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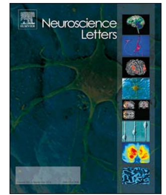

Research paper

Electroacupuncture suppresses morphine reward-seeking behavior: Lateral hypothalamic orexin neurons implicated

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ABSTRACT

The orexin system specifically located in the lateral hypothalamus (LH) has been shown to play a unique role in reward seeking, drug relapse, and addiction. In this study, we investigated the effects of electroacupuncture (EA) on morphine conditioned place preference (CPP), reinstatement of extinguished CPP, and the expression of orexin in the LH. A two-chamber CPP model was used to measure the rewarding properties of morphine. 2 Hz EA at points BL23 and ST36 was carried out for 30 min daily before morphine injection on three conditioning days. The extinguished CPP was reinstated by systemic morphine injection. Immunohistochemistry was used to detect orexin-positive nuclei in the LH. The findings indicated that the expression of morphine-induced CPP can be effectively suppressed by EA. EA suppressed orexin-positive nuclei in the LH compared with morphine-conditioned rats. A combination of EA and orexin A antagonist (10 mg/kg, i.p.) produced a significant decrease in the morphine reinstatement scores. The results indicate the possibility that orexin may be involved in the acupuncture effect on drug-seeking behavior and thereby has an important role in modulating acupuncture. The synergetic use of a protocol consisting of EA and low-dose orexin A antagonist may provide a novel treatment strategy for drug reward seeking and relapse.

1. Introduction

Acupuncture and electroacupuncture (EA), as nonpharmacologic interventions that activate self-healing mechanisms, have been applied to attenuate various conditions related to drug addiction. In an animal model, acupuncture and EA at ST36 acupoints have been found to suppress behavioral signs of morphine withdrawal, morphine conditioned place preference (CPP) and behavioral sensitization [1–3]. Consistent with this, additional recent work have shown that acupuncture at ST36 or HT7 can suppress morphine and ethanol self-administration, as well as attenuate the reinstatement of cue-induced heroin-seeking behaviors [4,5,24]. Our previous study showed that EA at BL23, located on the back and commonly used for analgesia and sedation in our clinic, attenuates morphine withdrawal signs and c-Fos expression in the central nucleus of the amygdale [25]. These findings have provided important evidence that EA or acupuncture might have a therapeutic effect on drug reward seeking, addiction, and relapse.

The neuropeptides orexin A and orexin B are recently discovered peptides that are produced from a prepro-orexin molecule specifically located in hypothalamic neurons. They target neurons throughout the central nervous system, such as the ventral tegmental area (VTA) and the nucleus accumbens (NAc), through two G protein–coupled receptors. A number of studies have shown a novel and important role of the orexin neuronal system in reward processing and addiction [6–8]. The stimulation of lateral hypothalamus (LH) orexin neurons drives morphine conditioned place preference and the reinstatement of an extinguished CPP for morphine [8]. Orexin receptor A antagonists reduce heroin or cocaine self-administration and cue-induced reinstatement of drug seeking [9]. Anatomically, orexin neurons in the LH heavily innervate the dopamine-rich VTA and NAc, and their receptors are expressed at high levels in the VTA and the NAc. A recent study showed that the orexin system in the LH is involved in neuronal plasticity and the potentiation of synaptic transmission in the VTA [10]. In the VTA, orexin A acutely potentiates the responses of NMDA receptors, which in turn leads to late-phase AMPA receptor–mediated plasticity in VTA dopaminergic neurons. These data indicate that orexin release associated with exposure to drugs of abuse might alter the synaptic plasticity within the VTA.

Acupuncture and EA have been well known to activate specific neural pathways and various endogenous transmitters, such as opioid peptides. Interestingly, neurobiological studies on acupuncture have identified the hypothalamus and limbic system as the important brain areas in mediating acupuncture effects [11,12]. Orexin neurons are specifically located in the LH. Feng et al. reported that EA increased the

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orexin A levels in the hypothalamus, PAG, and spinal cord in a rat model of post-laparotomy pain [16]. We hypothesize that orexin neurons in the hypothalamus might be important in understanding the underlying neurobiological mechanisms of acupuncture and EA in reward-seeking behavior. In the present study, we used orexin immunohistochemistry to determine whether orexin neurons were stimulated during the expression of preference for morphine rewards and the effect of EA stimulation. Furthermore, an animal model of morphine reinstatement was used to evaluate the synergetic effects of EA and an orexin antagonist on morphine reinstatement. Considering that arousal is most closely associated with orexin B activation, and drug reward with orexin A activation [6], the present study focused on the involvement of orexin A in the effects of EA and in reward-seeking behavior.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (250–300 g) from the Experimental Animals Center of Shanghai University of Traditional Chinese Medicine were used. All animal treatments were carried out in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The rats were randomly assigned and housed collectively (four per cage). They were allowed to acclimate to the housing facilities for 7 days and were maintained on a 12:12 h light/dark schedule at a constant room temperature of 22 °C, with free access to food and water. All experiments were done during the light cycle.

2.2. Drugs

Morphine hydrochloride, purchased from the First Pharmaceutical Factory of Shenyang, China, was dissolved in 0.9% sterile saline and given through intraperitoneal (i.p.) injection (10 mg/kg). The orexin A antagonist SB-334867 (SB; Tocris Bioscience, Minneapolis, MN) was dissolved in 25% dimethyl sulfoxide [DMSO] (10 and 30 mg/kg i.p.).

2.3. Place conditioning procedure

A two-chamber CCP model was used to measure the rewarding properties of morphine. One compartment had a grid floor with black walls, and the other had a mesh floor with black-and-white stripes on the walls. Each compartment was equipped with photocells to automatically record the time. On the preconditioning day (day 1 of the procedure), the rats were allowed to freely explore all the apparatus for 15 min, and the amount of time spent in each compartment was recorded. On the next 3 days, systemic morphine injection with either saline or morphine (10 mg/kg i.p.) was given in the morning and afternoon. The rats were confined to one side of the box for 30 min after each injection. Morphine and saline treatments were alternated in the morning and afternoon sessions for every conditioned animal. Briefly, the rats were given morphine in the morning and then were given saline in the opposite compartment in the afternoon. On subsequent days, the rats received saline in the morning and morphine in the afternoon. The morning and afternoon injections were at least four hours apart. Three days after the conditioning, a preference test was carried out. The animals were given free access to the apparatus for 15 min. The amount of time spent in each compartment and the amount of activity done were recorded. The preference scores were obtained as the amount of time the animals spent in the morphine reward–associated chamber minus the time they spent in the non-rewarded chamber.

2.4. Extinction and reinstatement procedure

The morphine preference of the rats was extinguished by repeatedly exposing the animals to the chambers without morphine administration. As previously reported [8], the animals were exposed daily until their preference levels for the morphine-paired chamber decreased to less than a 70 s difference between the two chambers for two consecutive days. The extinguished morphine preference was reinstated by systemic morphine injection (10 mg/kg i.p.).

2.5. EA stimulation

The rats were kept in special holders. Two stainless steel needles were bilaterally inserted into BL23 (at a depth of 5 mm and width of one or two ribs lateral to the caudal border of the spinous process of the second lumbar vertebra) or ST36 (at a depth of 5 mm near the knee joint, between the tibialis anterior and extensor digitorum longus muscles) as previously described [5]. For the EA at non-acupoint group, needle was placed into non-acupoints (1/5 tail length from the proximal region of the tail). The other needle was inserted into the anterior tibial muscle 3 mm distal to ST36. Constant current squarewave electric stimulation produced by a G-6805-2 electroacupuncture apparatus (Shanghai Medical Electronic Apparatus, China) was given by using two needles. The intensity of the stimulation was increased stepwise from 1.0 to 1.5 and then to 3.0 mA, with each step lasting for 15 min. The frequency of stimulation was 2 Hz. For the sham EA group, the needles were inserted only into the bilateral ST36 and BL23 points, with no current stimulation.

2.6. Orexin immunohistochemistry

One and a half hours after the morphine conditioning, the rats were deeply anesthetized with an overdose of sodium pentobarbital (100 mg/kg i.p.) and perfused transcardially with paraformaldehyde. According to the atlas of Paxinos and Watson [13], coronal sections that were 40 μm in thickness and −3.30 mm from the bregma were cut on a cryostat at −25 °C. The sections were incubated in PBS containing 5% normal goat serum and 0.3% Triton X-100 for 30 min and then in orexin A antibody (rabbit polyclonal antibody (1:500 dilution with PBS; Santa Cruz, USA) at 4 °C for 48 h. After being rinsed thrice with PBS, the sections were incubated in biotinylated goat anti-rabbit secondary antibody (1:200 dilution with PBS; Sigma, USA) for 2 h and then washed thrice. Next, the brain sections were placed in an avidin-biotin-peroxidase complex solution for 60 min. Finally, DAB was applied for visualization. The slices were mounted on glass slides, dehydrated in alcohol, and cover-slipped. For counting, orexin stained the cytoplasm brown. Two sections at same levels were chosen from each animal in each group. The sections were scanned by using a BX51 microscope (Olympus) and were quantified with the aid of the image analysis system Microimage (Olympus Optical, Europa).We took pictures with 40X and then count the cells. The number of orexin-positive neurons on the saved image were counted by a blind observer and averaged across sections for each animal.

2.7. Experimental protocols

In experiment 1, the effect of EA on the expressions of morphine-induced CPP and orexin was observed. The rats were divided randomly into five groups: the morphine-conditioned, the EA, the sham EA, the EA at non-acupoint, and the control. In the morphine-conditioned group (mor conditioned, n = 8), the rats were given only the morphine CPP training and test, without EA stimulation. In the EA group (n = 8), EA was carried out for 30 min before morphine injection during 3 days of morphine conditioning and on the preference test day. In the EA at non-acupoint group (EA nonacu, n = 7), EA was conducted at non-acupoint. In the sham EA group (n = 8), the same experimental procedures used in the EA group were applied, but the needles were inserted only into the bilateral ST36 and BL23 points, with no current stimulation. In the control group (n = 6), the same experimental procedures used in the control rats were used, except that the morphine

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