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Evidence of altered membrane phospholipid metabolism in the anterior cingulate cortex and striatum of patients with bipolar disorder I: A multi-voxel ^{1}H MRS study

Bo Cao [a, *], Jeffrey A. Stanley [b], Sudhakar Selvaraj [a], Benson Mwangi [a], Ives Cavalcante Passos [a], Giovana B. Zunta-Soares [a], Jair C. Soares [a]

[a] UT Center of Excellence on Mood Disorders, Department of Psychiatry and Behavioral Sciences, University of Texas Health Science Center at Houston, TX 77054, USA
[b] Department of Psychiatry & Behavioral Neurosciences, School of Medicine, Wayne State University, Detroit, MI 48201, USA

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ABSTRACT

Background: Previous proton magnetic resonance spectroscopy (^{1}H MRS) studies have reported elevated glycerophosphocholine plus phosphocholine (GPC+PC) in the basal ganglia of patients with bipolar disorders (BD), which implicates an imbalance between synthesis and degradation activity of neuronal and glia membrane phospholipids (MPLs). However, the full extent of altered metabolites of MPLs in subareas within the basal ganglia, such as caudate and putamen, as well as anterior cingulate cortex (ACC) of BD patients is poorly understood.

Methods: Multi-voxel ^{1}H MRS measurements were acquired in 50 type-one BD (BD-I) and 44 healthy controls (HC) on a 3-T MRI scanner. Four different anatomically defined voxels covering ACC, caudate and putamen were systematically extracted and quantified using LCModel. Group differences in absolute GPC+PC and other metabolites were tested with age and gender as covariates.

Results: BD-I patients had higher GPC+PC levels in the anterior-dorsal ACC ($p = 0.037$), caudate ($p = 0.005$) and putamen ($p = 0.004$) compared to HC. GPC+PC levels in the caudate were elevated most significantly in currently unmediated BD-I patients ($p = 0.022$) and were positively correlated with HAM-D scores ($r = 0.51$, $p = 0.005$). PCr+Cr and myo-inositol levels were also significantly higher in the caudate head ($F(1,45) = 6.010$, $p = 0.018$) of patients compared to HC. NAA and glutamate levels were not significantly different between BD-I and HC in these regions ($p > 0.05$).

Conclusion: The increased GPC+PC in BD-I patients may reflect an imbalance in the MPL metabolism. Caudate GPC+PC levels may be a potential biomarker for depressive symptoms in BD.

1. Introduction

Bipolar disorder (BD) has a worldwide prevalence of 2.1% (Merikangas et al., 2007), but its pathophysiology is not fully understood. Magnetic Resonance Imaging (MRI) studies consistently show anatomical alterations in cortical and subcortical brain areas of patients with BD (Selvaraj et al., 2012; Soares, 2003; Strakowski et al., 2005). However, the exact nature of the biochemical underpinnings of neuroanatomical alterations in BD is not fully understood with implicated mechanisms.

Magnetic resonance spectroscopy (MRS) is the only available non-invasive technique that can assess *in vivo* biochemical alterations in the brain (Stanley, 2002; Stanley et al., 2000). Specifically, proton (^{1}H) MRS can detect key metabolites that are implicated in healthy neuronal and glial function, such as N-acetylaspartate (NAA), phosphocreatine plus creatine (PCr+Cr), and choline-containing compounds or specifically glycerophosphocholine plus phosphocholine (GPC+PC). Both GPC and PC are metabolites associated with the metabolism of cell membrane phospholipids (MPLs), where PC is a precursor and GPC a breakdown product of MPLs. PC is also a precursor of sphingomyelin, which is active during myelination of white matter (WM) fibers (Govindaraju et al., 2000; Mcclure et al., 1994a; Stork and Renshaw, 2005). Increased GPC+PC levels has been associated with either increased synthesis

* Corresponding author.
E-mail address: cloudbocao@gmail.com (B. Cao).

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or degradation of MPLs in Alzheimer and multiple sclerosis (McClure et al., 1994b; Tartaglia et al., 2002).

The anterior cingulate cortex (ACC) and basal ganglia are crucial components within the neural circuitry that is associated with mood regulations and reward processing (Bush et al., 2000; Phillips et al., 2008; Sesack and Grace, 2010). Animal studies found that lithium, one of the commonly used mood stabilizers, was most active and accumulated in a region including ACC and striatum (Ramaprasad et al., 2005; Spirtes, 1976). In human *in vivo* ^{1}H MRS studies, ACC and basal ganglia have been extensively investigated in patients with BD. Early studies reported both higher GPC+PC ratios relative to PCr+Cr (Kato et al., 1996) and GPC+PC absolute levels without using the metabolite ratios (Hamakawa et al., 1998) in the basal ganglia of BD patients. However, recent GPC+PC findings in basal ganglia of BD patients have been mixed with either no differences (Ohara et al., 1998; Scherk et al., 2008; Shahana et al., 2011), or increases (Dager et al., 2004) or decreases (Port et al., 2008) levels, especially when subregions, such as the caudate and putamen, were investigated. GPC+PC findings in ACC have been mostly negative (Amaral et al., 2006; Ehrlich et al., 2015; Frye et al., 2007a, 2007b; Öngür et al., 2008; Port et al., 2008; Zhong et al., 2014) except one study that found higher GPC+PC to PCr+Cr ratios in ACC of BD-I patients (Moore et al., 2000). Six of these 12 studies reported GPC+PC results as a metabolite ratio relative to PCr+Cr, while the other studies reported absolute GPC+PC levels. In the case of reporting metabolite ratios, the assumption is that the denominator is constant between contrasting groups. However, significant differences in absolute PCr+Cr levels have been noted in patients with psychiatric disorders including BD compared to healthy subjects (Ongür et al., 2009), which may account, in part, for the inconsistencies between studies in the literature and stresses the importance of reporting absolute metabolite levels such as GPC+PC (Stanley et al., 2000). In addition, eight of the 12 studies reported GPC+PC alterations from localizing a single region of interest or voxel (e.g., ACC and striatum) and therefore, it is unclear whether GPC+PC alterations are widespread in multiple brain areas within the same subjects.

The main purpose of this study was to investigate the extent of GPC+PC alterations and other ^{1}H MRS metabolites in subareas of key mood processing regions, such as ACC, caudate and putamen in a relatively large sample of BD-I patients compared to healthy controls (HC) using a multi-voxel ^{1}H MRS approach with high spatial resolution and with absolute quantification. We hypothesized the absolute GPC+PC level to be higher in BD-I than in HC in ACC, caudate and putamen. The findings of this study would contribute to resolve the inconsistency about choline-containing compound levels in patients with BD, which will potentially be a useful biomarker of neuronal and glial membrane function in BD.

2. Materials and methods

2.1. Participants

Ninety-four adult participants were recruited in the study: 44 healthy control subjects (HC; age range 19–65 years; mean age = 35.4 years and SD = 13.4; 24 females) and 50 type-I bipolar disorder patients (BD-I; age range 19–63 years; mean age = 35.7 years and SD = 12.3; 29 females). Patients were recruited from outpatient clinics at the University of North Carolina at Chapel Hill. HC were recruited through local media advertisements and flyers. All patients met the DSM-IV-R criteria for type-I bipolar disorder, which was implemented with the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders Axis I (SCID I) by an independent psychiatrist or trained research assistant. Mood symptoms were assessed using the Young Mania Rating Scale (YMRS) (Young et al., 1978) and Hamilton Rating Scale for Depression (HAM-D) (Hamilton, 1980). Any participant with a history of substance abuse in the previous 6-months or chronic medical issues including cardiovascular and neurological disorders was excluded. HC with a history of any Axis I disorder in the first-degree relatives or use of psychoactive medication less than two-weeks prior to the start of the study were also excluded. All participants had a negative urine drug screen on the scan day and all female participants had a negative urine pregnancy test on the scan day. The study protocol was approved by the local Institutional Review Board and informed consent was obtained from all the participants.

For the 50 BD-I patients, 12 (24%) were euthymic at the time of interview, 29 (58%) were depressed, two (4%) were manic, one (2%) was hypomanic, and four (8%) were showing mixed symptoms. Two patients (4%) did not have any valid mood state documented. Eighteen (36%) BD-I patients were on medication at the time of interview. Eleven (22%) BD-I patients were on anticonvulsants, nine (18%) were on antidepressants, five (10%) were on Lithium. Twenty (58%) BD-I patients were not taking any medication for at least two weeks at the time of interview (Tables 1a and 1b).

2.2. *In vivo* ^{1}H MRS acquisition

A three dimensional (3D) multi-voxel acquisition scheme was used to acquire ^{1}H MRS data on a 3-T Siemens Allegra MR scanner (Siemens Medical Solutions, Erlangen, Germany). The point resolved spectroscopy (PRESS) combined with the chemical shift imaging (CSI) sequence was used with the following parameters: TR = 1410 msec, TE = 80 msec, FOV = 160 × 160 × 80 mm^3, PRESS volume of interest (VOI) dimension = 70 × 50 × 40 mm^3 covering a region from the anterior part of ACC to the head of the striatum for frontal lobe voxels and VOI dimension = 100 × 65 × 60 mm^3 covering a region from the head of striatum to the thalamus for temporal lobe voxels, nominal pixel dimension = 10 × 10 × 10 mm^3, one acquisition each for water suppressed and unsuppressed scans. The TE was selected based on a previous study that optimized the estimation of glutamate levels while maintained the accuracy of the estimation for GPC+PC, NAA and PCr+Cr (Schubert et al., 2004). A 3D T_1-weighted image was also acquired using magnetization prepared rapid acquisition gradient-echo (MPRAGE) sequence on the axial plane for each subject (TR = 1750 msec, TE = 4.38 msec, flip angle = 8°, field of view = 256 mm, slice thickness = 1 mm, number of excitations = 1, matrix size = 256 × 208) for MRS voxel localization and tissue segmentation that was utilized to calibrate the absolute quantification of the metabolites in each voxel.

2.3. Data processing

The post-processing included extracting and quantifying the ^{1}H MRS signal from the different voxel locations and estimating the tissue fraction of the extracted ^{1}H MRS voxels [i.e., the percent grey matter (GM), WM and cerebrospinal fluid (CSF)]. The procedure from start to finish was 100% automated (i.e., fully independent of

Table 1a
Demographics of BD-I and HC.

	BD-I (n = 50)	HC (n = 44)	F/X^2	p-value
Age (years)	35.7 ± 12.3	35.4 ± 13.4	0.02	0.90
Gender	21M + 29F	20M + 24F	0.11	0.74
Education (years)	14.3 ± 2.4	16.6 ± 3.8	12.00	0.001
YMRS	5.1 ± 5.3	0.4 ± 0.7	33.78	<0.001
HAM-D	13.0 ± 9.0	0.4 ± 0.9	79.50	<0.001