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Research article

Intra-LC microinjection of orexin type-1 receptor antagonist SB-334867 attenuates the expression of glutamate-induced opiate withdrawal like signs during the active phase in rats

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HIGHLIGHTS

- Intra-LC glutamate microinjection induces the expression of opiate withdrawal-like behaviors.
- SB-334867 did not affect glutamate-induced withdrawal-like signs during the rest phase.
- SB-334867 attenuated glutamate-induced withdrawal-like signs during the active phase.

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ABSTRACT

Opiate withdrawal syndrome is temporally associated with the hyperactivity of locus coeruleus neurons. Previous studies have shown that this hyperactivity, at least in part, results from the activity of excitatory afferents which mainly include the orexinergic neurons of hypothalamus and glutamatergic neurons of paragigantocellularis (PGi) nucleus. The effect of intra LC orexin type 1 receptor antagonism was investigated on expression of glutamate-induced morphine withdrawal-like signs in rats. Regarding the involvement of both orexin and LC in modulation of circadian rhythm, experimental procedures were performed during the rest (day) and the active (night) phases. Male Wistar rats (250–300 g) received escalating doses (6, 16, 26, 36, 46, 56, 66 mg/kg, 2 ml/kg) of morphine sulfate subcutaneously for 7 days. Then, glutamate (100 nM, 200 nl) was microinjected into the LC region and the subsequent behavioral manifestations were visually monitored in both rest and active phases. SB-334867 (as a selective orexin type 1 receptor antagonist) was microinjected into the LC prior to glutamate administration. Results indicate that intra-LC microinjection of glutamate elicits morphine withdrawal-like behavioral signs in rats. It is noteworthy that this effect was significantly suppressed in rats pretreated with SB-334867 only during the active phase. It could be concluded that orexin-A plays a role in expression of glutamate-induced opiate withdrawal-like signs and differential orexinergic tone during the rest and active phases might explain the observed difference in activity of LC neurons.

1. Introduction

Electrophysiological, biochemical and behavioral studies have shown that the nucleus locus coeruleus (LC), located in the ventrolateral border of the 4th ventricle, is involved in development of opiate dependence. In addition, it has been demonstrated that hyperactivity of LC is temporally associated with the expression of opiate withdrawal signs [32]. In this regard, systemic or intra-LC administration of clonidine (an α_2 adrenergic receptor agonist) has been reported to suppress both the increased firing rate of LC neurons [2] and the expression of some opiate withdrawal signs [18]. Furthermore, LC lesions results in attenuation of withdrawal signs, whereas, electrical stimulation of LC elicits opiate withdrawal-like behavioral manifestations [13,26].

Several intrinsic and extrinsic excitatory factors modulate the mentioned hyperactivity of LC neurons during opiate withdrawal. Among these, the nucleus paragigantocellularis (PGi), which sends the main excitatory projections to LC is of particular significance [29]. Previous studies have reported that intracerebroventricular (i.c.v) or intra-LC injection of glutamate and N-methyl-D-aspartate

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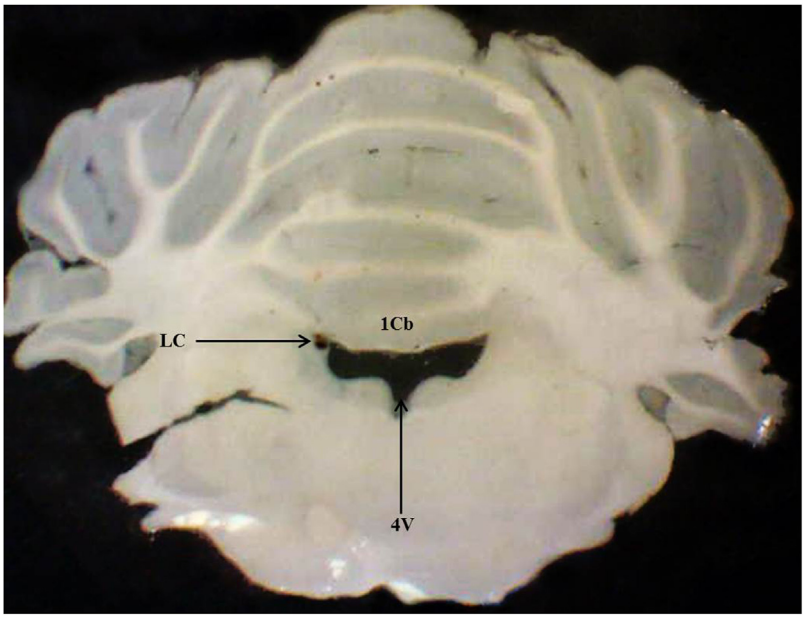

Fig 1. Histological verification of injection site. Figure indicates the site of intra-LC microinjection of 200 nl pontamine sky blue 2% in a coronal (300 μm thick) section of rat brain. The stereotaxic coordinates for LC region were as follows: 9.8 mm posterior to bregma, 1.3 mm lateral to midline and 7.2 mm vertical from the Dura. LC, locus coeruleus; 4 V, forth ventricle; 1 Cb, 1st cerebellar lobe. Scale represents 1 mm.

(NMDA) antagonists attenuate opiate withdrawal-induced hyperactivity of LC neurons [33,34]. Consistent to this, it has been shown that i.c.v. or intra-LC administration of NMDA or glutamate in morphine dependent rats induces opiate withdrawal signs and this effect could be fully prevented by MK-801 (an uncompetitive NMDA antagonist) pretreatment [22,24,25].

Another extrinsic source of excitatory inputs to LC is the orexinergic fibers of lateral hypothalamus which mainly originate from the dorsomedial part of this brain region (DMH) and modulate the effect of circadian rhythm on the activity of LC neurons [3]. Lesions of DMH have been shown to disturb such regulatory control [5]. Orexin neuropeptides activate two types of G-protein coupled receptors [36], orexin type 1 and orexin type 2 (OX1R and OX2R respectively) among which OX1Rs are highly expressed in LC [19]. Orexin excites LC neurons through activating OX1Rs and as aforementioned this increased activity has been shown to be associated with the expression of opiate withdrawal signs [14]. Previous reports support the idea that the activity of orexinergic system is essential for development of opiate withdrawal syndrome. For example, orexin knock out (KO) rats express less somatic withdrawal signs compared to wild type animals. This indicates the important role of endogenous orexin in modulating the opioid-induced responses [11]. It has been reported that intraperitoneal (i.p.) injection of SB-334867 (a selective OX1R antagonist) significantly suppresses the expression of naloxone-induced opiate withdrawal signs [30].

There are several lines of evidence indicating that the excitatory amino acid glutamate might have a role (at least in part) in excitatory effect of orexin on LC neurons [1,39]. For example, orexin can modulate glutamatergic transmission through pre-and post-synaptic mechanisms [35]. Also, i.c.v. injection of orexin promotes the glutamate release from LC neurons and application of orexin-A on brain slices containing hypothalamus has been shown to enhance glutamate release [37]. It has already been reported that around 50% of orexinergic neurons express the vesicular glutamate transporters (VGLUTs) mRNA [28]. On the other hand, Borgland et al. have demonstrated that orexin neuropeptides enhance the expression of NMDA receptors in post-synaptic terminals [7]. In vivo extracellular recording studies have shown that the discharge rate of LC neurons in active (night) phase differs from that in the rest (day) phase [4] and orexin, as mentioned, is involved in relaying the effect of circadian rhythm on LC neuronal activity [12]. Taking these together, the present study was designed to investigate the role of OX1R in mediating the expression of glutamate-induced withdrawal-like behaviors in morphine dependent rats during the active and rest phases.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–300 g, Pasteur Institute, Tehran, Iran) were used in this study. Animals received water and regular pelleted chow ad libitum and were housed under climate-controlled conditions with a 12 h light/dark cycle (the light period started at 7 am). Experimental procedures were performed during rest (10 am–2 pm) and active (8 pm–12 pm) phases. In order to reduce animal stress, rats were carefully handled for several days prior to the experiments. Attempt was made to minimize the number of animals used and avoid any possible discomfort. All procedures were approved by the "Ethical Committee of Faculty of Medical Sciences, Tarbiat Modares University", which are based on the "NIH Guide for the Care and Use of Laboratory Animals".

2.2. Drugs

The following chemical substances were used in our experiments: morphine sulfate (Temad, Tehran, Iran), glutamate (Sigma-Aldrich, St. Louis, USA), SB-334867 (Tocris, Bristol, UK), SB-334867 vehicle (β-cyclodextrin 2% DMSO 7%), ketamine (Trittau, Germany), xylazine (Alfasan, The Netherlands).

2.3. Experimental groups

Each experimental phase (rest/active) included 4 groups of morphine dependent rats as follows: Groups 1 and 2: morphine dependent rats received intra-LC microinjection of glutamate (100 nM, 200 nl) or its vehicle (aCSF, 200 nl) respectively. Groups