Physiology & Behavior 139 (2015) 321–327

Contents lists available at ScienceDirect

Physiology & Behavior

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

The oxytocin receptor impairs ethanol reward in mice

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HIGHLIGHTS

- Ethanol was used to establish conditioned place preference (CPP) in mice.
- CPP acquisition was reduced by the oxytocin analog Carbetocin and LV–OxtR.
- CPP extinction was accelerated by the oxytocin analog Carbetocin and LV–OxtR.
- CPP reinstatement was reduced by the oxytocin analog Carbetocin and LV–OxtR.

ARTICLE INFO

Article history:
Received 29 May 2014
Received in revised form 13 November 2014
Accepted 14 November 2014
Available online 20 November 2014

Keywords:
Acquisition
Carbetocin
CPP
Extinction
Oxytocin
Reinstatement
Viral vectors

ABSTRACT

It is well established that oxytocin, and its receptor (*OxtR*), play a crucial role in addiction and that the stimulation of oxytocin neurotransmission reduces addictive behaviors to ethanol in laboratory animals. However, the impact of *OxtR* modulation on acquisition, extinction and reinstatement of drug-elicited ethanol-conditioned place preference (EtOH-CPP) has not yet been investigated. In this study, we evaluated the effects of *OxtR* pharmacological modulation, using the oxytocin analog Carbetocin, and genetic overexpression in the nucleus accumbens (NAcc), using lentiviral-mediated gene transfer technology, of the *OxtR* on acquisition, extinction and reinstatement of drug-elicited EtOH-CPP in mice. In the first experiment, results showed that Carbetocin administration and NAcc *OxtR*-overexpression (LV–OxtR) reduced EtOH-CPP establishment. In the second experiment, systemic Carbetocin treatment and *OxtR* overexpression resulted in decreased time spent in the ethanol-paired compartment following completion of a 7-day extinction protocol. Finally, the third experiment showed that Carbetocin and LV–OxtR suppressed primed reinstatement of EtOH-CPP. It is concluded that pharmacological and genetic modulation of the *OxtR* can modulate the acquisition, extinction, and reinstatement of conditioned reinforcing effects of ethanol. Taken together, the current findings add to the growing literature on oxytocin neurotransmission modulation in the pharmacotherapy of ethanol addiction and alcoholism.

1. Introduction

Addiction to alcohol results in serious medical, societal and economic problems around the world. However, only a few medications have been approved by the U.S. Food and Drug Administration for the treatment of alcoholism [for review, see Ref. [1]]. Although a great deal of evidence supports the importance of the mesolimbic dopaminergic system in drug addiction as most of addictive drugs, including ethanol, increase extracellular dopamine in the Nucleus Accumbens (NAcc) [2–4], accumulating evidence implicates the endogenous oxytocin neurotransmission system in the retrieval of memories that underlie some conditioned effects of drugs of abuse in general [5–9] and ethanol in particular [10,11]. Previous studies demonstrate that oxytocin system neurotransmission can prevent the development of psychostimulants-induced addictive behavior in rodents. In fact, oxytocin reduced methamphetamine-induced dopamine efflux in the accumbens [12]. Furthermore, oxytocin pretreatment significantly reduced methamphetamine-induced *Fos* expression in the subthalamic nucleus and the NAcc-core in male Sprague–Dawley rats [13].

Apart from the role of the oxytocin system in psychostimulants conditioning, this system has been traditionally seen as a mediator of the rewarding properties of ethanol. In fact, it has been reported that when alcohol-preferring "P" rats were given a choice between a sweet alcohol-containing beverage (Raspberry Vodka Cruiser, 5% ethanol *v*/*v*) and a non-alcoholic sweet solution (3% sucrose) in daily sessions, a single administration of oxytocin (1 mg/kg) resulted in a long lasting decline in the preference for the alcoholic beverage relative to sucrose [10]. In addition, Silva and co-workers used immunohistochemistry and in situ hybridization techniques to show that chronic ethanol treatment resulted in a significant decrease in the number of oxytocin-immunoreactive

Abbreviations: CBT, Carbetocin; EtOH, ethanol; LV, lentiviral vector; *OxtR*, oxytocin receptor; PVN, paraventricular nucleus

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http://dx.doi.org/10.1016/j.physbeh.2014.11.046
0031-9384/

magnocellular neurons of the paraventricular nucleus (PVN) that was attributable to cell death [14]. In post-mortem brains of patients afflicted with chronic alcoholic disease, Sivukhina and colleagues showed a decrease in oxytocin-immunoreactivity in the supraoptic nucleus (SON) and increased in oxytocin immunoreactivity in the PVN [15]. Thus, the underlying mechanisms through which oxytocin can exert the protective effect against ethanol-induced reinforcing properties remained elusive.

Oxytocin is a nine amino acid neuropeptide that is synthesized in the PVN and SON of the hypothalamus [16,17] and released into the central nervous system as a modulator of neuronal transmission. The central actions of oxytocin are mediated via oxytocin receptors (*OxtR*) that are widely distributed in the brain. The *OxtR* is a typical rhodopsin-type class I G protein-coupled receptor (GPCR) that is primarily coupled via G_q proteins to phospholipase C. The seven transmembrane α-helices are most highly conserved among the GPCR family members [18–20]. A great deal of evidence supports the existence of *OxtR* in emotion-related regions, including the basal ganglia (Caudoputamen, Ventral pallidum, Globus pallidus, Nucleus accumbens), the limbic system (Lateral septal nucleus, Bed nucleus of stria terminalis, Amygdala, Hippocampus) and the thalamus and hypothalamus (Paraventricular nucleus (PVN), Paraventricular thalamic nucleus, Ventromedial hypothalamic nucleus) (for review, see Ref. [17]). Therefore, dysregulation of *OxtR* has been linked to many diseases including depression, anxiety, schizophrenia, drug addiction, stress-related behavior, memory and learning, sexual and social behavior, obsessive–compulsive disorder, eating disorders as well as neurodegenerative diseases (for review, see Ref. [21]). Taken together, oxytocin neurotransmission targeting agents could be advantageous for managing emotions-related disorders.

In this study, we used the oxytocin analog Carbetocin (CBT) and NAcc viral-mediated overexpression to assess the role of *OxtR* in the acquisition, extinction, and reinstatement of ethanol-induced CPP. Given that pharmacological studies have suggested that the oxytocin neurotransmission would participate in the retrieval of the memories and their consolidation [10], we hypothesized that CBT administration and NAcc *OxtR*-overexpression would modulate acquisition and facilitate extinction the of the EtOH-CPP response. Additionally, we examined the effects of CBT treatment and LV–OxtR overexpression on the reinstatement of drug-elicited extinguished EtOH-CPP, hypothesizing that as demonstrated before, it would prevent priming-induced reinstatement of EtOH-CPP.

2. Materials & methods

2.1. Animals

Male C57BL/6 mice obtained from the central animal breeding facility of the College of Medicine & Health Sciences were used. Mice weighing 23–30 g were kept under environmentally controlled conditions (ambient temperature, approximately 22 C; humidity, 50–60%) on a 12 h light/dark cycle (light on at 6:00 AM and light off at 6:00 PM) with food and water available ad libitum. The standard rodents chow diet was obtained from the National Feed and Flour Production and Marketing Company LLC. (Abu Dhabi, UAE). All procedures were approved by the College of Medicine & Health Sciences Animal Research, Care and Use Ethics Committee. All efforts were made to minimize suffering and the number of animals.

2.2. Drugs

Ethanol (10% *v*/*v* in physiological saline 0.9% sodium chloride and sterile water) was purchased from Panreac Quimica SAU (Barcelona, Spain) and used for intraperitoneal (i.p.) injections at dose of 2 g/kg. This dose was based on previous studies from our laboratory showing ethanol CPP with C57BL/6 mice [22–24]. The oxytocin analog Carbetocin acetate (CBT) (6.4 mg/kg; i.p.) purchased from Sigma-Aldrich (St. Louis, MO, USA) was dissolved in physiological saline and administered i.p. at a volume of 10 ml per kg of body weight. Doses of CBT were selected based on recent report [6].

2.3. Construction of OxtR overexpressing vectors and lentivirus production

Replication-defective, lentiviral vectors (LV) expressing the *OxtR* were created as described in our previous studies with minor modifications [25–28]. Briefly, using rat cDNA as a template, the *OxtR* coding sequence gene was amplified using specific primers capped with BamHI and XhoI recognition sequences. This fragment was then inserted at a unique BamHI and XhoI sites in the pTK431 transfer vector to construct the pTK–OxtR plasmid. For viral particles preparation, HEK293T cells were transfected by pTK–OxtR, pΔNRF and pMDG–VSV-G. The virus supernatant was collected after 48 h of cultivation. A concentrated solution of virus was made with ultra-centrifugation.

2.4. Stereotaxic surgery

A total of 48 adult male C57BL/6 mice, were assigned into one normal control group and one experimental group with stereotaxic injections of lentivirus, containing *OxtR* (LV–OxtR) or negative empty virus (LV-Mock), n = 24 mice for each group. For intra-cranial stereotaxic injection of viral particles, the mice were anesthetized with a combination of ketamine (100 mg/kg; i.p.) and xylazine (10 mg/kg; i.p.) and placed in stereotaxic apparatus. Using a 26-G Hamilton syringe, the viral solution was bilaterally injected through a burr hole into the NAcc using the following coordinates: anteroposterior, +1.7 mm from bregma; mediolateral, ±0.8 mm; dorsoventral, −4.5 mm [29]. The viral stock was injected at a rate of 0.2 μl/min (total volume 1 μl). The needle was left in place for an additional 10 min to permit time for the vector to diffuse from the needle tip and minimize upward flow of viral solution. The Hamilton syringe was rinsed with saline before being refilled for the next injection. After the surgical intervention, animals were injected subcutaneously with 2.5 ml pre-warmed isotonic saline and before waking, mice were allowed to recover in a heated cage. Behavioral experiment started 8 days later.

2.5. Ethanol-induced conditioned place preference (EtOH-CPP)

The EtOH-CPP was conducted as described previously with minor modifications [22–24,30]. In brief, eight identical wooden boxes with two equally sized chambers (30 cm × 30 cm × 30 cm) separated by a removable guillotine door were used for place conditioning. The chambers had different colored walls (black vs. white) and distinct floor textures (narrow vs. wide stainless-steel mesh). The time spent in each chamber was manually scored using an overhead camera. EtOH-CPP consisted of three phases and took place during the light cycle following an unbiased procedure in terms of initial spontaneous preference [22–24,30]. In brief, during the 1st phase (preconditioning/Pre-CPP) the mice were allowed access to both compartments of the apparatus and the time spent by the animal in each chamber during a 15-min period was recorded. Animals showing a strong unconditioned aversion (less than 35% of the time spent in both compartments) or preference (more than 65%) for any chamber were dismissed from testing. In the 2nd phase (acquisition), conditioning was performed for 5 consecutive days with twice daily injections. The first injection was performed in the morning with either administration of ethanol (2 g/kg; i.p.) or saline (same volume), and the mice were confined to one conditioning chamber for 30 min after the injection and then returned to their home cages. The second injection was performed in the afternoon with administration of saline, and the mice were confined to the other chamber for 30 min and then returned to their home cages. In each group, half the animals received the drug or vehicle in one chamber and the other half in the other chamber. During the 3rd phase, or post-conditioning (Post-CPP), the guillotine door separating the two chambers was