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The effects of GSK-3β blockade on ketamine self-administration and relapse to drug-seeking behavior in rats

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ABSTRACT

Rationale: The role of glycogen synthase kinase-3 (GSK-3) has recently been implicated in the neurochemical mechanism underlying ketamine-induced neuronal toxicity and behavioral disturbance.
Objectives: The primary goal of the present study was to determine the role of GSK-3β in ketamine self-administration (SA) and relapse to drug-seeking behavior after abstinence.
Methods: In Experiment 1, the level of phosphorylated GSK-3β (p-GSK-3β) and total GSK-3β (t-GSK-3β) was determined in various brain areas following 14 days of ketamine SA. In Experiments 2 and 3, the effects of a GSK-3β inhibitor, SB216763 (2 and 4 mg/kg) and a GSK-3 inhibitor, lithium (LiCl, 100 mg/kg) on the responding maintained by 0.5 mg/kg/infusion ketamine SA were evaluated. In Experiments 4 and 5, rats underwent ketamine SA for 14 days followed by a 10-day abstinence period. The animals were treated with 2 or 4 mg/kg GSK-3β inhibitor, or 100 mg/kg LiCl during the cue-induced relapse test. Seven days later, animals received the same drug treatment and underwent the drug-induced relapse test. Finally, the effect of saline and DMSO on locomotor activity was evaluated in Experiment 6.
Results: Ketamine SA significantly decreased the ratio p-GSK-3β and t-GSK-3β (p-GSK-3β:t-GSK-3β) in the caudate putamen, nucleus accumbens, and ventral tegmental area. Both SB216763 and LiCl decreased responding on a progressive ratio schedule, but not on a fixed ratio schedule. Cue-induced relapse was suppressed only by 4 mg/kg SB216763, whereas drug-induced relapse was inhibited by 2, 4 mg/kg SB216763 and LiCl. However, inactive responses were also suppressed by LiCl during progressive ratio and drug-induced relapse testing.
Conclusions: SB216763 was effective at decreasing ketamine SA under the PR schedule and reducing drug-seeking behavior after abstinence.

1. Introduction

Glycogen synthase kinase-3 (GSK-3) belongs to the protein-serine kinase super family and was originally characterized as a regulator of glycogen metabolism (Embi et al., 1980; Rylatt et al., 1980; Plyte et al., 1992). A wide range of data indicate that GSK-3 may be involved in a host of normal and pathological processes, including the control of glycogen metabolism, regulation of cell proliferation and apoptosis (Woodgett, 1990; Cohen and Frame, 2001). There are two isoforms, GSK-3α and GSK-3β, which are monomeric enzymes and encoded by different genes (Woodgett, 1990). While there is approximately 98% sequence homology between the isoforms, they are not functionally equivalent (Woodgett, 1990). GSK-3α and GSK-3β are constitutively active and become rapidly inactive when phosphorylated in response to insulin (Cohen et al., 1978; Parker et al., 1983; Frame and Cohen, 2001). Therefore, their activity is negatively correlated with the level of their phosphorylation (Cohen and Frame, 2001; Frame et al., 2001). GSK-3β has been found to be widely distributed in various regions of bovine (Woodgett, 1990), rat (Leroy and Brion, 1999), and mouse (Yao et al., 2002) brains. The abundant expression of GSK-3β in the brain implies its importance in the functions of the central nervous system.

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GSK-3β has been suggested to be involved in the pathology of several brain disorders, including Alzheimer's disease (Pei et al., 1999; DaRocha-Souto et al., 2012; Ly et al., 2013), schizophrenia (Kozlovsky et al., 2000; Emamian et al., 2004; Freyberg et al., 2010), developmental neuropsychiatric disease (Willi et al., 2013), major depression (Saus et al., 2010; Diniz et al., 2011), and bipolar disorders (Nishiguchi et al., 2006; Benedetti et al., 2013). These findings have led researchers to conclude that GSK-3β could be a valid target for developing new therapeutic interventions for neuronal degenerative and mood disorders (Mathew et al., 2008; Li and Jope, 2010; Maes et al., 2012).

Recent studies have revealed that GSK-3β may contribute to diseases that arise from dysfunctional dopamine, glutamate and serotonin transmission in the brain (Li and Jope, 2010; Li and Gao, 2011; Beaulieu, 2012). Given the overwhelming data demonstrating a role for these neurotransmitters in the neurobiology of drug addiction (Thomas et al., 2008; Kalivas et al., 2009; Muller and Homberg, 2014), GSK-3β has also gained attention in the field of drug addiction, primarily with respect to the effects of psychostimulants. For example, a single injection of cocaine has been demonstrated to produce decreased phosphorylation of GSK-3β (p-GSK-3β) in the caudate putamen (CPU) of rats, suggesting an increase in the activity of GSK-3β (Miller et al., 2009). Similarly, following repeated methamphetamine injections, decreased levels of p-GSK-3β have been reported in the limbic forebrain of mice (Chen et al., 2007). The activity of p-GSK-3β in the core of nucleus accumbens (NAc) has also been found to be decreased in rats sensitized to repeated cocaine and methamphetamine injections (Xu et al., 2009, 2011). Binge cocaine self-administration in both adolescent and adult rats has also been demonstrated to alter the level of p-GSK-3β in amygdala (Perrine et al., 2008; Sillivan et al., 2011). In line with the neurochemical data, behavioral studies have shown that pharmacological inhibition of GSK-3β attenuates the behavioral effects of cocaine (Perrine et al., 2008; Xu et al., 2009) and methamphetamine (Xu et al., 2011). Interestingly, GSK-3β has been suggested to regulate diurnal variations in cocaine-induced conditioned place preference (Li et al., 2013) and reconsolidation of cocaine cue memories (Wu et al., 2011). These studies underline the relationship between the activity of GSK-3β in various brain regions and the behavioral effects of psychostimulants.

In China alone, the use of ketamine has continued to increase annually and ketamine has remained the third most commonly used illicit drugs in China in the last five years (UNODC, 2013). There are now increasing concerns about the harmful social and legal consequences of repeated misuse of ketamine (Copeland and Dillon, 2005; Morgan et al., 2010). Exposure to ketamine is known to induce neuronal apoptosis and schizophrenia-like behavioral abnormalities (Umbricht et al., 2000; Takadera et al., 2006). GSK-3β has been implicated recently in the neurochemical mechanism underlying ketamine-induced neuronal toxicity and behavioral disturbance. For example, decreased p-GSK-3β activity has been found in rat pups exhibiting ketamine-induced apoptosis (Liu et al., 2013). Behavioral studies have also demonstrated that GSK-3β activation contributes to the motor, sensorimotor, and cognitive abnormalities induced by ketamine (Chan et al., 2012). On the other hand, inactivation of GSK-3β has been shown to underlie the neuroprotective effect of erythropoietin in ketamine-induced neurotoxicity in primary cortical neurons (Shang et al., 2007). In a clinical setting, acute treatment with ketamine significantly increased the plasma level of p-GSK-3β in patients with major depressive disorder (Yang et al., 2013). These data support an important role for GSK-3β in behavioral alterations induced by ketamine.

Drug addiction is behaviorally characterized by voluntary drug use and repeated relapse to drug use after periods of abstinence (Dackis and O'Brien, 2001; Cami and Farre, 2003). The reinforcing and motivational effects of drugs are of great importance to the development of drug addiction (Bergman and Paronis, 2006; Roberts et al., 2007). The drug self-administration (SA) model mimics voluntary drug-taking behavior in animals and has been used widely to assess the reinforcing and motivational aspects of drugs (Spealman and Goldberg, 1978; Panlilio et al., 2003; Bossert et al., 2013). To date, it remains unknown whether GSK-3β affects the reinforcing and motivational effects of addictive drugs and drug-seeking behavior after abstinence. The primary goal of the present study, therefore, was to determine the role of GSK-3β in the reinforcing and motivational effects of ketamine, using a SA model. The current study investigated changes in the activity of GSK-3β in various brain regions following chronic ketamine SA. Furthermore, pharmacological tools were used to determine whether blockade of GSK-3β altered the reinforcing and motivational effects of ketamine using fixed and progressive ratio procedures. Finally, the effects of GSK-3β on cue- and drug-induced seeking behaviors after abstinence were assessed.

2. Methods

2.1. Animals

The subjects were male Sprague-Dawley rats (Zhejiang Academy of Medical Sciences, Hangzhou, Zhejiang, China), weighing approximately 300–350 g at the beginning of the experiment. The animals were housed in a temperature- and humidity-controlled room with a reverse 12 hour (h) light/dark cycle (lights off at 7:00 am). The temperature was maintained at 20–25 °C and humidity levels were stable (50–70%). Experimental sessions were performed during the dark cycle. Food and water were available ad libitum. The experimental protocol was approved by an Institutional Review Committee for the use of Animal Subjects. All experiments were conducted in accordance with the guidelines of the Institutional Laboratory Animal Care and Use of Ningbo University. "Principles of laboratory animal care" were followed.

2.2. Surgery

After a minimum acclimation period of 7 days, animals were anesthetized with a combination of ketamine HCl (50 mg/kg, i.m.) and xylazine (7.5 mg/kg, i.m.). Each rat was subsequently implanted with a cannula into the right jugular vein. The cannula was passed subcutaneously to the dorsal surface of the scapulae and attached to an infusion pump via Tygon tubing (width = 0.05 cm, inner diameter = 0.05 cm; Saint Gobain Performance, Valley Forge, PA, USA) through a spring tether. Catheters were flushed daily with sterile saline containing heparin sodium (0.4%) and penicillin sodium to preserve catheter patency and to prevent infection. A total of nine animals were excluded due to failure to acquire ketamine SA ($n = 4$) or catheter failure ($n = 5$). A total of 51 animals completed the ketamine SA.

2.3. Ketamine SA

All experiments were conducted in operant response test chambers (AniLab Software Instruments Co., Ltd. Ningbo, China). Following approximately 5 days of recovery from surgery, rats began ketamine SA training. Sessions began at approximately 8 am every day while the house-lights were off, and illumination of the light above the left nose-poke hole signaled the availability of drug. A nose-poke response in the left hole was immediately reinforced with an injection of 0.5 mg/kg/infusion ketamine. Ketamine was delivered via the pump over approximately 5 seconds (s), depending on the weight of the animal. Each ketamine infusion triggered a timeout period of 20 s during which the house-light was turned on. A nose-poke response in the right hole was considered as an inactive response which had no programmed consequences. During the acquisition phase, the animals were allowed to self-administer ketamine (0.5 mg/kg/infusion) daily on a fixed ratio 1 (FR1) schedule of reinforcement for 4 h. Animals were considered to have acquired ketamine self-administration when they had received a minimum of 40 infusions during one training session and the number of infusions remained stable for three consecutive training sessions (±10%).

Following the acquisition, rats were allowed to self-administer ketamine daily under an FR1 schedule for 2 h. A progressive ratio (PR) schedule of reinforcement was introduced following FR1 testing (Richardson and Roberts, 1996). Briefly, each daily PR session was 6 h in duration. On the PR schedule, a progressively increasing number of responses (1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145,178, 219, 268, 328, 402, 492, and 603) were required for the next injection of ketamine. Prior to drug testing, breakpoints maintained by ketamine were generated from at least three consecutive sessions of stable responding (no more than three breakpoint difference across 3 consecutive days).