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Nucleus accumbens lentiviral-mediated gain of function of the oxytocin receptor regulates anxiety- and ethanol-related behaviors in adult mice

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HIGHLIGHTS

- Oxytocin receptor (OxtR) overexpression led to anxiolytic-like behavior.
- OxtR in the nucleus accumbens decreased ethanol consumption and preference.
- OxtR overexpression had no effect on saccharin nor quinine intake and preference.
- OxtR overexpression increased resistance to ethanol-induced sedation.
- OxtR overexpression attenuated ethanol-induced motor impairment.

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ABSTRACT

Anxiety is believed to influence ethanol use human in alcoholics. Studies using laboratory animals suggested an interaction between oxytocin and the behavioral effects of ethanol. Our previous study implicated a potential role for the oxytocin receptor (OxtR) in regulating ethanol-conditioned place preference. Here, we examined anxiety and the behavioral responses to ethanol in C57BL/6 mice stereotaxically injected in the nucleus accumbens (NAcc) with lentiviral vectors expressing an empty vector (Mock) or the OxtR cDNA. For anxiety we used the elevated-plus maze, the open-field and the marble-burying tests and for ethanol we used the two-bottle choice paradigm, the wire-hanging and ethanol-induced loss-of-righting-reflex tests. We found that, compared to Mock, OxtR overexpression led to anxiolytic-like behavior without altering spontaneous locomotor activity. Most importantly, we found that, relative to Mock controls, increased expression of the OxtR in the NAcc led to decreased ethanol consumption and preference in the two-bottle choice protocol and increased resistance to ethanol-induced sedation. We also compared the consequence of OxtR modulation on the consumption and preference of saccharin and quinine and found that the two experimental groups did not differ for any tastant. These results provide further evidence that the oxytocin system contributes to the regulation of ethanol drinking and sensitivity and position OxtR as a central molecular mediator of ethanol's effects within the mesolimbic system. Taken together, the current findings suggest that OxtR manipulation may be a relevant strategy to address ethanol use disorders.

1. Introduction

The neuropeptide, oxytocin is a circular nonapeptide member of the arginine vasotocin ubiquitous family. It is one of the most abundant peptides in the central nervous system [1]. It is well established that altered oxytocin neurotransmission in the reward system of the brain is associated with alcoholism [2]. Oxytocin neurotransmission is thought to contribute to the activating and reinforcing effects of ethanol and other drugs of abuse [for review, see [3,4]]. Altered oxytocin neurotransmission has been observed in human alcoholics [5–7] and in several animal models of alcohol dependence [2,8,9]. It is believed that the oxytocin receptor (OxtR) mediates the involvement of the oxytocin system in psychiatric disorders in general and addiction in particular. In fact, there is increasing evidence that OxtR may be implicated in a number of psychiatric disorders [for review, see [10]].

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 [11–13] widely distributed in the brain, with the highest levels of OxtR mRNA present in emotion-related regions,

Abbreviations: EPM, elevated-plus maze; LORR, loss-of-righting-reflex; MBT, marble-burying test; OF, open-field; OxtR, oxytocin receptor.

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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) [14,15]. As such, 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 [15]].

The role of oxytocin/OxtR in anxiety-related behaviors has been investigated in a number of behavioral tests. Intracerebroventricular (icv.) administration of an OxtR antagonist (des Gly-NH2d(CH2)5[Tyr(Me)2,Thr4]OVT; 0.75 μg/5 μL) decreases anxiety-related behaviors, in the EPM test, in both female pregnant and lactating rats without altering locomotor activity [16]. However, no effect was observed in either male or non-pregnant female rats [17]. In contrast, infusion of the same OxtR antagonist, into the ventrocaudal periaqueductal gray (cPAGv) reduced the percentage of time dams spent in the open arms. However, after separating dams from their litters for 4 h to increase anxiety, oxytocin (2 ng) infused into each hemisphere of the cPAGv doubled the percentage of time dams spent in open arms, which is indicative of decreased levels of anxiety [18]. More importantly, Mak and co-workers have shown recently that central but not systemic administration of the oxytocin analog carbetocin (CBT) produced pronounced anxiolytic-like behavioral changes after acute as well as repeated administration [19]. In the same line, it has been reported that oxytocin and CBT increased locomotion, in an open filed (OF) test, indicating attenuated anxiety levels. Interestingly, the increase of the activity induced by OXY and CBT indicating anxiolytic-like effects was blocked by oxytocin antagonists [20].

Interestingly, there is increasing evidence that individuals with anxiety disorders were self-medicating their symptoms with alcohol which can lead to abuse [21]. There is also evidence from the literature to suggest that anxiety and alcoholism may share some of the same underlying genetic risk factors. Data from family and twin studies indicated that the phenotypic correlation between alcohol and anxiety could be explained by common genetic factors [22,23]. Therefore, we suggest that alterations in the oxytocinergic signaling may contribute to anxiety-induced excessive drinking.

One possibility is that oxytocin, acting within the CNS, may modulate the role of dopamine in the reward circuitry [for review, see [3]]. In fact, it has been reported that the oxytocin neurotransmission inhibits tolerance to morphine [24] and ethanol [25,26]. Importantly, McGregor and Bowen reported lasting reduction (at least 6 weeks) in "Vodka Cruiser" preference (relative to 3% sucrose) in male and female alcohol preferring rats following a single administration of oxytocin (1 mg/kg) [4]. Recently, Peters and colleagues reported that, following 2 weeks of a two-bottle free choice continuous access of increasing concentrations of ethanol, systemic oxytocin (10 mg/kg) administration reduced the alcohol intake in single housed mice but not in stressed counterparts [27]. In humans, and compared to placebo, intranasal oxytocin given twice daily for 3 days in alcohol-dependent subjects, during a double-blind clinical trial, reduced alcohol withdrawal symptoms and reduced the amount of lorazepam administered "as needed" during detoxification [6]. Taken together, oxytocin neurotransmission targeting agents could be advantageous managing anxiety-induced alcohol intake in humans.

This study was the continuation of our previous investigation in which we reported that pharmacological and genetic modulation of the OxtR can modulate the acquisition, extinction, and reinstatement of conditioned reinforcing effects of ethanol [28]. The purpose of the current work was to determine if altered OxtR levels using a lentiviral expression vector into the Nucleus Accumbens (NAcc) would alter anxiety-related behaviors, and if so, what role does the OxtR plays in the regulation of ethanol voluntary consumption and preference in mice. We also investigated the effects of OxtR overexpression on ethanol-induced locomotor impairment and sedation. It was predicted that lentiviral-mediated OxtR overexpression in the NAcc would decrease anxiety-related behaviors and, consequently, reduces ethanol consumption and preference in a standard two-bottle continuous access drinking paradigm. Additionally, we anticipated that ectopic OxtR expression would attenuate ethanol-induced motor alterations and hypnotic sedation.

2. Materials and methods

2.1. Animals

Adult male C57BL/6 mice, obtained from the central breeding facility of the College of Science and Health Sciences, were housed in groups of five and allowed to adapt to this environment for a period of 7 days before the stereotaxic injections. Animals were given water and rodent chow diet, obtained from the National Feed and Flour Production and Marketing Company LLC (Abu Dhabi, UAE), ad libitum and maintained on a 12 h light/dark cycle (lights on at 6:00 A.M.). All experiments and procedures were approved by the institutional Animal Research Ethics Committee.

2.2. OxtR vector construction and lentiviral production

The OxtR expressing plasmid (pTK431-OxtR) was described in our previous study [28]. In brief, mouse OxtR cDNA was amplified using specific primers capped with *Bam*HI and *Xho*I restriction sites. The amplicon was then inserted into pTK431 previously digested with the same enzymes and the ligation product was amplified and confirmed by sequencing. For the replication-defective lentiviral vectors preparation, HEK293T cells were transfected by empty pTK431 (LV-Mock) or pTK-OxtR (LV-OxtR), pΔNRF and pMDG-VSV-G using the calcium phosphate method. The virus supernatant was collected after 48 h of cultivation. A concentrated solution of virus was made with ultra-centrifugation as described previously [29–37].

2.3. Stereotaxic injection and lentiviral vectors delivery

Viral transduction was performed as previously described [28–33,36]. Briefly, after anesthesia using a combination of ketamine (100 mg/kg; i.p.) and xylazine (10 mg/kg; i.p.), animals were mounted into a stereotactic frame (David Kopf Instruments, Tujuna, CA) in flat skull position. After the scalp of the mouse was shaved and then sterilized with 30% betadine and 70% ethanol solution, an incision was made down the midline of the head and the skin was pulled back to access the skull. As in our previous study [28], using a dental drill with a 0.75 mm burr two holes were drilled to access the NAcc bilaterally at coordinates 1.7 mm anterior to bregma; 0.8 mm medial lateral on each side and 4.5 mm ventral from the dural surface as determined using a stereotaxic atlas [38]. Using a 5 μL Hamilton syringe with a 26 gauge needle, 1 μL of the lentiviral constructs were delivered to the NAcc (0.5 μL/hemisphere) area at a rate of 0.2 μL/min. After microinjection was completed, the needle was left for an additional 5 min before its withdrawal to reduce the backflow of injected liquid along the injection tract, and then the incision was closed with interrupted sutures. The mouse was allowed to recover under a heat lamp until it was ambulating and ready to be returned and single housed in its cage. All of the behavioral experiments started 7 days after injection to allow sufficient time for viral vector transduction.

2.4. Anxiety-like behavioral experiments

All tests were conducted in a sound-attenuated room between 9:00 A.M and 3:00 P.M. Animals were habituated to the room for 1 h before starting the respective test. All behavioral tests were performed in a