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AUTS2 IN THE NUCLEUS ACCUMBENS IS ESSENTIAL FOR HEROIN-INDUCED BEHAVIORAL SENSITIZATION

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Abstract—Autism susceptibility candidate 2 (AUTS2) is a gene associated with autism and mental retardation. Recent studies have suggested an association of the *AUTS2* gene with heroin dependence, and reduced *AUTS2* gene expression may confer increased susceptibility to heroin dependence. However, the functional role of the AUTS2 protein in regulating enduring neuroadaptations in response to heroin exposure has not been established. Here, we investigated the effects of acute and chronic heroin exposure on AUTS2 mRNA and protein expression in the nucleus accumbens (NAc) and caudate-putamen (CPu) to determine whether changes in AUTS2 expression are associated with heroin-induced locomotor sensitization in mice. Moreover, we explored whether AUST2 knockdown affects heroin-induced locomotor sensitization. AUTS2 mRNA and protein expression in the NAc, but not the CPu, was decreased after chronic heroin (1 mg/kg) administration. In the NAc, the expression of heroin-induced locomotor sensitization was enhanced through the lentiviral-AUTS2-shRNA-mediated knockdown of AUTS2, while the overexpression of AUTS2 attenuated the locomotor-stimulant effects of heroin. Together, these results indicate that AUTS2 in the NAc, but not the CPu, suppresses the initiation and expression of heroin-induced behavioral sensitization, suggesting that AUST2 may be a potential target for the treatment of heroin dependence.

Key words: autism susceptibility candidate 2, nucleus accumbens, behavioral sensitization.

INTRODUCTION

Heroin abuse is a major health and social problem of modern society worldwide. From a neurobiological point of view, heroin addiction can be characterized by long-lasting changes in behavior that persist despite prolonged abstinence; however, the underlying mechanisms are complex. Extensive studies have suggested that alterations in gene expression in the reward circuitry contribute to the long-term adaptations in brain structure and function that accompany chronic exposure to heroin. In the past decade, epigenetic mechanisms, including alterations in the accessibility of genes within the native chromatin structure of these molecules induced through histone tail modifications and DNA methylation, have been implicated in mediating the long-term adaptations that underlie opiate addiction.

Polycomb group (PcG) proteins maintain repressive forms of chromatin and appropriate patterns of gene repression through epigenetic mechanisms. Alterations of PcG protein complexes, including Polycomb repressive complex 1 (PRC1) and 2 (PRC2), at *Bdnf* promoters in the rat ventral tegmental area (VTA) have been implicated in mediating neural and behavioral plasticity in response to chronic morphine administration (Koo et al., 2015). Previous studies have revealed a physical link between novel PRC1 complexes, comprising Polycomb group RINF fingers (PCGFs) 3 and 5, and autism susceptibility candidate 2 (AUTS2) (Gao et al., 2012). A recent study indicated that PRC1-AUTS2 neutralizes PRC1 repressive activity, whereas AUTS2-mediated recruitment of P300 results in gene activation through promoter association (Gao et al., 2014). In addition, the conditional targeting of *Auts2* in the mouse central nervous system (CNS) leads to various developmental impairments (Gao et al., 2014).

The *Auts2* gene, located on chromosome 7q11.2, encodes a nuclear protein and is frequently associated with multiple neurological diseases, including autism. In addition, *Auts2* has been implicated as an important gene in human-specific evolution (Sultana et al., 2002; Oksenberg and Ahituv, 2013). A recent functional analysis of this gene revealed a potential role for AUTS2 in the regulation of alcohol consumption and heroin addiction (Schumann et al., 2011; Chen et al., 2013; Chojnicka et al., 2013), as well as neurodevelopment (Beunders et al., 2013; Oksenberg et al., 2013; Gao et al., 2014); however, the precise function and signaling pathways associated with AUTS2 in adult brains and opiate addiction have not been established. Here, we report

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Abbreviations: AUTS2, autism susceptibility candidate 2; CPu, caudate-putamen; NAc, nucleus accumbens; PcG, polycomb group; PRC, polycomb repressive complex.

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that decreased AUTS2 mRNA and protein expression in the nucleus accumbens (NAc), but not the caudate-putamen (CPu), is associated with repeated heroin exposure. Furthermore, AUTS2 expression levels in the NAc are reduced during the initiation and expression of heroin-induced behavioral sensitization. The specific overexpression of AUTS2 in the mouse NAc blocks the locomotor-stimulant effects of heroin, while AUTS2 knockdown enhances heroin-induced hyperlocomotion.

EXPERIMENTAL PROCEDURES

Subjects

Male C57BL/6J mice (weighing 18–20 g upon arrival) were obtained from the Laboratory Animal Center, Xi'an Jiaotong University. The mice were housed in groups of four in a temperature-controlled (23 ± 2 °C) and humidity-controlled (50 ± 5%) room with food and water provided *ad libitum* in the home cage. The animals were maintained on a reverse 12-h/12-h light/dark cycle (lights off at 8:00 AM). All animal procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the procedures were approved by the local Animal Care and Use Committee.

Drugs

Heroin hydrochloride (purity 80%) was provided by the Drug Intelligence and Forensic Center, Ministry of Public Security from PR China. The drugs were dissolved in a vehicle (saline with 0.005% acetic acid), and an injection volume of 10 ml/kg body weight was freshly prepared prior to each experiment.

Acute and repeated heroin treatment paradigm

For the acute injections, the mice were subcutaneously (s.c.) injected with 1 mg/kg of heroin (n = 8) or vehicle (vehicle, n = 8) at 1 h prior to sacrifice. For repeated injections, the mice (n = 8 per group) were injected twice a day (at 11:00 h and 16:00 h) for five consecutive days with heroin (1 mg/kg, s.c.). The mice were sacrificed 1 h after the last injection.

Heroin-induced behavioral sensitization

Heroin-induced behavioral sensitization was conducted according to previous studies, with some modifications (D'Este et al., 2002; Ranaldi et al., 2009; Wu et al., 2015). Briefly, heroin (HRI; 1.0 mg/kg, s.c.) or vehicle (SAL) was injected twice a day (at 8:00 h and 20:00 h) for five consecutive days (Day 1 to Day 5). Three days after the last injection (Day 8), the mice were challenged with an injection of heroin (0.5 mg/kg, s.c., at 20:00 h), and the locomotor activity was measured for 60 min after the challenge injection. To examine the influence of AUTS2 on the development of behavioral sensitization, lentiviral-AUTS2-shRNA was administered 1 week prior to the twice-daily heroin regimen.

Locomotor responses were determined in individual activity chambers (43 × 43 × 43 cm) as previously described (Zhu et al., 2012). Following a 2-h habituation period, the mice were injected, and locomotor activity was monitored using a Smart Video Tracking System (version 2.5; PalnIab Technology for Bioresearch, Spain). The cages were carefully cleaned with a 0.1% acetic acid solution to prevent any odor traces.

Plasmid constructions and preparation of viral stocks

For lentivirus expression, cDNAs encoding AUTS2-shRNA or control-shRNA (Hori et al., 2014) and a fusion AUTS2 protein with the V5 tag at the C-terminal (AUTS2 overexpression) were ligated into the lentiviral vector plenti6/V5-topo (Life Technologies, Paisley, UK), bicistronically expressing enhanced green fluorescent protein (EGFP). Recombinant lentiviruses were produced using the ViraPower Lentiviral Expression System (Invitrogen, K4970-00). The virus was concentrated 10–15 times through centrifugation in a Centricon Plus-20 filter (Millipore) according to the manufacturer's instructions. Aliquots were stored at −80 °C. All virus preparations were titered according to the Virapower protocol and contained 4×10^8 TU/ml.

Surgery and intracranial injections

The mice (weighing 18–20 g at the time of surgery) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and placed in a stereotaxic apparatus (David Kopf Instruments). Lentiviral-AUTS2-shRNA (knockdown) or lentiviral-AUTS2 (overexpression) (approximately 1.0×10^6 infectious units of virus per μl) was bilaterally injected into the NAc (from the bregma: AP, +1.6 mm; ML, ±1.5 mm; DV, −4.4 mm) using a Hamilton syringe (needle gauge 31) at a speed of 0.1 μl/min to a total volume of 0.5 μl. The needle remained in place for an additional 5 min. The behavioral tests were performed 7 days after the viral injection, and the infected NAc areas were collected after sacrifice for western blotting.

Tissue sample preparation and western blot assays

Tissue sample preparation and western blotting were performed as described previously (Wang et al., 2015; Zhao et al., 2015). Briefly, the NAc and CPu were isolated on ice using a 26-gauge needle under a dissecting microscope. The isolated tissue was stored at −80 °C until further processing for western blotting. The proteins were separated on 7.5% sodium dodecyl sulfate–polyacrylamide gels and transferred to nitrocellulose membranes (Invitrogen). The membranes were incubated with blocking solution containing 5% dried non-fat milk in TBST (0.1% Tween-20 in TBS buffer) for 1 h. The membranes were incubated in primary antibody diluted in blocking solution overnight at 4 °C. The membranes were rinsed in TBST buffer (3 × 20 min) and subsequently incubated in secondary antibody for 1 h at room temperature. The following primary antibodies were used: rabbit anti-AUTS2 (1:1000, ab92326, Abcam, Cambridge, UK) and mouse anti-β-actin (1:10,000, A5316, Sigma–Aldrich, St. Louis, MO, USA). The blots were developed with enhanced chemiluminescence (ECL) reagent (Amersham), and the