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Cocaine modifies brain lipidome in mice

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ABSTRACT

Lipids are predominant components of the brain and key regulators for neural structure and function. The neuropsychopharmacological effect of cocaine has been intensively investigated; however, the impact of cocaine on brain lipid profiles is largely unknown. In this study, we used a LC-MS-based lipidomic approach to investigate the impact of cocaine on brain lipidome in two mouse models, cocaine-conditioned place preference (CPP) and hyperlocomotor models and the lipidome was profoundly modified in the nucleus accumbens (NAc) and striatum respectively. We comprehensively analyzed the lipids among 21 subclasses across 7 lipid classes and found that cocaine profoundly modified brain lipidome. Notably, the lipid metabolites significantly modified were sphingolipids and glycerophospholipids in the NAc, showing a decrease in ceramide and an increase in its up/downstream metabolites levels, and decrease lysophosphatidylcholine (LPC) and lysophosphoethanolamine (LPE) and increase phosphatidylcholine (PC) and phosphatidylethanolamines (PE) levels, respectively. Moreover, long and polyunsaturated fatty acid phospholipids were also markedly increased in the NAc. Our results show that cocaine can markedly modify brain lipidomic profiling. These findings reveal a link between the modified lipidome and psychopharmacological effect of cocaine, providing a new insight into the mechanism of cocaine addiction.

1. Introduction

Cocaine addiction has become an insidious and hard public health problem (Pomara et al., 2012). Evidences have shown that cocaine affects synaptic plasticity in the mesolimbic dopamine system of brain, which is critical to brain reward processing (Ungless et al., 2001). The prefrontal cortex (PFC), nucleus accumbens (NAc), hippocampus and striatum consist of the reward circuit. Although studies have made efforts to explore the mechanisms of cocaine-induced dysregulation of brain function (Bocklisch et al., 2013; Kupchik et al., 2014), few studies have investigated the impact of cocaine on the lipidomic profiling of brain. The lipidome remodeling of brain induced by cocaine is largely unknown.

It has been known that lipids play critical roles in various biological processes and functions in brain, including membrane structure and trafficking, signal transmission, and synaptogenesis (Cermenati et al., 2015). Brain contains three major class of lipid: phospholipids, sphingolipids (SPs), and cholesterol. Phospholipids are the backbone of neural membranes, and its acyl chains determine the thickness and packing pattern of the bilayer membranes, fluidity, and ion permeability (Farooqui et al., 2004). Brain cholesterol contributes to synapse formation, dendrite differentiation, axonal elongation, and long-term potentiation, which is necessary for normal neuronal function and morphology (Zhang and Liu, 2015). SPs are enriched in neuronal membranes, and gangliosides (GMs), a subclass of SPs, participate in the development and differentiation of neurons. Inhibition of SPs synthesis affects neuronal growth and dampens axonal branching (Harel and Futerman, 1993; Schwarz et al., 1995).

Drug-induced synaptic plasticity is closely related to the modulation of neuronal lipid composition and structural organization of brain membrane and synapses (Sebastiao et al., 2013). Neuronal lipid composition can regulate the trafficking and/or activity of the membrane-

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bound receptors, transporters, ion channel, and enzymes (Lippincott-Schwartz and Phair, 2010; Poveda et al., 2014; Simons and Sampaio, 2011). Studies have suggested the link between lipid metabolism and drug addiction. For instance, phospholipase A2 (PLA2) activity is reduced in the striatum of rat treated with chronic cocaine (Ross and Turenne, 2002). The activity of phosphocholine cytidylyltransferase, a rate-limiting enzyme of phosphocholine synthesis, is reduced in cocaine users (Ross et al., 2002). Changes in fatty acid levels in the blood are correlated with relapse, suggesting that blood lipids can serve as biomarkers of cocaine-induced neurological dysfunction (Buydens-Branchey et al., 2003). A recent study focusing on defined lipid species showed cocaine can modify some phospholipids, such as phosphatidylethanolamines (PE), phosphatidylserines (PS) and phosphatidylcholines (PC), in the hippocampus and cerebellum of cocaine-treated rats (Cummings et al., 2015). However, there are very few studies conducted to explore the global changes of lipidome in addiction model. This situation has been caused mainly by the limited analytical capacity and discovery power of the traditional targeted metabolite analysis approach.

Recent technological developments have made a simultaneous and quantitative examination of hundreds of lipids in various biological tissues. Recently, the detector of liquid chromatography tandem mass spectrometry (LC/MS-MS) has facilitated the precise profiling of lipid species, even those low abundance lipid classes that function in cell signaling and membrane stability (Wenk, 2010). The use of lipidomics strategies, which provide broad and unbiased coverage of the lipids, can be used to understand the multitude of effects of cocaine on brain lipid metabolism and to identify the novel lipid species that may guide subsequent mechanistic investigations of cocaine addiction.

In this study, by using reverse phase liquid chromatography-quadrupole time of flight mass spectrometry (Q-TOF/MS), we comprehensively analyzed the brain lipidome of mice with cocaine exposure. Our results reveal that cocaine can induce a profound remodeling of lipidome within specific brain regions. These findings contribute to the understanding of how cocaine alters lipid metabolites and provide a novel insight into the neuropsychological effect of cocaine.

2. Materials and methods

2.1. Drug

Cocaine hydrochloride was provided by National Institutes for Food and Drug Control (Beijing, China).

2.2. Experimental animals and administration

Male C57BL/6J mice (6–8 weeks old, 18–22 g weights) were purchased from Beijing Marshall Biotechnology Co., Ltd. (Beijing, China) and acclimated for a week before drug administration. Every mouse was individually housed in cages (lights were on 7:30 to 19:30 h; room temperature 23 ± 3 °C). All experimental procedures and use of the animals were approved by the Institutional Animal Care and Use Committee of Sichuan University, and complied to National Institutes of Health guide for the care and use of laboratory animals. Twenty-four mice were randomly divided into control and cocaine-treated groups. Mice in cocaine-treated group were administered intraperitoneally with cocaine (20 mg/kg), and control mice were administered with saline.

2.3. Conditioned place preference (CPP)

CPP test was performed as previous report (Itzhak and Martin, 2002). Briefly, CPP test was composed of a pre-conditioning preference test, a conditioning phase and a post-conditioning preference test. Mouse behavior was recorded by overhead cameras and analyzed by the Anymaze Software v4.81 (Wood Dale, IL, USA). For pre/post-CPP tests, mice were placed in the central chamber, and had access to all three compartments for 15 min. The time spent in each chamber was recorded. The preference was defined as the difference between the times spent in one side chamber and the other. In conditioning phase, the mice in cocaine group were administered with cocaine on days 1, 3, 5, and then immediately confined in the non-preferred chamber for 30 min. On days 2, 4, 6, mice were given one injection of saline, and confined in the preferred chamber for 30 min. After conditioning mice were tested for their post-conditioning preference without an injection. CPP score is time spent in the preferred chamber minus time spent in the non-preferred chamber.

2.4. Locomotor sensitization

Spontaneous locomotor activity was performed as previous report (Heller et al., 2016). Briefly, each mouse was assessed in clear plastic cages, and recorded and analyzed using a video tracking system (Wageningen, The Netherlands). Cocaine-treated mice were intraperitoneally administered with cocaine for 7 consecutive days. After injection, individual mouse was immediately placed in the testing cages, and the distance traveled was monitored for 15 min.

2.5. Sample preparation

Mice were sacrificed by cervical dislocation within 2 h after the end of locomotor activity test or CPP posttest, and brain was excised. Bilateral hippocampus, NAc, PFC and striatum were immediately dissected according to the brain atlas (Paxinos and Franklin, 2004) and frozen in liquid nitrogen before storage at − 80 °C.

2.6. Lipid extraction

Total lipids were extracted with methyl-tert-butyl ether (MTBE) (Matyash et al., 2008). Methanol (150 μl) (stored at − 20 °C) was added to tissues (25–30 mg), and then MTBE (450 μl) was added. Brain samples were incubated for 10 min at room temperature, followed by homogenization by sonication. Phase separation was induced by adding 300 μl 25% methanol. After mixing vigorously and centrifugation at 14000*g* for 10 min, the upper aqueous layer (organic phase) was collected and dried under a gentle stream of nitrogen and stored at − 80 °C until use.

2.7. Lipid metabolite analysis

Lipid samples were separated by an ACQUITY Ultra Performance LC mass spectrometry system (Milford MA, US) and analyzed by electrospray ionization (ESI)-Q-TOF (quadrupole-time-of-flight) Premier mass spectrometer (Milford MA, US). The dried samples were redissolved in acetonitrile/isopropanol (v/v, 7:3). The injection volume was fixed at 3 μl, and an ACQUITY UPLC HSS T3 column (1.8 μm, 2.1 × 100 mm; Milford MA, US) was used for separation. The column was maintained at 55 °C. The flow rate of the mobile phase was 0.4 ml/min. Mobile phase A consisted of acetonitrile/water = 4/6 (10 mmol ammonium