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Cocaine represses protein phosphatase-1Cβ through DNA methylation and Methyl-CpG Binding Protein-2 recruitment in adult rat brain

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ABSTRACT

Repeated cocaine exposure induces epigenetic factors such as DNA methyl-binding proteins, indicating that resulting changes in gene expression are mediated by alterations in brain DNA methylation. While the activity of protein phosphatase type-1 (PP1) is involved in cocaine effects and in brain plasticity, the expression of the *PP1Cβ* catalytic subunit gene was identified here as modulated by cocaine. Its expression was induced together with that of *PP1Cγ* in the brain of Methyl-CpG Binding Protein-2 (Mecp2) mutant mice, whereas *PP1Cα* expression was not affected, illustrating a different regulation of PP1C isoforms. Repeated cocaine administration was found to increase DNA methylation at the *PP1Cβ* gene together with its binding to Mecp2 in rat caudate putamen, establishing a link between two genes involved in cocaine-related effects and in learning and memory processes. Cocaine also increased *DNMT3* expression, resulting in *PP1Cβ* repression that did not occur in the presence of DNMT inhibitor. Cocaine-induced PP1Cβ repression was observed in several brain structures, as evaluated by RT-qPCR, immunohistochemistry and Western blot, but did not occur after a single cocaine injection. Our data demonstrate that *PP1Cβ* is a direct MeCP2-target gene *in vivo*. They suggest that its repression may participate to behavioral adaptations triggered by the drug.

1. Introduction

Acute and repeated cocaine exposures have been shown to induce changes in the expression of many genes in the brain (Robison and Nestler, 2011). Such transcriptional modifications can be rapid and transient, or persistent and account for long term behavioral changes following repeated exposure. Drug-induced behaviors have been attributed at least in part to epigenetic mechanisms that represent attractive candidates to explain long lasting behaviors, such as relapse after long periods of abstinence (Colvis et al., 2005; Graff et al., 2011). However, while histone post-translational modifications by psychostimulants have been well documented, only recently it has been shown that cocaine also acts on DNA methylation involving DNA methyltransferases (DNMTs) and Methyl-CpG Binding Domain Proteins (MBDs) (Cassel et al., 2006; Carouge et al., 2010; Deng et al., 2010; Im et al., 2010; LaPlant et al., 2010; Host et al., 2011).

DNA methylation is usually considered as a stable covalent modification and involves the transfer of a methyl group to cytosines in CpG sites. Traditionally, DNA methylation in gene promoters is associated with transcriptional repression by preventing the binding of transcription factors to their cognate sites or by recruiting MBD proteins such as Methyl-CpG Binding Protein-2 (MeCP2). MeCP2 possesses repressive chromatin-remodeling activity (Deaton and Bird, 2011) and is considered as a negative or positive regulator of gene transcription (McGraw et al., 2011). Deviations from the normal DNA methylation pattern contribute to various diseases and have been observed in response to certain dietary components or drugs of abuse (Alegria-Torres et al., 2011; Graff et al., 2011). However, only a limited number of genes has been identified in adult brain as modulated by DNA methylation in response to drugs of abuse.

Abbreviations: CGI, CpG Island; ChIP, chromatin immunoprecipitation; CPP, conditioned place preference; CPu, caudate putamen; CREB, cAMP response element-binding protein; DARPP-32, dopamine- and cyclic AMP-regulated phosphoprotein-32; 5-dAZA, 5-Aza-2′-deoxycytidine; DNMT, DNA methyltransferase; 5-hmC, 5-hydroxymethylcytosine; 5-mC, 5-methylcytosine; MBD, Methyl-CpG Binding Proteins; Mecp2, Methyl-CpG Binding Protein-2; MeDIP, methylated DNA immunoprecipitation; NAc, nucleus accumbens; PFCx, prefrontal cortex; PKA, cAMP-dependent protein kinase; PP1C, protein phosphatase type-1 catalytic subunit; RT-qPCR, reverse transcription-quantitative PCR.

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We have previously shown that cocaine and the antidepressant fluoxetine influence chromatin remodeling *via* Methyl-CpG Binding Proteins (MBD) and act through mechanisms involving DNA methylation (Cassel et al., 2006). Indeed, cocaine was found to induce the expression of Mecp2 and MBD1 and we provided the first evidence for its effect on gene DNA methylation occurring in mature brain (Carouge et al., 2010). Thereafter, the striatal Mecp2 knockdown was reported to block the development of escalating cocaine intake and to reverse the long-term trajectory of cocaine-taking behavior in rats with extended access to the drug (Im et al., 2010). Consistent with the latter findings, both conditioned place preference (CPP) and enhanced locomotion following repeated amphetamine administration were no longer observed in Mecp2$^{308/Y}$ mutant mice (Deng et al., 2010) lacking Mecp2 domains essential for mediating chromatin folding and nucleosome compaction (Ghosh et al., 2010). Interestingly, overexpression of DNMT3A in the nucleus accumbens (NAc) reduced cocaine CPP and enhanced depression-like behavior, suggesting that DNMT3A mediates both cocaine- and stress-induced behavioral changes (LaPlant et al., 2010). In addition, injections of DNMT inhibitors in brain structures involved in learning and memory processes were shown to block acquisition or expression of cocaine-induced CPP (Han et al., 2010), further underlining the importance of DNA methylation in cocaine-related behavior.

By using microarrays, we have identified the β catalytic subunit of the protein phosphatase type-1 gene (*PP1Cβ*) as being repressed by chronic cocaine treatment. Three highly related catalytic subunit genes (*PP1Cα*, *PP1Cβ/δ* and *PP1Cγ*) have been reported, and alternative splicing generates the γ1 and γ2 isoforms (da Cruz e Silva et al., 1995). They associate with a host of divergent regulatory or targeting subunits controlling their differential cellular localization (Virshup and Shenolikar, 2009). In the brain, PP1C isoforms are present in dendritic spines or in the periphery of the soma (Allen et al., 1997; Nakanishi et al., 1997; Strack et al., 1999) and are also present in the nucleus (Moorhead et al., 2007). Their subcellular localization patterns allow independent roles for each isoform in regulating cellular processes, but their involvement in drug-induced behavior (Hiroi et al., 1999; Zachariou et al., 2002) or in learning and memory processes (Genoux et al., 2002; Graff et al., 2010) has been mainly studied by manipulating endogenous PP1 inhibitors that do not discriminate between PP1C isoforms. While cocaine inhibits PP1 activity through at least dopamine D1 receptors and dopamine- and cyclic AMP-regulated phosphoprotein (DARPP-32) phosphorylation (Svenningsson et al., 2004), very little is known about the mechanism by which the expression of each PP1C isoform is regulated.

In the present study, we investigated whether cocaine could modulate PP1 expression by a mechanism involving DNA methylation in brain structures related to the reward system. Since Mecp2 expression critically regulates a wide range of psychostimulant effects (Feng and Nestler, 2010) and is required for proper learning and memory processes (Moretti et al., 2006; Pelka et al., 2006), the possible involvement of Mecp2 in the regulation of PP1C isoforms was addressed. We showed that PP1Cβ expression is repressed following repeated cocaine treatment, as illustrated by RT-qPCR, Western blot and immunohistochemistry. The repression was associated with increased levels of *DNMT* and increased DNA methylation at the *PP1Cβ* gene together with its binding to Mecp2. Our data underline *PP1Cβ* gene as one of the rare genes identified so far in the brain as being regulated by DNA methylation in response to cocaine.

2. Materials and methods

2.1. Animals

Male Wistar rats (Janvier, France), 8–9 weeks old at their arrival in the laboratory, were housed in standard home cages in temperature- and humidity-controlled rooms with a 12 h/12 h light/dark cycle (lights on at 7.00 am). Animals had access to food and water *ad libitum*. They were allowed to acclimate to laboratory conditions and were handled at least one week before experimental procedures. They were then intraperitoneally (i.p.) injected either once or repeatedly (1 injection per day) for 10 days with either 20 mg/kg cocaine hydrochloride (Cooper, Melun, France) or an equivalent volume of saline (0.9% NaCl). Mecp2$^{308/Y}$ and Mecp2$^{X/Y}$ males were from breeding pairs of Mecp2−308 mice backcrossed to C57BL/6J mice. Original mice were from Jackson Laboratories (B6-129S-Mecp2tm1Hzo/J, stock number: 005439, USA). 13 weeks-old mice were killed by cervical dislocation and their brain was dissected. All procedures involving animal care were conducted in compliance with current laws and policies (Council directive 87848, 1987, Service Vétérinaire de la Santé et de la Protection animale, permission 67–165 to JZ).

2.2. Intracerebroventricular cannula implantation and 5-dAZA infusion

Rats were anesthetized by intraperitoneal injection (1 ml/kg) of a 5:1 (v/v) mixture containing 0.1 g/ml ketamine (Imalgene 1000, Merial) and 2% xylazine (Rompun, Bayer). 23-gauge stainless steel guide cannulae were implanted bilaterally using the stereotaxic coordinates: −0.9 mm AP, ±1.8 mm ML and −3 mm DV from the skull surface (Paxinos, 2007). For intracerebroventricular infusion, the infusion cannula extended 1 mm beyond the tip of the guide cannula. Guide cannulae were secured to the skull with dental cement and steel screws. Experiments were performed 5–7 days after implantation. 5-Aza-2′-deoxycytidine (5-dAZA) (Sigma–Aldrich) was dissolved at a concentration of 1 μg/μl in a sterile saline solution containing 10% DMSO and was stored at −20 °C. 5-dAZA (5 μg in 5 μl) or vehicle was infused daily 20 min before cocaine administration over a 2-min period using a 10 μl Hamilton microsyringe. Infusion cannulae remained in place for 1 min after infusion to allow drug diffusion.

2.3. Reverse transcription-quantitative PCR analysis

Animals were sacrificed 5 h, 10 h or 15 h after the last i.p. injection and brain structures of interest were dissected, as previously described (Carouge et al., 2010). RNA was extracted (Ludwig et al., 2000) from the rat medial prefrontal cortex (PFCx) and the caudate putamen (CPu) and first strand cDNA was generated from 0.5 μg of total RNA using random primers and Superscript II Reverse Transcriptase (Invitrogen). RT-qPCR was performed as previously described (Carouge et al., 2010). Primers for rat and mouse genes were from Sigma–Aldrich Co. and are listed in Table 1. Primers were designed with Primer 3 software (http://frodo.wi.mit.edu/primer3/input.htm) used under stringent conditions and amplicons were selected to span exon borders to exclude false positive detection of genomic contaminations. Results were normalized to 36B4 used as an internal control. Cycling conditions were: 95 °C for 14 min, then 40 cycles of 95 °C for 14 s, 60 °C for 18 s and 72 °C for 18 s. The specificity of each PCR product was verified by melting curve analysis and confirmed by 2% agarose gel electrophoresis. Real time PCR was conducted three times for each gene of interest, with samples in triplicates.

2.4. Methylated DNA immunoprecipitation assay

Animals were sacrificed 12 h after the last i.p. injection and the caudate putamen (CPu) was dissected, as previously described (Carouge et al., 2010). Methylated DNA immunoprecipitation (MeDIP) was performed using Methylamp™ Methylated DNA Capture Kit (Epigentek Group, Brooklyn, NY). Genomic DNA was extracted from rat CPu and 0.5 μg DNA aliquots were sheared by sonication in 150 μl (Vibra^{m} Cell™ 75041, Bioblock Scientific) with 3 cycles of 12 s ON, 40 s OFF at 22% power. Sonicated fragments ranging from 200 bp to 1000 bp were divided into immunoprecipitated (IP) and input (Inp) portions. IP DNA was incubated with anti-5-methylcytosine antibody and the negative control was normal mouse IgG. The DNA fragments recovered were then used as templates for real time PCR using *PP1Cβ* FS2 R3 and FEx1S4 REx1R3 primer sets (Table 1), generating PCR fragments of 139 bp and 103 bp, respectively. Cycling conditions were: 95 °C for 15 min, then 60 cycles of 95 °C for 15 s, 58 °C for 20 s and 72 °C for 20 s. The specificity of the PCR product was verified by melting curve analysis and by 2% agarose gel electrophoresis. Real time PCR was conducted three times, with samples in duplicates. Relative enrichment of the target *PP1Cβ* sequence after MeDIP was estimated as the ratio of the signal in IP DNA divided by that in Inp DNA.

2.5. Chromatin immunoprecipitation assay

Animals were sacrificed 12 h after the last injection and the CPu was dissected, as previously described (Carouge et al., 2010). Cross-linking proteins to genomic DNA and ChIP assay have been essentially performed as previously described with an antibody raised against Mecp2 (Carouge et al., 2010). Precipitated chromatin sonicated DNA fragments ranging from 0.3 to 1.2 kb were quantified by real time PCR using three different sets of primers for *PP1Cβ* (FS2 R3, FEx1S1 REx1R5 and FEx1S4 REx1R3; Table 1). Cycling conditions were: 95 °C for 15 min, then 50 cycles of 95 °C for 15 s, 58–62 °C (depending on the primers) for 20 s and 72 °C for 20 s. Single PCR products were verified both by assessing their melting temperature and by viewing them on agarose gel. Real time PCR was conducted three times. Immunoprecipitated