Methamphetamine Downregulates Striatal Glutamate Receptors via Diverse Epigenetic Mechanisms

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Background: Chronic methamphetamine (METH) exposure causes neuroadaptations at glutamatergic synapses.

Methods: To identify the METH-induced epigenetic underpinnings of these neuroadaptations, we injected increasing METH doses to rats for 2 weeks and measured striatal glutamate receptor expression. We then quantified the effects of METH exposure on histone acetylation. We also measured METH-induced changes in DNA methylation and DNA hydroxymethylation.

Results: Chronic METH decreased transcript and protein expression of GluA1 and GluA2 alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) and GluN1 *N*-methyl-D-aspartate receptor subunits. These changes were associated with altered electrophysiological glutamatergic responses in striatal neurons. Chromatin immunoprecipitation-polymerase chain reaction revealed that METH decreased enrichment of acetylated histone H4 on GluA1, GluA2, and GluN1 promoters. Methamphetamine exposure also increased repressor element-1 silencing transcription factor (REST) corepressor 1, methylated CpG binding protein 2, and histone deacetylase 2 enrichment, but not of sirtuin 1 or sirtuin 2, onto GluA1 and GluA2 gene sequences. Moreover, METH caused interactions of REST corepressor 1 and methylated CpG binding protein 2 with histone deacetylase 2 and of REST with histone deacetylase 1. Surprisingly, methylated DNA immunoprecipitation and hydroxymethylated DNA immunoprecipitation-polymerase chain reaction revealed METH-induced decreased enrichment of 5-methylcytosine and 5-hydroxymethylcytosine at GluA1 and GluA2 promoter sequences. Importantly, the histone deacetylase inhibitor, valproic acid, blocked METH-induced decreased expression of AMPAR and *N*-methyl-D-aspartate receptor subunits. Finally, valproic acid also attenuated METH-induced decrease H4K16Ac recruitment on AMPAR gene sequences.

Conclusions: These observations suggest that histone H4 hypoacetylation may be the main determinant of METH-induced decreased striatal glutamate receptor expression.

Key Words: Addiction, AMPAR, CoREST, HDAC2, MeCP2, NMDAR, REST, valproic acid

Addictions are neuropsychiatric disorders that are secondary, in part, to altered synaptic plasticity in mesostriatal and corticostriatal projection areas (1–3). The dorsal striatum is important in the neural circuitry of addiction because the nidus of control for drug taking appears to shift from the ventral to the dorsal striatum as drug taking becomes habitual (4–7). Repeated psychostimulant injections can produce biochemical, molecular, and physiological alterations at striatal glutamatergic synapses (1,8). Specifically, cocaine administration is accompanied by changes in the expression or trafficking of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) in the mesolimbic system (1). Both contingent and noncontingent administration of cocaine is associated with increased expression of AMPARs on neuronal membranes (9), increased expression of GluA2-lacking AMPARs (10,11), and physiological evidence of differential AMPAR expression (12) in the ventral striatum. Therefore, dynamic alterations in AMPAR subunit composition might be involved in the maintenance of drug seeking and/or in the occurrence of relapses (10,11). Parenthetically, very little is known about the effects of methamphetamine (METH) on the expression of these receptors. In rodents, injections of increasing doses of METH (10–30 mg/kg) for 7 consecutive days produced increased AMPA GluA2 protein expression in the dorsal striatum (13). Nevertheless, the transcriptional effects of METH on GluA1 or GluA2 or the epigenetic bases for any potential METH-induced changes in striatal AMPAR expression are unknown.

Gene transcription is regulated by complex epigenetic changes including posttranslational histone modifications and DNA methylation that regulate diverse genomic functions (14,15). Eukaryotic DNA is packaged into chromatin, whose basic unit, the nucleosome, contains four core histones that form an octamer surrounded by 147 base pair of DNA. The N-tails of histones possess lysine residues that can be reversibly acetylated or deacetylated by histone acetyltransferases or histone deacetylases (HDACs), respectively (16). Because epigenetic phenomena are involved in the clinical manifestations of neuropsychiatric diseases, including addiction (17), we thought it is likely that METH could engender transcriptional and epigenetic changes that are unique to this clinically devastating drug (18). Studies of the transcriptional effects of METH on AMPAR expression are important because its biochemical effects are different from those of cocaine. Specifically, METH interacts with vesicular monoamine transporter and causes release of dopamine by reverse transport (18,19), whereas cocaine inhibits monoamine reuptake (20,21). The two main purposes of this study were to characterize the effects of METH exposure on striatal AMPAR expression and to identify potential epigenetic bases for any changes in receptor expression.

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Methods and Materials

Animals and Drug Treatment

All animal treatments and procedures were approved by the National Institute on Drug Abuse Animal Care and Use Committee and followed the *Guide for the Care and Use of Laboratory Animals* (ISBN 0-309-05377-3). Male Sprague-Dawley rats (Charles River Labs, Wilmington, Massachusetts), weighing 250 g to 300 g, were housed in a humidity- and temperature-controlled (22.2°C ± .2°C) room with free access to food and water. Following habituation, rats were assigned to two groups (eight rats each) and were injected daily for 2 weeks with either saline or METH, as shown in Table S1 in Supplement 1. The animals were euthanized 16 hours after the last saline or METH injection. This METH regimen was meant to mimic the patterns of METH abuse by human abusers who start at low to moderate doses (10–50 mg) and progressed to higher doses (22,23). This pattern of METH administration to rats does not cause any striatal toxicity (24) (Figure S1 in Supplement 1).

For co-treatment with HDAC inhibitor, rats received intraperitoneal sodium valproate (VPA) (300 mg/kg, dissolved in water; Sigma, Valencia, California) injections twice a day 30 minutes before either saline or METH injections. We chose VPA, a well-tolerated agent with extensive clinical use, recognizing its varied effects on the brain (25). The VPA dose was based on the published literature (26). There were four groups for the co-treatment experiments: vehicle/saline (control); vehicle/METH (METH); VPA/saline (VPA); and VPA/METH (VPA + METH).

Quantitative Polymerase Chain Reaction Analysis of Messenger RNA Levels

Total RNA was isolated from one striatal hemisphere using RNeasy Mini kit (Santa Cruz Biotechnology, Santa Cruz, California) from eight rats per group. Quantitative polymerase chain reaction (PCR) was carried out essentially as described by us (27).

Subcellular Fractionation

Separation of nuclear, cell membrane, and cytoplasmic fractions from striatal tissues was performed by differential centrifugation at 4°C. Details are provided in Supplement 1.

Immunoblot Analysis

Striatal protein lysates ($n = 6$) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and electrophoretically transferred on polyvinylidene difluoride membranes, essentially as described by us (see Supplement 1 for details). The membranes were incubated overnight at 4°C with specific antibodies against GluA1, GluA2, GluN1/NR1, histone deacetylase 1 (HDAC1), and histone deacetylase 2 (HDAC2) (Santa Cruz); H4K5ac, H4K12ac, and H4K16ac (Millipore, Billerica, California); and sirtuin 1 (SIRT1), sirtuin 2 (SIRT2), and methylated CpG binding protein 2 (MeCP2) (Cell Signaling, Danvers, Massachusetts).

Co-immunoprecipitation

Nuclear extracts were prepared from the striatum of saline- and METH-treated rats according to Barrett *et al.* (28) with minor modifications. Details are included in Supplement 1.

Chromatin Immunoprecipitation Assays

Striatal tissue was processed for acetyl H4, repressor element 1 silencing transcription factor (REST), REST corepressor 1 (CoREST), HDAC1, HDAC2, and MeCP2 chromatin immunoprecipitation (ChIP) (29) or methylated DNA immunoprecipitation and hydroxymethylated DNA immunoprecipitation (30,31) according to published protocols. Details are provided in Supplement 1.

Enrichment of various proteins at GluA1, GluA2, and GluN1 promoters were determined by quantitative real-time PCR using specific ChIP primers designed to amplify proximal or distal sequences from the transcription start site (TSS). Each PCR reaction was repeated at least twice. The specific primers used are listed under Table S2 in Supplement 1.

Electrophysiology

Perfused rat dorsal striatum was used in the electrophysiology experiments. Details of the electrophysiological experiments were essentially as described by Britt *et al.* (32) and are provided in the Supplement 1.

Statistical Analysis

All the quantitative data are presented as mean + SEM. For data comparing control and METH-treated groups, unpaired Student *t* test was used (StatView version 4.02, St. Louis, Missouri). For the experiments involving VPA co-treatment, two-way analysis of variance (ANOVA) was used followed by Bonferroni post hoc. For electrophysiology, data were assessed using one-way ANOVA for multiple group comparisons, with a Bonferroni post hoc. For all experiments, the null hypothesis was rejected at $p < .05$.

Results

Chronic METH Administration Causes Decreased Striatal AMPAR Expression and Function

To identify the effects of METH on AMPAR expression, we treated rats with either saline or increasing METH doses as described above (Table S1 in Supplement 1). Chronic METH decreased striatal messenger RNA (mRNA) expression of GluA1 (Figure 1A) and GluA2 (Figure 1B). Methamphetamine also caused decreased GluA1 (Figure 1C) and GluA2 (Figure 1D) protein levels.

To determine whether these changes in AMPAR subtypes alter excitatory synaptic transmission, ex vivo whole-cell patch clamp recordings were performed on striatal medium spiny neurons. Sixteen hours after the last METH or saline injection, rats were sacrificed and coronal slices containing the striatum were obtained. Miniature excitatory postsynaptic currents on medium spiny neurons (Figure 2) were measured blindly according to previous descriptions (32). Unexpectedly, chronic METH did not cause significant changes in miniature excitatory postsynaptic current amplitude or frequency (Figure 2A,B), in contrast to published observations with cocaine (12,33). We also increased stimulus intensities and measured evoked excitatory postsynaptic currents. We found that the input–output relationship between evoked excitatory postsynaptic currents and increasing stimulus intensities was significantly decreased in the METH group in comparison with control animals (Figure 2C). Surprisingly, we found that the ratio of peak AMPAR- to peak *N*-methyl-D-aspartate receptor (NMDAR)-mediated evoked currents, a measure of glutamate synaptic plasticity (34), was significantly increased in the chronic METH-treated group (Figure 2D). The METH-induced increases in AMPAR/NMDAR ratios appear to be related, in part, to METH-induced decreased mRNA (Figure 2E) and protein (Figure 2F) levels of the obligatory *N*-methyl-D-aspartate (NMDA) receptor, GluN1/NR1, because the percentage decrease in AMPA protein expression (−22% to 26%) was less than that of GluN1 (−45%) (compare Figures 1C and 1D with