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Research report

Electroacupuncture attenuates morphine withdrawal signs and c-Fos expression in the central nucleus of the amygdala in freely moving rats

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Abstract

Experimental efforts for understanding the mechanisms of electroacupuncture (EA) for opiate addiction are partially hampered by restraint stress. In unrestrained animals, it is difficult to perform EA stimulation at acupuncture points frequently selected on the four limbs. The present study was performed to evaluate the effect of EA at the acupuncture point Shen-Shu (BL.23) on morphine withdrawal signs and c-Fos expression of the amygdala in freely moving rats or restrained rats. We applied immunohistochemistry to detect c-Fos-positive nuclei. Corticosterone levels and behavioral responses were measured during EA stimulation. The needles were bilaterally inserted and fixed at BL.23, and 100-Hz electric stimulation was conducted 30 min before naloxone-precipitated withdrawal. In both freely moving rats and restrained rats, EA significantly reduced the signs of morphine withdrawal. Notably, EA stimulation in freely moving rats attenuated c-Fos expression in the central nucleus of the amygdala while EA in restrained animals increased this response. In addition, the restrained rats emitted greater levels of vocalization and facial expression than freely moving rats during EA stimulation. Corticosterone levels were also significantly higher in restrained animals after EA stimulation. The new EA paradigm demonstrated in the present study might help the analysis of certain physiological responses induced by EA that would otherwise have been hindered by restraint stress.

Theme: Neural basis of behavior
Topic: Drugs of abuse: opioids and others

Keywords: Acupuncture point BL.23; Morphine withdrawal; The amygdala; c-Fos immunohistochemistry; Restraint stress

1. Introduction

Abrupt cessation of chronic opiate use results in a characterized withdrawal syndrome that includes nausea, dysphoria, and anxiety [23]. These consequences of abstinence are thought to be important factors contributing to opiate addiction. Acupuncture and electroacupuncture (EA) have been applied with great success to attenuate behavioral signs of morphine withdrawal in addicts [8,30,39]. The effects of acupuncture on drug addiction have also been verified by animal experiments. The withdrawal syndrome observed in morphine-dependent rats can be effectively suppressed by 100-Hz EA [20,47]. Morphine-induced conditioned place preference can be successfully suppressed by 2- or 100-Hz EA [38,42]. These animal studies have provided important information for understanding the underlying neurobiological mechanisms of acupuncture and EA in the treatment of opiate addiction.

However, experimental efforts are, at least partially, hampered by several limitations of laboratory animals. The most important one is that laboratory animals must be restrained for several minutes to complete acupuncture treatment. This has the following inherent problems: (1) acute restraint stress itself can interfere with acupuncture stimulation [27]; (2) both a single restraint session and a repeated restraint stress similarly enhance the effects of

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morphine on locomotor activity [37]. Those rats with high locomotor activity are more vulnerable to drug addiction [34]; (3) restraint stress induces the release of endogenous opioids [3] and produces opioid-like effects [2,32]; (4) restraint stress can activate several brain regions [10], representing a major confounding variable for the assessment of EA-induced c-Fos expression in brain areas.

In animal studies, the acupuncture points most frequently selected are Zusanli (ST.36) and Sanyinjiao (SP.6) located on the leg. It is difficult to perform the needle manipulation at these acupuncture points, which are located on the four limbs, in unrestrained animals. In fact, according to the theory of traditional Chinese medicine, some acupuncture points represent discrete locations on the body, where manual or electrical stimulation can exhibit similar, if not identical, therapeutic effects [4]. This raises the possibility that some acupuncture points located on the back and head have similar therapeutic effects as Zusanli (ST.36) and Sanyinjiao (SP.6). An important acupuncture point, Shen-Shu (BL.23), is located on the back and is commonly used for analgesia and sedation in our clinic. It has been shown previously that EA at BL.23 has similar antinociceptive effects as EA at ST.36 [4,17]. We hypothesized that some acupuncture points that induce analgesia could play a role in the treatment of opioid addiction.

The goals of our present study were to evaluate the effect of EA at BL.23 on morphine withdrawal syndrome in both freely moving and restrained rats. We compared the behavioral responses during EA stimulation. To determine whether EA exhibits different activation within certain brain regions in restrained or unrestrained rats, we initially applied c-Fos immunomapping to investigate functional activation of the amygdala during precipitated withdrawal from morphine and EA stimulation. The amygdala is thought to be important in mediating the behavioral, autonomic, and endocrine responses to stressors [11,12,22]. Functional neuroimaging studies demonstrate that the human amygdala is activated during negative affective states [11]. Furthermore, the amygdala is well known to be involved in opioid withdrawal [23,28]. A number of studies have shown that both morphine withdrawal and restraint stress induce the activation of c-Fos in the amygdala [5,16,18,35,41]. By excluding or minimizing interference from restraint stress, the present study assessed the "real" effects of EA stimulation on c-Fos expression of the amygdala in morphine withdrawal rats.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (250–300 g) from the Zhejiang Center of Experimental Animals were used. They were randomly assigned and housed collectively (four per cage) under controlled environmental conditions (22 °C, 12-h light/dark cycle) with free access to food and water. All animal treatments were performed in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All experiments were conducted during the light cycle.

2.2. Drug and withdrawal behavioral scores

Morphine treatment and precipitation of withdrawal were performed according to published procedures [48]. Rats were subcutaneously injected with morphine–HCI twice daily. The dose of each injection was 10 mg/kg in the first day and increased by 10 mg/kg each day thereafter (10, 20, 30, 40, 50 mg/kg). On day 6, morphine withdrawal syndrome was precipitated by an intraperitoneal (ip) injection of 4 mg/kg naloxone hydrochloride (Sigma, USA), 4 h after one single injection of 50 mg/kg morphine.

The withdrawal behavioral scores were calculated during the following 45 min (divided into three 15-min periods for scoring) in a quiet room by one observer who did not know what experimental treatment had been administered. The scored signs are based on published criteria [26,36,48], with minor modifications. The scores obtained for each 15-min period were noted on the score sheet and were summed over the 45-min observation period. Withdrawal scores included teeth chattering, wet dog shake, diarrhea, irritability, salivation, and abnormal posture including writhing, digging, and hunching. The amount of weight loss experienced by the animal during the observation period was also analyzed. Weight loss during the 45-min observation period was scored as 1 for a loss of <2%, 5 for a loss of <4%, 10 for a loss of <6%, 15 for a loss of <8%, and 20 for a loss of >8%.

2.3. EA stimulation

Electric stimulation was conducted for 30 min before naloxone-precipitated withdrawal. Stainless steel needles were bilaterally inserted to a depth of 5 mm into BL.23 (one to two rib's width lateral to the caudal border of the spinous process of the second lumbar vertebra) or ST.36 (located near the knee joint, between the muscle anterior tibialis and muscle extensor digitorum longus). Constant current square-wave electric stimulation produced by an electroacupuncture apparatus (Model G-6805-2, Shanghai Medical Electronic Apparatus, China) was administered via the two needles. The frequency of stimulation used was 100 Hz (0.2 ms pulse width). The intensity of the stimulation was increased stepwise from 1.5 to 2 mA, with each step lasting for 15 min.

2.4. c-Fos immunohistochemistry

Rats were deeply anesthetized with sodium pentobarbital (60 mg/kg, ip) and killed by transcardial perfusion of 200 ml saline followed by 200 ml 4% paraformaldehyde in