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Cocaine-induced epigenetic DNA modification in mouse addiction-specific and non-specific tissues

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ABSTRACT

Cocaine-related DNA methylation studies have primarily focused on the specific brain regions associated with drug addiction (e.g., the *nucleus accumbens,* NAc). To date, no studies have focused on the complex role of both DNA methylation and demethylation in the mechanisms of psychostimulant-induced addiction in the brain and peripheral tissues. Therefore, in this study, we evaluated cocaine treatment and withdrawal (animals were withdrawn from seven days of repeated injections of cocaine that caused behavioral sensitization) effects on epigenetic DNA modifiers (i.e., DNA methyltransferases, [DNMTs] and ten-eleven translocation enzymes [TETs]) in an addiction-specific brain region (NAc), a structure outside the mesolimbic dopaminergic system (cerebellum, Cer), and in peripheral blood cells (PBCs). Using a mouse behavioral sensitization model, we demonstrated that acute cocaine (AC; 0.5 h) treatment significantly decreased *Dnmt1, Dnmt3a*, *Tet1,* and *Tet2* mRNA levels in the NAc and PBC, whereas at 24 h after AC treatment, *Dnmt* mRNA expression and enzyme activity levels were significantly increased. Acute procaine treatment caused the opposite effect on the *Dnmt3a* mRNA level in PBCs; this outcome suggests that the inhibition of voltage-gated sodium channels may be the mechanism that alters *Dnmt* expression in PBCs. Cocaine withdrawal is associated with increased expression of *Dnmts* in the NAc, Cer and PBCs and the decreased expression of *Tet1* and *Tet3* in the NAc. Additionally, cocaine withdrawal increased DNMT but decreased TET activity levels, and these changes were associated with enhanced global and selected candidate gene promoter-region DNA methylation and hydroxymethylation levels in the NAc and PBCs. Together, these data indicate that cocaine treatment and withdrawal affect the expression of epigenetic DNA modifiers in both addiction-specific brain structures and structures outside of the mesolimbic dopaminergic system and PBCs.

1. Introduction

Psychostimulants, such as cocaine and amphetamine, change neuronal structure and function in specific brain regions, resulting in persistent changes at the molecular, cellular, and behavioral levels (Koob and Le Moal, 2001; Koob and Simon, 2009; Nestler, 2001; Nestler, 2013; Paulson et al., 1991). The neurobiology of psychostimulant-induced addiction remains poorly understood. It has been hypothesized that persistent alterations in gene expression could be responsible for the long-term behavioral and structural changes (Nestler and Aghajanian, 1997; Nestler, 2013; Robinson and Nestler, 2011). Accumulating data suggest that epigenetic modifications of transcription, such as DNA methylation, histone modification, and microRNAs, are the critical regulators of gene expression changes in the adult central nervous system (CNS) under normal and pathological conditions (Cholewa-Waclaw et al., 2016; Jaenisch and Bird, 2003). Many reports have demonstrated that DNA methylation contributes to psychostimulant-induced changes in gene expression and may regulate mechanisms underlying addictive behaviors (Anier et al., 2010; LaPlant et al., 2010; Massart et al., 2015; Nielsen et al., 2012; Pol Bodetto et al., 2013; Tian et al., 2012).

DNA methylation is an epigenetic modification by which methyl groups are added to the 5th position of cytosine (5-mC),

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predominantly at cytosine-phospho-guanine dinucleotides (Holliday and Pugh, 1975; Jaenisch and Bird, 2003; Klose and Bird, 2006). DNA methylation is catalyzed by a family of enzymes called DNA methyltransferases (DNMTs) that mediate the transfer of a methyl group from S-adenosylmethionine to cytosine, resulting in 5-mC (Goll and Bestor, 2005; Jin et al., 2011). There are three major DNMTs with enzyme activity in mammals: DNMT1 (maintenance DNMT), DNMT3A, and DNMT3B (*de novo* DNMTs) (Bestor, 2000; Goll and Bestor, 2005; Okano et al., 1999).

Several studies have shown that ten-eleven translocation enzymes (TET1-3), which add a hydroxyl group onto the methyl group of 5-methylcytosine (5-mC) to form 5-hydroxymethylcytosine (5-hmC), participate in the active DNA demethylation process (Ito et al., 2011; Kohli and Zhang, 2013; Kriaucionis and Heintz, 2009; Tahiliani et al., 2009). Recent findings suggest that 5-hmC is not merely a DNA demethylation intermediate but that it also functions as a stable epigenetic mark that is enriched within gene promoters, gene bodies and transcription factor binding sites and that it may influence the expressions of several genes (Hahn et al., 2013; Kaas et al., 2013; Mellén et al., 2012; Szulwach et al., 2011).

Thus far, psychostimulant-related epigenetic studies have mainly focused on specific regions of the brain associated with drug addiction, e.g., the nucleus accumbens (NAc), prefrontal cortex, and hippocampus (Anier et al., 2010; Day et al., 2013; Han et al., 2010; Kumar et al., 2005; LaPlant et al., 2010; Massart et al., 2015; Pol Bodetto et al., 2013; Renthal et al., 2007; Robison et al., 2013; Shen et al., 2008). Given the difficulty of obtaining human brain tissue, a key question in molecular psychiatry concerns the extent to which epigenetic signatures measured in more accessible tissues, such as the blood, can serve as a surrogate marker for the brain (Walton et al., 2016). Therefore, the aim of our work was to assess how cocaine exerts complex effects on both DNA methylation and demethylation in an addiction-specific brain region, such as the NAc, a structure outside of the mesolimbic dopaminergic system (the cerebellum, Cer), and an easily accessible non-CNS tissue (peripheral blood cells, PBCs).

2. Material and methods

Detailed protocols for RNA isolation, qPCR, and quantification of 5-mC and 5-hmC levels are described in the Supplementary Materials and Methods section.

2.1. Animals

Adult male C57BL/6NTac mice (25–32 g, 3–4 months old) were obtained from Taconic Biosciences (Denmark) and were housed in standard polypropylene cages in temperature- and humidity-controlled rooms (12-h light-dark cycle). The animals were habituated for at least 7 days before behavioral testing. All behavioral experiments were performed during the light cycle in an isolated experimental room. All experiments were performed in accordance with EU guidelines (Directive 2010/63/EU) on the ethical use of animals and using an experimental protocol that was approved by the Animal Welfare committee of the Ministry of Rural Affairs, Republic of Estonia.

2.2. Acute cocaine (AC) treatment

Mice received (0.1 ml/10 g body weight) a single intraperitoneal (i.p.) injection of saline or cocaine hydrochloride (15 mg/kg; Sigma-Aldrich, St. Louis, MO, USA). Tissues (NAc and Cer) and peripheral blood were collected at 0.5, 1.5, 3 and 24 h after saline or acute cocaine (AC) treatment. Serial 1-mm brain sections were cut using a brain matrix and were placed on chilled microscope slides. The NAc was dissected out using a round-shape puncher (inner diameter of 1.0 mm) on the chilled microscope slides and then frozen immediately in liquid nitrogen. The tissues were stored at −80 °C until RNA and DNA extracts and protein lysates were prepared.

2.3. Procaine treatment

Mice received a single injection (0.1 ml/10 g body weight, i.p.) of saline or procaine hydrochloride (15 mg/kg or 45 mg/kg; Sigma-Aldrich, St. Louis, MO, USA). The NAc and Cer tissues and peripheral blood were collected at 0.5 and 24 h after saline or procaine treatment. Serial 1-mm brain sections were cut using a brain matrix and were placed on chilled microscope slides. The NAc was dissected out using a round-shape puncher (inner diameter of 1.5 mm) on the chilled microscope slides and then frozen immediately in liquid nitrogen. The tissues were stored at −80 °C until RNA isolation and used for qPCR analysis.

2.4. Cocaine treatment and locomotor activity measurement

Induction of behavioral sensitization. Mice were treated (0.1 ml/10 g body weight) for 7 consecutive days with sterile saline or cocaine hydrochloride (15 mg/kg) via i.p. injection.

Expression of behavioral sensitization. Animals were assigned to one of the following treatment groups: (1) Saline + Saline (SAL) – the mice were treated for 7 days with saline and they received saline on the 28th day; (2) Saline + Acute Cocaine (AC) – the mice received saline for 7 days and were treated with a challenge dose (8 mg/kg; i.p.) of cocaine on the 28th day; (3) Cocaine + Saline (RS) – the mice received cocaine for 7 days, were withdrawn from cocaine for 21 days and were then challenged with saline on day 28; or (4) Cocaine + Cocaine (RC) – the mice received cocaine for 7 days, were withdrawn from cocaine for 21 days and were then challenged with cocaine (8 mg/kg; i.p.) on day 28.

Locomotor activity measurement. Horizontal locomotor activity was monitored with a PhenoTyper (Noldus Information Technology, Netherlands) for 60 min after the i.p. injections on days 1–7 and 28. The animals were euthanized at 24 h after the end of the treatment, and blood and brain tissues (NAc, Cer) were collected.

2.5. DNMT and TET activity measurements

Nuclear proteins were extracted from the NAc, Cer and PBCs of mice according to the manufacturer's protocol (Nuclear Extraction Kit; Abcam, Cambridge, UK). DNMT activity was determined using an EpiQuik DNMT activity assay kit (Epigentek Group, Brooklyn, NY, USA), and a TET Hydroxylase Activity Quantification kit (Abcam, Cambridge, UK) was used to measure TET activity according to the instructions of the manufacturers. DNMT and TET activity levels (OD/h/mg) were calculated according to the manufacturers' protocols. Each experiment was performed in duplicate, and 5 μg of nuclear protein extracts were used for assays.

2.6. DNA hydrolysis, 5-mC and 5-hmC quantification

Genomic DNA was extracted from the NAc using a QIAamp DNA Micro kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA samples were hydrolyzed using DNA Degradase Plus (Zymo Research, Irvine, CA, USA). Ten microliters of the DNA hydrolysis samples were used for liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) to measure the levels of 5-mC and 5-hmC (see Supplementary Materials and Methods for details). Quantifications of 5-mC and 5-hmC were performed as previously reported (Kaas et al., 2013).