Priority Communication

Biological Psychiatry

BRG1 in the Nucleus Accumbens Regulates Cocaine-Seeking Behavior

Zi-Jun Wang, Jennifer A. Martin, Lauren E. Mueller, Aaron Caccamise, Craig T. Werner, Rachael L. Neve, Amy M. Gancarz, Jun-Xu Li, and David M. Dietz

ABSTRACT

BACKGROUND: Drug addiction is defined as a chronic disease characterized by compulsive drug seeking and episodes of relapse despite prolonged periods of drug abstinence. Neurobiological adaptations, including transcriptional and epigenetic alterations in the nucleus accumbens, are thought to contribute to this life-long disease state. We previously demonstrated that the transcription factor SMAD3 is increased after 7 days of withdrawal from cocaine self-administration. However, it is still unknown which additional factors participate in the process of chromatin remodeling and facilitate the binding of SMAD3 to promoter regions of target genes. Here, we examined the possible interaction of BRG1—also known as SMARCA4, an adenosine triphosphatase–containing chromatin remodeler—and SMAD3 in response to cocaine exposure.

METHODS: The expression of BRG1, as well as its binding to SMAD3 and target gene promoter regions, was evaluated in the nucleus accumbens and dorsal striatum of rats using western blotting, co-immunoprecipitation, and chromatin immunoprecipitation following abstinence from cocaine self-administration. Rats were assessed for cocaine-seeking behaviors after either intra-accumbal injections of the BRG1 inhibitor PFI3 or viral-mediated overexpression of BRG1.

RESULTS: After withdrawal from cocaine self-administration, BRG1 expression and complex formation with SMAD3 are increased in the nucleus accumbens, resulting in increased binding of BRG1 to the promoter regions of *Ctnnb1*, *Mef2d*, and *Dbn1*. Intra-accumbal infusion of PFI3 attenuated, whereas viral overexpression of *Brg1* enhanced, cocaine-reinstatement behavior.

CONCLUSIONS: BRG1 is a key mediator of the SMAD3-dependent regulation of cellular and behavioral plasticity that mediates cocaine seeking after a period of withdrawal.

Keywords: BRG1, Chromatin remodeling, Cocaine, Cue-induced reinstatement, Self-administration, SMAD3

http://dx.doi.org/10.1016/j.biopsych.2016.04.020

Drug addiction is one of the most debilitating psychiatric disorders and is characterized by compulsive drug seeking and episodes of relapse despite prolonged periods of abstinence. In drug addicts, relapse and craving during abstinence are often triggered by environmental cues that were previously associated with drug use (1). There are few effective treatments for drug addiction, in part because of a lack of detailed pathophysiology of the disease (2). The clinical scenario can be modeled by testing for reinstatement of drug seeking in laboratory animals after exposure to discrete cues (3–5). Such studies have shown that neurobiological adaptations leading to changes in neuronal plasticity in key brain areas, such as the nucleus accumbens (NAc), contribute to increased drug seeking and craving (6–8).

Accumulating evidence suggests that the long-term changes in cellular and behavioral function after drug exposure are mediated by alterations in gene transcription (9,10). We previously identified SMAD3, a transcription factor within the transforming growth factor-β signaling pathway, as an essential mediator of cocaine-induced plasticity and craving behavior that regulates the expression of β-catenin (*Ctnnb1*), adenylyl cyclase-associated protein 2 (*Cap2*), and drebrin (*Dbn1*) (11). Binding of SMAD3 to DNA and the subsequent regulation of gene expression are thought to require interactions with adenosine triphosphate (ATP)-dependent swItch/sucrose nonfermentable (SWI/SNF) nucleosome repositioning complexes (12,13). A core subunit of SWI/SNF family complexes is BRG1 (also known as SMARCA4), which incorporates into the transcriptional complexes formed by SMADs (12,14,15). Although a role of these transcriptional complexes has been documented in regulation of cognitive processes, such as memory-related synaptic plasticity (16), the role of the BRG1-SMAD3 complex in drug addiction remains unknown. The data presented here provide evidence that this complex is involved in drug seeking after a period of abstinence. Withdrawal from cocaine self-administration in rats resulted in increased expression and functional interaction of BRG1 and SMAD3 in the NAc. Furthermore, pharmacologic inhibition of BRG1 attenuated its interaction with SMAD3 and suppressed cue-induced reinstatement of cocaine seeking, whereas overexpression of BRG1 exacerbated cocaine-seeking behavior. These results implicate

SEE COMMENTARY ON PAGE e77

 ISSN: 0006-3223

the SMAD3 and BRG1 transcriptional complex in the regulation of gene expression and behavioral changes after cocaine exposure, providing a novel mechanism for potential therapeutic targets in the treatment of cocaine addiction.

METHODS AND MATERIALS

Subjects

Male Sprague Dawley rats (300–375 g; Harlan, Indianapolis, IN) were allowed to habituate to the colony room for 7 days on arrival. Rats had ad libitum access to food and water and were singly housed after surgery and for the duration of the self-administration phase of the experiments to protect the catheter/harness assembly. Behavioral testing took place during the dark phase of the 12-hour light/dark cycle. This study was conducted in accordance with the guidelines set up by the Institutional Animal Care and Use Committee of the State University of New York at Buffalo.

Self-administration Test Chambers

Twenty-four standard experimental test chambers (MED Associates, Inc., St. Albans, VT) were used for self-administration and reinstatement experiments. The test chambers contain two snout-poke holes located on one wall, with a stimulus light mounted above each, and a house light mounted in the middle of the back wall. Snout pokes were monitored with infrared detectors. Test chambers were computer controlled by a MED Associates interface with MED-PC.

Drugs

(–)-Cocaine hydrochloride, generously supplied by the National Institute on Drug Abuse drug supply program, was dissolved in sterile 0.9% saline. Cocaine solutions (4.5 mg/mL) were prepared on a weekly basis and were delivered by a syringe pump. Pump durations/injection volumes were adjusted on a daily basis according to body weight to deliver 1.0 mg/kg per infusion. The BRG1 inhibitor PFI3 (Tocris, Minneapolis, MN) was dissolved in a mixture of 1 part absolute ethanol, 1 part Emulphor-620 (Rhodia of Solvay; S.A., Brussels, Belgium), and 18 parts saline (17) at a concentration of 30 mmol/L, based on a previous study (18).

Jugular Catheterization and Patency Testing

Rats were implanted with chronic indwelling jugular catheters as previously described (19,20). Rats were allowed 7 days to recover after surgery. The catheters were flushed daily with 0.2 mL of a solution of enrofloxacin (4 mg/mL) in heparinized saline (50 IU/mL in 0.9% sterile saline) to preserve catheter patency. At the end of behavioral testing, each animal received an intravenous infusion of ketamine hydrochloride (0.5 mg/kg in 0.05 mL), and the behavioral response was observed to verify catheter patency. Loss of muscle tone and righting reflexes served as behavioral indicators of patency.

Self-administration and Cue-Induced Reinstatement

Self-administration procedures were conducted as previously described (11,21,22). After the 7-day recovery from jugular catheter surgery, rats were assigned to self-administer either saline or 1.0 mg/kg cocaine per infusion. Rats were subjected to 10 test sessions (2 hours per session), during which time responses to the active alternative snout-poke hole resulted in intravenous injections of cocaine or saline according to a fixed-ratio 1 (FR1) schedule of reinforcement, followed by a 30-second time-out period. Infusions were accompanied by a 5-second illumination of the stimulus light above the active snout-poke hole, and the house light was extinguished for the duration of the time-out period. Responses to the inactive hole resulted in no programmed consequences. The criterion for acquisition of cocaine self-administration was an average of 10 cocaine infusions per day during the 10-session test phase.

Extinction tests were initiated 24 hours after the last self-administration session. Rats were exposed to multiple within-session extinction sessions, during which time the chambers were dark and responses were recorded but resulted in no programmed consequences. Extinction sessions were 1 hour in duration, separated by 5 minutes, and were continued until responding levels fell to fewer than 20 responses per session (8–10 sessions) (23,24). Animals were then allowed an additional 6 days of abstinence, followed by 1-hour test of cue-induced reinstatement, during which time active responses produced cues previously paired with drug delivery.

Tissue Collection

After a 7-day withdrawal (or after cue-induced reinstatement for animals receiving PFI3 microinfusions), animals were euthanized by rapid decapitation, brains were removed and sliced into 1-mm-thick sections using a brain matrix (Braintree Scientific, Inc., Braintree, MA), and 2-mm-diameter tissue punches from the NAc and dorsal striatum/caudoputamen (CPU) were collected and rapidly frozen on dry ice.

Western Blotting and Immunoprecipitation

NAc or CPU tissue punches from each rat that self-administered cocaine or saline (n = 6 per group) were homogenized in 25 mmol/L Tris (pH 8.0) and 0.25 mol/L sucrose buffer. Total protein was extracted, and 30-μg samples were loaded onto 10% Tris-sodium dodecyl sulfate (SDS) polyacrylamide gels for electrophoresis separation, then transferred to nitrocellulose membranes and blocked with 5% nonfat milk in phosphate-buffered saline. Membranes were incubated overnight at 4°C with primary antibodies diluted in blocking buffer (Rockland Immunochemicals, Inc., Limerick, PA), including rabbit anti-BRG1 (1:2,000; Abcam, Cambridge, MA), rabbit anti–phospho(p)-SMAD3 (1:500; Calbiochem of Millipore Corp., Billerica, MA), and mouse anti–β-actin (1:10,000; Cell Signaling Technologies, Inc., Danvers, MA). Membranes were incubated with IRDye secondary antibodies (1:5,000; LI-COR, Inc., Lincoln, NE) for 1 hour at room temperature. The blots were imaged using the Odyssey Infrared Imaging system (LI-COR, Inc.) and quantified by densitometry using ImageJ (National Institutes of Health, Bethesda, MD). β-Actin was used as a loading control.

For immunoprecipitation, NAc or CPU tissue punches from rats (n = 8 for the saline group, n = 7 for the cocaine group) were homogenized in 500 μL of homogenization buffer that contained 50 mmol/L β-glycerol phosphate, 1 mmol/L dithiothreitol (DTT), 2 mmol/L ethylene glycol tetraacetic acid