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Original research article

The influence of electrical stimulation on dorsal raphe nucleus with different current intensities on morphine-induced conditioned place preference in male rats

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ABSTRACT

Introduction: The dorsal raphe nucleus (DRN) influences a wide range of behavioral and physiological Q3 processes. The purpose of the present study was to test the effects of electrical stimulation of the DRN with different current intensities on morphine-induced conditioned place preference (CPP).
Materials and methods: Male Wistar rats were divided for experimental groups ($n = 7$). Stimulating electrodes were stereotaxically implanted into the DRN in anesthetized rats. We investigated the influences of electrical stimulation of the DRN with different current intensities with ineffective and effective dose of morphine (0.5 and 2.5 mg/kg, respectively) on morphine-induced CPP.
Result: Subcutaneous administration of morphine 2.5 mg/kg produced significant CPP in comparison with saline group. The stimulation of the DRN with different current intensities (10, 25, 50 and 100 μA) in combination with an effective dose of morphine did not show significant differences on acquisition phases, whereas there were significant decreases on expression phases *versus* to the morphine group on Q4 CPP only in current intensity 100 μA. The stimulation of the DRN with different current intensities in combination with an ineffective dose of morphine showed significant increases in current intensities (50 and 100 μA) on acquisition phases of CPP, but did not show significant differences on expression phases *versus* to the morphine group on CPP.
Conclusion: It is possible that electrical stimulation of the DRN with changes in concentration of serotonin or involving other transmitters such as glutamate and GABA would be involved with these changes of CPP.

Introduction

Q5 The dorsal raphe nucleus (DRN) influences a wide range of behavioral and physiological processes such as depression, sleep, aggression, anxiety, reward, and memory [1,2]. The DRN is the chief source of serotonergic (5-HT) projections to the nucleus accumbens (NAc) [3] and stimulates the release of dopamine from this nucleus [4] and is implicated in behavioral effects of opioids [5]. Nonetheless, the physiological mechanisms of the serotonin in the behaviors related to addiction are not well understood and have been focused mainly on motor behavior and sleep–wake cycle [6]. Moreover, there is evidence for reports that dysfunctions of serotonin and dopamine neurotransmission are involved in the pathophysiology of various neuropsychiatric disorders, including depression, schizophrenia, and drug abuse [7,8]. Also it was reported that addiction may be related to an imbalance between the excitatory glutamate (Glu) and inhibitory (GABA) neurotransmitters [9,10]. Endogenous opioids and their receptors are present in the DRN [11]. Opioids increase the level of extracellular 5-HT in the DRN and nucleus accumbens [12]. The previous studies indicated that acute systemic administration of morphine, enhancement 5-HT turnover in the CNS, but this effect was attenuated after prolonged opiate treatment [8]. The conditioned Q6 place preference (CPP) is one of the routine experimental protocols which used widely to study drug reward in laboratory [13]. It is known that agents such as eating delicious food, sweetened solutions, electrical brain stimulation and drugs abused could increase the conditioning time in this protocol [14]. CPP also can be

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induced by morphine in animals (monkeys and rabbits) [15–17]. Like many other abused drugs, administration of morphine, will induce preferences for distinctive environments conditioned [18]. Previous studies showed that electrical stimulation of medial prefrontal cortex suppressed morphine-induced CPP [18]. There are few studies which discussed the role of the dorsal raphe nucleus and its relation to the various phases CPP. In a clinical study lesions and inhibition of the DRN prevent stressor potentiation of morphine conditioned place preference in rats [19]. Moreover Xiang et al. indicated that peripheral electrical stimulation (PES) could be successfully used in the treatment of drug abuse [20]. Previous studies reported that electrical stimulation of the DRN decreased using of morphine self-administration [21]. Therefore we expected that the DRN stimulation in this experimental model can suppress conditional response induced by morphine. The purpose of the present study was to test the effects of electrical stimulation of the DRN with different current intensities on morphine induced CPP.

Materials and methods

Animals

All experiments were accomplished on male Wistar rats, obtained from the Isfahan University, Isfahan, Iran, weighing 200–250 g at the beginning of the experiment. Animals were maintained in animal house in a 12-h light–12-h dark normal cycle with food and water available at all times. The laboratory temperature was maintained at 22–25 °C. In each group of experiments seven rats were used.

Drugs

Morphine sulfate was purchased daily from the Temad Factory of Tehran (Iran) and two doses: ineffective (0.5 mg/kg) and effective (2.5 mg/kg) are prepared for injection (subcutaneously) by dissolving in saline 0. 9%. The dose of 0.5 mg/kg had no significant effect on CPP; therefore, we sidelined it as a non-effective dose. The dose of 2.5 mg/kg was relatively induced CPP but other doses had smaller effect; therefore, we choose 2.5 mg/kg as an effective dose.

Apparatus

The condition place preference apparatus containing three chambers (**A**, **B**, **C**) that are being separated by guillotine doors. The two large conditioning chambers (**A** and **B**) are with the same size but in different colors. Chamber **A** has walls and floor, which are black and white, while the walls and floor of the chamber **B** are white. The **C** chamber that is located in front of the other chamber was smaller and guillotine door is related to **A** and **B** chambers.

Surgery

Rats were anesthetized with 400 mg/kg chloral hydrate. Each animal was implanted with a stimulating electrode in a stereotaxic instrument. All electrodes were aimed at the DRN, following the coordinates of Paxinos and Watson rat brain atlas (anterior −7.92 mm, lateral 0.2 mm, and vertical −6.4 mm) [22]. After insertion, the brain electrodes were anchored to the skull by dental cement and held by two metal screws fixed to the skull bones.

Electrical stimulation protocol

In order to obtain the optimal current intensity, each group received one of these four stimulating current intensities (10, 25, 50 and 100 μA) with a constant stimulation frequency at 25 Hz just 15 min prior to morphine administration during the conditioning phase and before starting *postconditioning phase* for 10 min (period of stimulation; 1 s and rest interval; 5 s) (Stimulator Isolator A360, WPI, USA) in the separate box which was related to the stimulator [18].

Behavioral testing

The CPP protocol is followed continuously for 5 days and it consisted of three distinct phases [23,24].

Preconditioning, *conditioning* and *postconditioning* test. A week before the beginning of the experiment, all animals were allowed to habituate to the apparatus.

After habituation, in the *preconditioning phase* (day 1) animals received a test in which they were placed in the **C** chamber while the guillotine doors are raised to allow access to the entire apparatus for 15 min. In this phase the time spent in each chamber was recorded.

During the *conditioning phase* (days 2, 3 and 4), rats need to receive morphine with one of the two chambers (**A** or **B**) (45 min) in the 'unbiased' procedure. Morphine and saline were injected alternatively, in morning and afternoon sessions for every animal, so that rats given morphine in the morning were given saline in the opposite chamber in the afternoon, and contrariwise on subsequent days. Between morning and afternoon injection sessions were with 6 h intervals.

In the *postconditioning trial* (day 5), a test for CPP was given. Animals were placed in the **C** chamber while the guillotine doors are removed and like the day 1, they are allowed free access to the entire apparatus for 15 min. In this phase also the time spent in each chamber was recorded. The change of preference was calculated as the difference (in second) between the times spent in morphine receiving chamber on the day 5 and the day 1 [24].

Experimental design

One week after surgery, rats were divided into two surgical groups: morphine group and morphine-stimulation group. Morphine group received morphine without any stimulation while morphine-stimulation group are trained with stimulation before injection of morphine.

Locomotor activity

For prevention of intervention in locomotor activity on CPP, the number of Compartment Crossings (NCC) was calculated.

Histology

After completion of all experiments, the rats were sacrificed with an overdose of chloral hydrate and perfused transcardiac with 0.9% normal saline followed by 10% buffered formalin. Brains were removed and placed in 10% formalin for 72 h sectioning and cut coronally in 60 μm sections to confirm the location of the stimulating electrode aimed for the DRN (Fig. 1). Only rats with correct electrode placements in the area were included in subsequent data analysis [18].

Statistical analysis

Results are presented as mean ± SEM. In order to analyze data, Student's *t*-test and one-way ANOVA following Tukey post-test were used. Calculations were performed using the SPSS statistical software 21.

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