride; Sigma-Aldrich, USA) was dissolved in the sterile normal saline. 0.5 μl of a 250 nM solution of carbachol was administered into the LH as a cholinergic agonist. 0.5 μl of 0.3, 1, 3 and 10 nM solutions of SB-334867 (Tocris Bioscience, Bristol, UK) were used into the CA1 as the orexin-1 receptor antagonist. Dimethyl sulfoxide (DMSO) 12% was used as a vehicle for SB-334867. Formalin 2.5% was prepared from formaldehyde 37% (Merck, Germany) diluted with normal saline.

A stainless steel injector (a 30 gauge needle) with a length of 1 mm longer than the guide cannula was used for injecting the drug into the LH and CA1. The injector was connected to a 1-μl Hamilton syringe using a polyethylene tube (PE-20). Intra-CA1 microinjection of SB-334867 or DMSO was carried out 5 min prior to microinjection of carbachol or saline into the LH. Following the second microinjection (5 min later), formalin test was performed. Each microinjection lasted about 60 s and the injector was left for an extra 60 s to facilitate the drug diffusion in order to prevent the drug backflow.

### 2.3. Experimental design

In the first set of experiments, the effect of drug (carbachol + different doses of SB-334867), vehicle and DMSO administration on locomotor activity were studied and compared to the Naïve group. Locomotor activity of animals was recorded by video tracking system and Ethovision software for 60 min.

In the second set of designed experiments, three control groups including intact, sham-operated and vehicle (DMSO-control) groups were defined (7–8 in each group; n = 23). The effect of formalin test was examined on the intact group. Sham-operated group underwent the surgery and after 5 to 7 days, formalin test was done. DMSO-control group underwent the surgery and after 5 to 7 days, animals received 0.5 μl DMSO as drug vehicle into the CA1, 5 min before intra-LH administration of 0.5 μl saline (n = 7). Then, 5 min after intra-LH microinjection of saline, 50 μl of formalin 2.5% was subcutaneously injected into the plantar surface of hind paw.

In the third part of study, in order to investigate the role of CA1 orexin-1 receptors in mediation of analgesia induced by LH stimulation, SB-334867 (0.3, 1, 3 and 10 nM) was microinjected into the CA1, 5 min prior to microinjection of effective dose of carbachol (250 nM) [16] into the LH (6 in each group; n = 24). DMSO-control group received 0.5 μl DMSO as drug vehicle into the CA1, 5 min before intra-LH administration of 0.5 μl saline (n = 7). Carbachol-control group received DMSO and carbachol (250 nM) into the CA1 and LH, respectively (n = 6). In all rats, 5 min after intra-LH microinjection of carbachol or saline, formalin test was performed.

The effect of carbachol injection into some brain regions surrounding the LH (n = 4) or the effect of carbachol administration into the LH in combination with SB-334867 (10 nM) into some brain regions surrounding the CA1 (n = 7) were also examined in order to distinguish between the results of drug injections into the LH and CA1, and those obtained from drug injections into the neighboring regions. During the experiments, the experimenter was blinded to the experimental conditions.

### 2.4. Experimental procedure

Experiments were carried out in accordance with the Guide for Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996) and were confirmed by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

#### 2.4.1. Stereotaxic surgery

A mixture of xylazine 2% (10 mg/kg) and ketamine 10% (100 mg/kg) were intraperitoneally injected for anesthetizing the rats. According to the rat brain atlas [32], the coordinates for perifornical part of LH (AP = 2.65 mm caudal to the bregma, Lat = ± 1.3 mm lateral to the midline and DV = 8.6 mm ventral to the skull surface) and CA1 (AP = 3.50 mm caudal to the bregma, Lat = ± 2.2 mm lateral to the midline and DV = 2.7 mm ventral to the skull surface) were determined. A mixture of lidocaine/epinephrine (0.2 ml) was injected around the surgery site to reduce the nociceptive responses and bleeding, respectively. In each rat, two 23-gauge, 12 and 8 mm-long stainless steel guide cannulae were unilaterally (based on the guidelines of our institute for pain management in animal study) inserted into the left or right side of the skull 1 mm above the LH and CA1, respectively. Formalin was injected into the right or left hind paw contralateral to the surgery side. The guide cannulae were unilaterally implanted and fixed in place using two stainless steel screws which anchored to the skull and dental acrylic cement. After the cement was completely dried and hardened, two stainless steel obturators were used to occlude the guide cannulae during the recovery period to prevent clogging. Penicillin-G 200,000 IU/ml (0.2–0.3 ml/rat, single dose, intramuscular) was immediately administered after surgery to prevent infection. Animals were individually housed and allowed to recover for 5–7 days before the experiments.

#### 2.4.2. Formalin test

A Plexiglas chamber (35 × 35 × 35 cm) with a mirror angled at 45° below the surface of chamber was used as the apparatus to observe animal's behavior during the formalin test. In all experiments, 5 min after carbachol or saline microinjection (second microinjection), 50 μl of formalin 2.5% was injected into the plantar surface of right or left hind paw contralateral to the surgery side. Then, animals were immediately placed in the apparatus. Formalin induces biphasic nociceptive responses. The first phase immediately starts after formalin injection and lasts for 3–5 min. Over the next 10–15 min, pain responses decrease because of activation of the descending pain inhibitory system. The second phase starts 15–20 min after formalin administration and lasts for 20–40 min [12,44]. Nociceptive behaviors were recorded and quantified as following: 0, when the distinction between the posture of injected paw and other hind paw was difficult; 1, when putting weight on the injected paw reduced; 2, the injected paw was elevated; 3, the injected paw was licked, shacked or bitten [12]. The time spent in each type of behavior was recorded in 5-min blocks for 60-min test period. For each 5-min block of time, the weighted nociceptive score with a range from 0 to 3 was calculated:

$$\text{Nociceptive score} = (t0\times0) + (t1\times1) + (t2\times2) + (t3\times3)/t0 + t1 + t2 + t3$$

Following the formalin test, histological verification of samples and data analysis was performed.