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Remodeling of brain lipidome in methamphetamine-sensitized mice

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ABSTRACT

Lipids are predominant components of the brain and key regulators for neural structure and function. The effect of methamphetamine (METH) on behavior, cognition as well as memory has been intensively investigated; however, the impact of METH on brain lipid profiles is largely unknown. Here, we used a global lipidomic approach to investigate brain lipidome of METH-sensitized mice. We found that repeated METH significantly modified the lipidome in the hippocampus, prefrontal cortex (PFC) and striatum. Interestingly, nucleus accumbens showed no obvious alteration in lipidomic profiling. Phospholipid and sphingolipid metabolisms were profoundly modified in the hippocampus of METH-sensitized mice, exhibiting increased phosphatidic acid and ether phosphatidylcholine but decreased lysophosphatidylethanolamine, lactosylceramide and triglycerides. The fatty acyl length of phospholipids and diacylglycerol longer than 40 carbon were clearly decreased in the hippocampus, and that 36 carbon was decreased in the PFC. These results indicate METH can profoundly affect the metabolism of phospholipids, sphingolipids and glycerolipids in the brain. Our findings reveal a link between remodeled brain lipidome and neurobehavior induced by METH.

1. Introduction

Methamphetamine (METH) is a strong central nervous system stimulant drug with a high potential for abuse, and increased METH use in many parts of the world has become a major public health concern (Mattson, 2013). Studies have intensively investigated the regulatory effect of METH on the expressions of addiction-related genes, neurotransmission and synaptic remodeling (Godino et al., 2015; Scheyer et al., 2016). Proteomic analysis reveals a handful of proteins that are differentially expressed in the brain of METH-treated rats (Wearne et al., 2015). Metabonomic studies show that dopaminergic, glutamatergic and/or GABAergic neurotransmissions are involved in the neurobehavioral effect of METH (Salo et al., 2007; Simoes et al., 2007). However, very few studies have investigated the impact of METH on brain lipidome in a global view.

Lipids represent an essential structural component of brain, and play crucial roles in neural function, including neurite growth, exocytosis, vesicle transportation, membrane formation and trafficking (Allen et al., 2007; Rohrbough and Broadie, 2005). By regulating biochemical properties of membrane, lipids can profoundly influence the location, activities, trafficking as well as signal transduction of receptors, ion channels, transporter and enzymes in plasma membrane of brain (Cen et al., 2008; Piomelli et al., 2007). Brain contains relatively high content of phospholipids (PLs), which forms the backbone of neural membrane. Alterations of PLs composition result in the changes in the fluidity and permeability of neural membrane (Lauwers et al., 2016). Sphingolipid (SL), a critical component of membrane lipid microdomains, is involved in cellular signaling and membrane trafficking (Allen et al., 2007). Glycosphingolipid (GSL), a subclass of SL, mainly includes glucosylceramide (GlcCer), lactosylceramide (LacCer), and ganglioside (GM), which play essential and diverse roles in synaptic transmission and neuron-glia interaction (Farooqui et al., 2000).

Drug-induced membrane remodeling is closely associated with the modulation in lipid composition and structural organization of brain membrane (Hong and Amara, 2010). The activities of membrane-bound receptors, transporters, ion channel as well as enzymes can be

Abbreviations: NAc, nucleus accumbens; PFC, prefrontal cortex; UPLC/Q-TOF-MS, ultra-performance liquid chromatography/quadrupole-time-of-flight mass spectrometry; OPLS-DA, orthogonal partial least squares discriminant analysis; CE, cholesterylester; Cer, ceramide; CerP, ceramide 1-phosphate; DG, diacylglycerol; GM3, ganglioside GM3; GlcCer, glucosylceramide; GalCer, galactosylceramides; LacCer, lactosylceramide; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; MG, monoradylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; Sulf, sulfatides; Sulf(2OH), 2-hydroxy *N*-acyl sulfatide; TG, triacylglycerol; PL, phospholipid; SL, sphingolipid; GL, glycerolipid; SMase, sphingomyelinase; PLA2, phospholipase A2; PLD, phospholipase D

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pathologically altered by modifying the composition of lipids in neural cells (Carta et al., 2014; Schneider et al., 2016). Studies have suggested a link between lipid metabolism and METH-induced brain dysfunction. For instance, calcium-stimulated phospholipase A2 (PLA2) activity is reduced selectively in the brain of human METH users (Ross et al., 2002). Repeated monosialoganglioside (GM1) administration attenuates behavioral sensitization induced by chronic METH exposure (Bellot et al., 1997). However, the impact of METH on brain lipidome is largely unknown. Giving the importance of lipids in the brain, an understanding of the comprehensive lipid profile in different brain regions will get an insight into the neuropsychological effect of METH.

It is a huge work to comprehensively identify and quantify the lipid molecules in complex tissues. However, recent technological developments have made a quantitative examination of the large scale profiling of lipidome in various biological tissues. Ultra-performance liquid chromatography/quadrupole-time-of-flight mass spectrometry (UPLC/Q-TOF-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 lipidomics strategies, which provide broad and unbiased coverage of the lipids, can be used to explore the impact of METH on brain lipidome and to identify the novel lipid species that may guide subsequent mechanistic investigations of METH effect.

In rodents, repeated administration of METH produces a progressive increase in locomotor activity (behavioral sensitization), which is believed to represent the neurochemical changes driving psychoses (O'Dell, 2012) In the present study, we performed a lipidomic approach based on UPLC/Q-TOF-MS to investigate lipid composition and metabolism in different brain regions of METH-sensitized mice.

2. Materials and methods

2.1. Drugs and chemical reagents

Methamphetamine-HCL was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methamphetamine-HCL was dissolved in 0.9% saline. Isopropanol, acetonitrile, ammonium acetate and formic acid were purchased from Sigma-Aldrich (USA).

2.2. Animals

Animals used in this study were adult male C57BL/6 mice (8–12 weeks old, weight 22–24 g). The mice were housed five per cage in clear plastic cages with wire grid lids in a colony with a 12 h light/dark cycle (lights on from 7:00 a.m. to 7:00 p.m.) at constant temperature (23 ± 3 °C). Access to food and water was unrestricted. The animals were acclimatized for 7 days before experiment. All animal protocols in this study were in accordance with the guidelines established by the Association for Assessment and Accreditation of Laboratory Animal Care.

Mice were randomly divided into two groups: control (n = 12) and METH (n = 12). Mice in the METH group received METH (3 mg/kg), via intraperitoneal injection dissolved in 0.9% saline once a day for 7 days, while the control group received the same volume of 0.9% saline for the same period.

2.3. Locomotor activity

Locomotor activity test was conducted as previous report (Lodge and Grace, 2008).

Briefly, each mouse was evaluated in four chambers (40.64 cm × 40.64 cm × 31 cm), and every chamber was separated with black plastic walls. The movement distance and route of each mouse were tracked and analyzed by a video tracking system and Ethovision XT software (Noldus Information Technology, The Netherlands) in every session. Each of mice was placed in the chamber after intraperitoneal injection of 3 mg/kg METH or 0.9% saline. Behaviors sessions were conducted at 2 pm every day for 7 days, and every session lasted 15 min per day. All data was measured by a video tracking system objectively.

2.4. Tissues isolation

Mice were euthanized by cervical dislocation within 2 h after the end of locomotor activity test. The brain was rapidly removed. According to the brain atlas (George Paxinnos, 2001), the nucleus accumbens (NAc), prefrontal cortex (PFC), hippocampus and striatum were dissected from brain slices on ice. 1 mg PFC, 1.5 mg NAc, 5 mg striatum and 15 mg hippocampus were precisely weighed, frozen at liquid nitrogen and stored at −80 °C until use.

2.5. Sample extraction

The lipids were extracted with methyl-tert-butyl ether (MTBE), and the extraction method was previously described (Bozek et al., 2015). Briefly, methanol (stored at −20 °C) was added to the tissue, and then MTBE was added, mixing gently after each addition. The samples were vortexed and incubated at 4 °C for 10 min. The mixtures were ultrasonicated in an ice-cooled bath. To separate the organic from the mixture, a 25% methanol/H_2O solution was added, followed by centrifugation at 4 °C for 10 min (14000*g*). The final ratio of methanol-MTBE- methanol/H_2O solution was 1:3:2. The upper aqueous layer (organic phase) was collected, dried under a gentle stream of nitrogen, and stored at −80 °C until use.

2.6. UPLC/Q-TOF-MS

The concentrated organic was re-suspended in 200 µl acetonitrile:isopropanol (7:3, v/v), ultrasonicated, and centrifuged at 14000*g* for 10 min. The supernatant (100 µl) was transferred to clear insert pipes (Agilent), of which 5 µl was loaded onto a UPLC system (Acquity, Waters) equipped with a HSS T3 column (100 mm × 2.1 mm × 1.8 µm particles, Waters), which was maintained at 55 °C. The mobile phase A consisted of acetonitrile and water with 10 mM ammonium acetate (40:60, v/v), the mobile phase B consisted of acetonitrile and isopropanol both containing 10 mM ammonium acetate (10:90, v/v). The flow rate of the mobile phase was 0.4 ml/min. A linear gradient was used as follows: 40–70% B at 0–3 min, 70–95% B at 3–14 min, 95% B at 14–15.5 min. The column was reequilibrated for 3.5 min, giving a total run 19 min time. The mass spectra was acquired using a G2-S QTof mass spectrometer. Electrospray positive and negative ionization modes both were detected in a resolution mode. The capillary voltage and the sample cone voltage were set to 2 kV and 30 kV separately. The desolvation gas was set to 900 l/h at a temperature of 550 °C. The cone gas was set to 50 l/h and the source temperature was 120 °C. The mass of 50–1200 Da was acquisition using altering full scan and all ion fragmentation scan mode. A lock mass is required for analyses to ensure accuracy. Leucine encephalin (4 ng/ml) was used for the positive and negative ion modes ($[M+H]^+$ = 556.2771 and $[M+H]^-$ = 554.2615). The data were collected in the continuous mode using MassLynx (Waters). 20 µl of each sample was added into one tube, mixed as the QC sample.

2.7. Data process

The original data from MS acquisition was aligned peaks, matched peaks and corrected peak intensities by Progenesis QI (version 2.0, Waters). Metabolites were identified by querying the Human Metabolome Database (http://www.hmdb.ca/) and the Lipid Maps (http://www.lipidmaps.org/). The software provided a weight score of mass error, fragment and isotope similarity. Data sheets from