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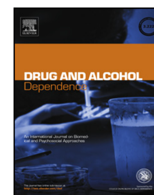

Differential effects of cocaine exposure on the abundance of phospholipid species in rat brain and blood☆

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ABSTRACT

Background: Lipid profiles in the blood are altered in human cocaine users, suggesting that cocaine exposure can induce lipid remodeling.
Methods: Lipid changes in the brain tissues of rats sensitized to cocaine were determined through shotgun lipidomics using electrospray ionization-mass spectrometry (ESI-MS). We also performed pairwise principal component analysis (PCA) to assess cocaine-induced changes in blood lipid profiles. Alterations in the abundance of phospholipid species were correlated with behavioral changes in the magnitude of either the initial response to the drug or locomotor sensitization.
Results: Behavioral sensitization altered the relative abundance of several phospholipid species in the hippocampus and cerebellum, measured one week following the final exposure to cocaine. In contrast, relatively few effects on phospholipids in either the dorsal or the ventral striatum were observed. PCA analysis demonstrated that cocaine altered the relative abundance of several glycerophospholipid species as compared to saline-injected controls in blood. Subsequent MS/MS analysis identified some of these lipids as phosphatidylethanolamines, phosphatidylserines and phosphatidylcholines. The relative abundance of some of these phospholipid species were well-correlated (R^2 of 0.7 or higher) with either the initial response to cocaine or locomotor sensitization.
Conclusion: Taken together, these data demonstrate that a cocaine-induced sensitization assay results in the remodeling of specific phospholipids in rat brain tissue in a region-specific manner and also alters the intensities of certain types of phospholipid species in rat blood. These results further suggest that such changes may serve as biomarkers to assess the neuroadaptations occurring following repeated exposure to cocaine.

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1. Introduction

The Substance Abuse and Mental Health Services Administration (2014) estimates 1.1 million people over the age 12 used cocaine in 2013, and that over 850,000 of these individuals met the Diagnostic and Statistical Manual of Mental Disorders criteria for dependence or abuse of cocaine (2014). Cocaine is responsible for more emergency room visits in the United States than any other illegal drug (http://www.oas.samhsa.gov/2k10/DAWN034/EDHighlights.htm) and there are currently no FDA-approved treatments for cocaine substance use disorder.

Although an extensive effort has been made to characterize and evaluate various mechanisms involved in cocaine-induced alterations of brain function, few studies have assessed the effect of cocaine use on brain lipid metabolism in humans (Ross et al., 1996, 2002), and none have identified the specific lipid species in the

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brain that are altered following drug exposure. It is known that a history of cocaine use alters blood levels of cholesterol (Buydens-Branchey and Branchey, 2003) and fatty acids (Buydens-Branchey et al., 2003). These findings are consistent with the premise that altered cell membrane remodeling activity is occurring in individuals exposed to cocaine. Given that lipid remodeling would be inherent to the changes in neuronal morphology and synaptic plasticity thought to underlie the neuroadaptations associated with substance use disorder, it is somewhat surprising that so few studies have assessed changes in lipid profiles after cocaine exposure, and that none have been described using animal models of addiction.

In addition to data demonstrating that cocaine can induce changes in the lipid profiles, one study reported a link between the brain dopaminergic and phospholipid catabolic systems (Ross and Turenne, 2002), suggesting that changes in phospholipid profiles in cocaine-treated subjects may mirror changes in dopaminergic signaling. Additionally, changes in fatty acid levels in the blood have been correlated with relapse (Buydens-Branchey et al., 2003), supporting the hypothesis that blood lipids can serve as biomarkers of cocaine-induced neurological dysfunction. The hypothesis that blood lipids can be related to neurological dysfunction and behavior is further bolstered by a recent finding in Alzheimer's patients demonstrating that select serum glycerophospholipids predicted cognitive impairment over 2–3 years with 90% accuracy (Mapstone et al., 2014). Finally, another study has demonstrated that cocaine-induced hepatotoxicity in mice resulted in concurrent changes in serum lipids (Shi et al., 2012).

While the aforementioned studies support the rationale for investigating cocaine-induced alterations in lipidomic profiles, a practical consideration impeding such studies is that thousands of lipids exist in biological tissues, making it difficult to identify specific changes in select lipid species. Furthermore, until recently, the methods used to isolate and characterize such lipids were somewhat laborious and time consuming. One productive approach has been to use matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) imaging in rat brain tissue sections to identify lipid species (Jackson et al., 2005a, 2005b). Subsequent studies have described discrete localization of lipids throughout both the rat (Delvolve et al., 2011; Mikawa et al., 2009) and the human brain (Veloso et al., 2011a, 2011b). These observations suggest that heterogeneity in the relative abundance of lipid species both within and between brain regions may have functional relevance.

We performed shotgun lipidomics using electrospray ionization–mass spectrometry (ESI–MS) to assess the persisting effects of cocaine on the relative abundance of phospholipid species in both brain tissues and blood of rats repeatedly exposed to the drug. The resulting data significantly contribute to our understanding of how cocaine alters lipid remodeling within specific brain regions and further suggests that changes in the blood lipidome may be useful as prognostic and/or diagnostic indicators of behavioral responses to cocaine and of drug exposure history.

2. Methods

2.1. Behavior assays

Cocaine conditioning and the induction of locomotor sensitization: Male Sprague-Dawley rats, 8 weeks of age (Harlan, Indianapolis, IN, USA) were housed in pairs in clear plastic cages and maintained on a 12 h light/dark cycle (0700/1900 h). Food and water was available ad libitum except during the behavioral sessions. Animals were allowed to adapt to the lab conditions for a week before behavioral testing began. Behavioral sessions were conducted daily between 0900 and 1600 h.

The apparatus and measurement of activity have been described in detail elsewhere (Gosnell, 2005; Seymour and Wagner, 2008). Briefly, locomotor (LM) activity was measured in four 43.2 × 43.2 cm chambers with clear plastic walls and a solid smooth floor (Med Associates, St. Albans, VT, USA). The chambers were individually housed in sound-attenuating cubicles equipped with a house light and a ventilation fan. Two banks of 16 infrared photobeams and detectors, mounted at right angles 3.5 cm above the floor, detected horizontal activity. Activity Monitor software (Med Associates) was used to count photobeam breaks.

Animals were subjected to a locomotor sensitization protocol over a period of twelve days (Fig. 1A). Following 3 days of habituation to experimenter handling, animals were placed in the center of the open field activity chamber on day 4 for 30 min to establish a baseline LM activity for each animal. After that time, they were given an i.p. injection of either 10 mg/kg cocaine or 0.9% saline and returned to the chamber for an additional 60 min of monitoring for this Activity day session (Fig. 1B, cocaine/saline). During conditioning sessions (days 5–8), animals were injected i.p. with either cocaine (15 mg/kg) or 0.9% saline using the same open field procedure. Seven days after the last conditioning session, on day 15, animals were again injected during the Challenge day session with either cocaine (10 mg/kg) or saline in the open field activity chamber as described above for protocol day 4. For correlation analysis, the initial response to cocaine was determined by the total horizontal counts over the first 30 min after exposure to cocaine on day 4 (e.g. Cocaine 40–70 min of Fig. 1B). Locomotor sensitization to cocaine was determined by subtracting the total horizontal counts over the first 30 min after exposure to cocaine on day 15 from the initial response measured on day 4.

Isolation of specific brain areas and blood: All experimental protocols were performed following approval by the University of Georgia Animal Care and Use Committee. Rats were anesthetized with halothane prior to decapitation. Brains were removed and cooled in ice-cold oxygenated (95% O_2/5% CO_2) dissection artificial cerebrospinal fluid containing 120 mM NaCl, 3 mM KCl, 4 mM $MgCl_2$, 1 mM NaH_2PO_4, 26 mM $NaHCO_3$ and 10 mM glucose. Brain regions were dissected and the tissue was either processed immediately with the Bligh–Dyer lipid extraction (see below) or flash frozen in liquid N_2. Blood was isolated via cardiac puncture prior to decapitation and immediately mixed, by vortexing, with 1 mL of methanol:water (2.0:0.8 v/v) and then placed at −20 °C until lipid extraction.

2.2. Lipidomic analysis

Lipid nomenclature: Phospholipids differ in terms of the numbers of carbons, double bonds and polar head groups. Typically, the nomenclature used to identify these traits is X:Y, where X = the number of carbons and Y = the number of double bonds; hence, 34:2 would indicate a lipid with 34 carbons and 2 double bonds. Polar head groups are referred to by their abbreviations.

Bligh–Dyer lipid extraction: Phospholipids were extracted using chloroform and methanol according to the method of Bligh and Dyer (1959). After treatment, tissue and blood were isolated as explained above, and tissue was washed with PBS and homogenized in 3 mL of methanol:water (2.0:0.8 v/v). Blood was suspended in 1.25 mL of methanol and 1.25 mL of chloroform. Tubes were vortexed for 30 s and allowed to sit for 10 min on ice. Tubes were centrifuged at 213 × g for 5 min and the bottom chloroform layer was transferred to a new test tube. The extraction steps were repeated a second time and the chloroform layers combined. The collected chloroform layers were dried under nitrogen,

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