Brain Research 1678 (2018) 419–431

Contents lists available at ScienceDirect

Brain Research

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

Research report

Effects of medial prefrontal cortex 5-HT$_7$ receptor knockdown on cognitive control after acute heroin administration

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ARTICLE INFO

Article history:
Received 15 February 2017
Received in revised form 28 October 2017
Accepted 1 November 2017
Available online 16 November 2017

Keywords:
Serotonin
Heroin
Medial prefrontal cortex
NMDA receptor
Cognition

ABSTRACT

Heroin abuse is linked to a deleterious effect on cognitive functioning in the individual. Recent evidences suggest that the serotonin$_7$ receptor (5-HT$_7$R) is engaged in the regulation of cognitive control and the drug use-associated behaviors. However, the role of 5-HT$_7$R in the cognitive control after acute heroin administration has not been studied. The present study aims to investigate whether the knockdown of the 5-HT$_7$R by virus-mediated gene silencing in the medial prefrontal cortex (mPFC) could ameliorate the acute heroin-induced cognitive impairments. The attentional function, impulsivity and compulsivity were assessed by the 5-choice serial reaction time task (5-CSRTT) in mice. The memory ability and locomotor activity were examined by the novel objects recognition (NOR), Y-maze and open-field test (OFT). Acute heroin administration at 5 mg/kg produced robust disruptions in attention, impulsivity and motivation in mice. 5-HT$_7$R knockdown in the mPFC did not affect the 5-CSRTT baseline performance, spatial working memory, visual episodic memory and locomotion. However, mPFC 5-HT$_7$R knockdown selectively ameliorated acute heroin-induced increase in omissions and premature responses under conditions of increased perceptual load. In addition, mPFC 5-HT$_7$R knockdown induced increases in perseverative responding observed across both saline and heroin-treated animals. Moreover, 5-HT$_7$R knockdown prevented the heroin-induced decrease in NR1/CaMKII phosphorylation in mPFC, thus suggesting that 5-HT$_7$R and *N*-methyl-D-aspartic acid (NMDA) receptor signaling may be involved in the cognitive outcomes of acute heroin administration. Altogether, these observations suggest modest and restricted effects of mPFC 5-HT7R knockdown on cognitive behaviors, both in the presence or absence of acute heroin treatment.

1. Introduction

Heroin abuse has been associated with a deleterious effect on cognitive functioning in numerous reports (Papageorgiou et al., 2004; Verdejo et al., 2005). Heavier use of heroin has been shown to be associated with a greater likelihood of cognitive impairment (Zhong et al., 2015). In the case of former heroin addicts, there is a slower performance and/or less accuracy in a variety of behavioral tests exploring cognitive abilities (Mintzer et al., 2005; Verdejo et al., 2005). Heroin also has a negative effect on impulsive control, while attention and mental flexibility/abstract reasoning ability are not affected (Pau et al., 2002). Alterations in memory circuit and inhibitory control were suggested to contribute to high relapse risk even after a period of heroin abstinence (Zhang et al., 2016). In comparison with numerous reports describing cognitive deficits after long-term heroin exposure, the cognitive changes after acute heroin administration is much more controversial. It has been reported that heroin administration acutely impairs stimulus-driven attentional function rather than having a specific effect on impulsive control (Schmidt et al., 2013). However, data from a clinical study suggested that acute heroin administration exacerbates impulsive characteristics of heroin-dependent participants (Jones et al., 2016). In this study, we investigated whether and how heroin

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https://doi.org/10.1016/j.brainres.2017.11.002
0006-8993/

administration acutely modulates response inhibition and stimulus-driven attention allocation.

Serotonin (5-HT), which closely interacts with the dopamine system (Korte et al., 2016), has been shown to be of essential importance not only to maintain synaptic plasticity (West et al., 1995), but also for the hedonic tone, motivational (Browne and Fletcher, 2016) and reinforcement processes (Zoratto et al., 2016), and for learning and memory (Mishra and Goel, 2016). The 5-HT_7 receptor (5-HT_7R) is a member of the 5-HT family of G-protein-coupled receptors. A wide body of evidence supports a role of 5-HT_7R in diverse disorders of the central nervous system, including depression, anxiety and schizophrenia (Hedlund, 2009; Matthys et al., 2011). Recent experimental data suggest that 5-HT_7R may also play a role in the establishment of drug use-associated behaviors. For example, the 5-HT_7R antagonist SB-269970 attenuated the increase in rearing and circling behavior in amphetamine-induced hyperlocomotion (Waters et al., 2012). Alcohol vapor exposure for 20 days led to an increase in 5-HT_7R mRNA expression in the striatum of mice (Yoshimoto et al., 2012). At present, less is known about the effects of the dysfunction of brain region-specific 5-HT_7R on the heroin-induced cognitive deficits.

The medial prefrontal cortex (mPFC) is the target area of the mesocorticolimbic pathway, in which the 5-HT_7 receptors are highly expressed (Ciranna and Catania, 2014). The mPFC plays a crucial role in higher cognitive functions involving motivation, emotion, learning and memory (Cassaday et al., 2014; Dalley et al., 2004). Accumulating evidence indicates that dysfunction in the mPFC is associated with heroin-seeking behavior and cognitive deficits (Doherty et al., 2013; Puig et al., 2014; Tammimaki et al., 2016). Some of these deficits can be assessed in animal models in a manner analogous to human tests. The 5-choice serial reaction time task (5-CSRTT) is a well-established test that allows for the simultaneous examination of multiple aspects of cognition, including attention, motivation, impulsivity and cognitive flexibility (Robbins, 2002). In this paradigm, mice earn reinforcement by detecting and correctly responding to brief visual stimuli randomly occurring in one of five spatial locations. Attention deficits are expressed as decreased response accuracy and increased omissions. Premature and perseverative responses (responding during an ITI and multiple responses to a single stimulus, respectively) gauge aspects of impulsivity and compulsivity. The motivation for the food reward is reflected by the total number of trials completed by the animal and the reward collection latency. Additionally, the load for these cognitive deficits can be further increased by using a variable ITI. Previous studies have indicated that agents affecting 5-HT receptor activity disrupt 5-CSRTT performance, and the behavioral effects are not always consistent (Nikiforuk et al., 2015; Robbins, 2002).

In the present study, we sought to utilize recombinant adeno-associated virus (rAAV)-mediated gene silencing to investigate whether knockdown of the 5-HT_7R in the mPFC alters the cognitive behaviors in the 5-CSRTT induced by acute heroin administration. The learning memory and locomotor activity were also examined by using the Y-maze test, novel objects recognition (NOR) test and open-field test (OFT). Moreover, the activated (phosphorylated) form of the obligatory NR1 subunit of the *N*-methyl-D-aspartic acid receptor (NMDAR) and its downstream modulator Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) in the brain were examined by Western blotting.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice were housed in temperature- and humidity-controlled rooms (22 ± 2 °C and 55 ± 5%) under a 12-h light-dark cycle, and water and laboratory chows were available *ad libitum*. All animals were experimentally naïve and used only once. All experimental procedures were performed under light conditions. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of the Ningxia Medical University and were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and to avoid suffering and distress.

2.2. rAAV production and microinjection

The rAAV constructs expressing shRNA directed against 5-HT_7R (shHTR7) were generated by Genechem (Shanghai, China). Briefly, a sequence targeting 5-HT_7R in the consensus coding region of the mRNA was chosen (GenBank accession NM_008315.2). For the scrambled shRNA (Scr), a random sequence of 26 bases with no similarity to any known mRNA was selected. These synthetic oligonucleotide duplexes were tested in cell culture using the serotonergic-like CA-77 cell line (Greene and Tischler, 1976). To achieve long-term in vivo knockdown, efficacious sh5-HT_7R or control Scr was incorporated into AAV2. The final construct was pAAV-U6-shHTR7-CMVβGlobin-EGFP-3Flag.

Before surgery, rAAV vectors were diluted in buffer (400 nM NaCl, 20 mM Tris base) to 1×10^{12} v.g/ml and dialyzed in 0.9% saline. Heparin (1000 U/ml) was added to the virus in a 1:7 ratio (v/v) to facilitate viral infection (Smith et al., 2004). Mice were anesthetized with sodium pentobarbital (80 mg/kg, i.p.) and were positioned and fixed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA). Two holes were drilled in the skull of each mouse above the intended site of injection, and a glass micropipette (tip O.D. 0.1 mm) was lowered into the mPFC (AP + 1.8 mm, ML ± 0.5 mm, DV − 2.5 mm) and held in place for 5 min before the infusion began. The virus was bilaterally delivered (2.0 μl per side) over the course of 5 min using a microinjection pump. The micropipette was left in place for 5 min before being removed. Mice were given buprenorphine (50 μg/kg, s.c.) every 8 h for 2 days. Ampicillin (150 mg/kg, s.c.) was administered twice daily for 3 days. Mice remained undisturbed in the vivarium for 7 days before the initiation of behavioral testing.

2.3. 5-CSRTT

Mice were trained on the 5-CSRTT as previously described, with minor modifications (Finlay et al., 2015). The apparatus, 5-CSRTT training procedure and behavioral measures are described in the Supplementary materials. Through a sequence of training, mice learned to collect milk reward by making a nosepoke into one of the 5 randomly illuminated holes within the stimulus duration (SD) or during a limited hold (LH). The retrieval of the milk then initiated a 5 s inter-trial interval (ITI). A timeout (5 s), signaled by illumination of the houselight, occurred if a mouse made a response during the ITI or failed to make a correct response. Sessions were terminated after 30 min or 100 trials.

Probe sessions began when a mouse attained ≥80% accuracy (number of correct responses/number of correct + incorrect responses) and ≤20% omissions (number of trials missed/number of trials presented) under the 0.8 s SD condition for 3 consecutive days. Probe sessions consisted of variable short ITIs (S-ITI, 2, 3, 4, and 5 s), long ITIs (L-ITI, 5, 6, 7, and 8 s), reduced SDs (RSD, 0.2, 0.4, 0.6, and 0.8 s), and reduced stimulus intensities (RSI, 30%, 40%, 50%, 70%, and 100%). Probe sessions were performed in the order listed above, and mice were returned to baseline parameters sessions (baseline 1–4, 5 s ITI and timeout; 0.8 s SD, 5.8 s LH) between each probe session. The accuracy (%), omissions (%), premature responses (total number of nosepokes during an ITI and