Neuroscience Letters 611 (2016) 21–27

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research paper

Alterations of prefrontal cortical microRNAs in methamphetamine self-administering rats: From controlled drug intake to escalated drug intake

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HIGHLIGHTS

- 28 differential miRNAs were screened in controlled or escalated methamphetamine use.
- miR-186 increased and miR-195, -329 decreased in PFC of controlled drug use rats.
- miR-127, -186, -222, -24 increased and miR-329 decreased in escalated drug use rats.
- Predicted targets functionally clustered in neural apoptosis and synaptic plasticity.

ARTICLE INFO

Article history:
Received 21 August 2015
Received in revised form
10 November 2015
Accepted 11 November 2015
Available online 1 December 2015

Keywords:
Methamphetamine addiction
Escalated drug use
Controlled drug use
Prefrontal cortex
microRNAs

ABSTRACT

Drug addiction is a process that transits from recreative and regular drug use into compulsive drug use. The two patterns of drug use, controlled drug intake and escalated drug intake, represent different stages in the development of drug addiction; and escalation of drug use is a hallmark of addiction. Accumulating studies indicate that microRNAs (miRNAs) play key regulatory roles in drug addiction. However, the molecular adaptations in escalation of drug use, as well as the difference in the adaptations between escalated and controlled drug use, remain unclear. In the present study, 28 altered miRNAs in the prefrontal cortex (PFC) were found in the groups of controlled methamphetamine self-administration (1 h/session) and escalated self-administration (6 h/session), and some of them were validated. Compared with saline control group, miR-186 was verified to be up-regulated while miR-195 and miR-329 were down-regulated in the rats with controlled methamphetamine use. In the rats with escalated drug use, miR-127, miR-186, miR-222 and miR-24 were verified to be up-regulated while miR-329 was down-regulated compared with controls. Furthermore, bioinformatic analysis indicated that the predicted targets of these verified miRNAs involved in the processes of neuronal apoptosis and synaptic plasticity. However, the putative regulated molecules may be different between controlled and escalated drug use groups. Taken together, we detected the altered miRNAs in rat PFC under the conditions of controlled methamphetamine use and escalated use respectively, which may extend our understanding of the molecular adaptations underlying the transition from controlled drug use to addiction.

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1. Introduction

Methamphetamine is a psychostimulant abused throughout the world. Acute use of methamphetamine produces euphoria, increased arousal and reduced fatigue, while long-term abuse results in addiction and neurotoxicity. The prefrontal cortex (PFC), a key brain region involved in cognitive and executive functions as well as a component of rewarding system, has been proved critical for drug addiction [12]. Accumulating studies have revealed the functional and structural abnormalities in PFC after long-term methamphetamine use, including the deficits in gray-matter and the abnormalities in white-matter microstructure of PFC in individuals addicted to methamphetamine [24,26,28], and the alterations of synaptic plasticity, neuronal apoptosis and gliogenesis in methamphetamine-treated animals [8,9,15]. These dysfunctions of PFC have been considered as pivotal neuropathological mechanisms underlying methamphetamine addiction.

Abbreviations: PFC, prefrontal cortex; miRNA, microRNA; LgA, long access; ShA, short access; BDNF, brain-derived neurotrophic factor; LIMK, LIM domain kinase; PAK, p21-activated kinase.

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http://dx.doi.org/10.1016/j.neulet.2015.11.016
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microRNAs (miRNAs) are a class of small non-coding RNAs that bind to 3′-untranslated regions of their target mRNAs to repress translation and silence gene expression [4]. Recently, increasing attention has been focused on the regulation of miRNAs in addiction to a variety of drugs, including cocaine, opioids, amphetamine-type stimulants and alcohol. For example, chronic exposure to cocaine increased miR-181a and decreased let-7d and miR-124 in the nucleus accumbens, hippocampus, ventral tegmental area or PFC [5]; while up-regulating let-7d and miR-124 or down-regulating miR-181a in the nucleus accumbens attenuated cocaine-induced conditioned place preference in rats [6]. Additionally, miR-212 in the dorsal striatum controlled cocaine intake in rats with extended access to the drug by influencing motivational property [10]. However, recent studies on miRNA modulating drug addiction have predominantly focused on cocaine or alcohol exposure, with less attention to amphetamine-type stimulants. Indeed, it is possible that distinct molecular adaptations occur in response to different addictive drugs.

Drug addiction is a process that transits from recreative and regular drug use to compulsive drug use. Recent studies showed that rats permitted to self-administer cocaine, heroin or methamphetamine extendedly produced a gradual escalation in daily drug intake, which was reminiscent of the loss of control over intake observed in drug addicts; whereas animals permitted restricted access to addictive drugs maintained regular and controlled pattern of drug intake [2,3,13]. Since many studies have shown that rats with a history of escalated drug self-administration display other characteristics of addictive behavior, such as increased motivation for the drug, compulsive drug use (resistance to punishment) and high liability to relapse [1,3,14,29], escalation of drug use is thought to be a hallmark of addiction. In fact, the two patterns of drug use, escalated drug intake and controlled drug intake, represent different stages in the development of drug addiction. However, the neuroadaptations in escalation of drug use, as well as the difference in the adaptations between escalated and controlled drug use, remain unclear. In the present study, therefore, the alterations of miRNAs expression in PFC were investigated in rats with escalated and controlled methamphetamine use, and the function of putative targets was analyzed using bioinformatic method.

2. Materials and methods

Male Sprague-Dawley (SD) rats (Vital River Laboratories, Beijing, China) weighing 250–300 g at the beginning of the experiment were individually housed under environmentally controlled conditions (ambient temperature, 22 ± 1 °C; humidity, 40–70%) on a 12:12 h light/dark cycle (8:00/20:00). Animals were provided with ad libitum access to food and water except during experimental sessions. All animal procedures were approved by the Institutional Animal Care and Use Committee and were conducted in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The surgical catheterisation in the jugular vein was performed according to our previous publication [30]. After recovery from surgery (5–7 days), rats received methamphetamine self-administration training. The procedure was in accordance with Kitamura et al. [13] with some modifications. For acquisition of drug intake, rats were trained with an FR1 schedule of methamphetamine self-administration with 1 h access to D-methamphetamine hydrochloride (0.05 mg/kg/infusion) for 12–13 consecutive days. Then the rats were divided into two groups balanced for methamphetamine consumption. During escalation phase, groups differed only with respect to methamphetamine availability. Drug access time of long access (LgA) group increased to 6 h daily, while that of short access (ShA) group still maintained at 1 h. The escalation training lasted for 14 days. Additionally, control (Con) group was trained the same as LgA group with 0.9% saline infusion.

2.1. RNA extraction and miRNA microarray assay

24 h after the last self-administration training, animals were sacrificed and PFC was separated. Total RNA was extracted from PFC using miRNeasy Mini kit (Qiagen, Dusseldorf, Germany) in accordance with the manufacturer's protocol. The RNA samples (RIN > 7, analyzed by high performance liquid chromatography) were used for subsequent microarray experiment. The miRNA microarray assay was performed by LC Sciences (Houston, USA). Three μParaflo™ microfluidic miRNA chips contained 679 probes of rat mature miRNAs (version No. miRRat_16.0, from miRBase, http://microrna.sanger.ac.uk/sequences/) were done for each group, with each microarray chip containing pooled RNA from two rats per group. Fluorescence labeling was Cy5 dye. Data were analyzed by first subtracting the background and then normalizing the signals using a LOWESS filter (Locally-weighted Regression). Altered miRNAs (1.5-fold up- or down-regulated, $P < 0.05$) were identified, and a heatmap (hierarchical clustering) was generated using MeV4.8.1 software.

2.2. Reverse transcription and real-time PCR

The differential expressions of some miRNAs were verified by quantitative reverse transcription-PCR (qRT-PCR). Total RNA (1 μg) was reverse transcribed in reverse transcriptase reaction (New England Biolabs, USA) using specific stem-loop RT primers. Real-time PCR was performed using QuantiTect SYBR Green PCR kit (Qiagen, Dusseldorf, Germany) with the ABI7500 system (Applied Biosystems, USA). Each sample was run in triplicate, and the small nuclear RNA U6 (U6-snRNA) was used as endogenous control gene. Relative quantification was calculated with the 2^{-ddCt} method. The sequences of RT primers and PCR primers are shown in Table S1.

2.3. miRNA target genes prediction and their functional analysis

TargetScan 6.2 (http://www.targetscan.org) and miRecods (http://c1.accurascience.com/miRecords/) were used to predict the target genes of verified altered miRNAs. In order to eliminate false positive predictions, the target genes selected to perform the subsequent functional analysis must not only overlap in both databases, but also exhibit a Total context+ score ≤ -0.2 in TargetScan 6.2. Gene ontology (GO) analysis and pathway enrichment analysis were done in DAVID website (http://david.abcc.ncifcrf.gov) for combined targets of ShA/Con and LgA/Con, respectively. GO category in this study included molecular function, while Kyoto Encyclopedia of Genes and Genomes (KEGG) was used to obtain an overview of metabolic and regulatory pathways. The top 10 GO terms and KEGG pathways with the most significant *P*-value were identified, respectively.

2.4. Statistical analysis

Data were expressed as mean±SEM. The data of methamphetamine self-administration in escalation phase were tested using two-way ANOVA with repeated measure followed by Bonferroni test, and data of total methamphetamine consumptions of ShA and LgA groups were tested with Student's *t*-test. In addition, statistical analyses of microarray and qRT-PCR data were performed with one-way ANOVA followed by Dunnett's *t*-test. The level of statistical significance was defined as $P < 0.05$.

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