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Increased platelet membrane phosphatidylinositol-4,5-bisphosphate in drug-free depressed bipolar patients

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Abstract

Prior investigations in bipolar disorder patients have suggested abnormalities in the cellular phosphoinositide second messenger system. This study was conducted to examine the levels of platelet membrane phosphoinositides in drug-free bipolar patients in the depressed state ($n = 9$) and healthy controls ($n = 19$). Bipolar patients had significantly increased levels of platelet membrane phosphatidylinositol-4,5-bisphosphate (PIP_2) compared to healthy individuals (0.67 ± 0.14 and 0.44 ± 0.17%, respectively, t-test = 3.71, d.f. = 26, $P = 0.001$). No significant differences in the levels of phosphatidylinositol-4-phosphate (PIP) (0.65 ± 0.17 and 0.58 ± 0.20%, respectively, t-test = 1.02; d.f. = 26; $P = 0.32$) or phosphatidylinositol (PI) (5.92 ± 1.23 and 5.56 ± 1.45%, respectively, t-test = 0.68; d.f. = 26; $P = 0.51$) were found. These findings provide the first demonstration of increased PIP_2 platelet levels in bipolar patients in the depressed state, and provide additional evidence that the phosphoinositide second messenger system may be a site of abnormality in bipolar disorder.

Keywords: Phosphoinositide; Platelet; Bipolar disorder; Affective disorders; Signal transduction; Second messenger; Pathophysiology

Bipolar disorder is a severe psychiatric disorder that has a prevalence of about 1–1.5% in the general population. Unfortunately, its pathophysiology is still largely not understood. In recent years, there has been a growing interest in research that attempts to identify the basic neurobiological mechanisms that might be involved in causation of this serious mental disorder [1]. There have been suggestions that abnormalities of cellular signal transduction mechanisms may be involved in its pathophysiology [6,8,10,12,16,20]. In particular, the phosphoinositide second messenger system has been examined as a possible site of abnormalities [7,15]. Because of the limited available means to directly examine this system in human subjects' brains in vivo, research in this area has primarily focused on peripheral models. In particular, platelets have been used as a model for second messenger and neurotransmitter function [4,13]. Our group has previously conducted studies examining platelet membrane phosphoinositide content, as an approach to study the functioning of the phosphoinositide second messenger system in relation to bipolar disorder. Following the initial demonstration of increased phosphatidylinositol-4,5-bisphosphate (PIP_2) levels in drug-free manics [2], we reported the case of a patient whose PIP_2 levels increased during a manic episode, and then returned to baseline levels after treatment with lithium [14]. Thereafter, we reported reduced PIP_2 levels in euthymic lithium-treated bipolar individuals compared to healthy controls [18], as well as decreased PIP_2 levels after 3 weeks of lithium at therapeutic concentrations in bipolar patients who were studied both prior to and during treatment [17]. Findings of elevated PIP_2 levels in the manic state, which seem to normalize with lithium treatment, suggest a state-related abnormality that may be implicated in the pathophysiology of bipolar disorder.

In order to further extend these studies, we investigated whether any abnormalities in the platelet membrane levels of phosphoinositides would be present in unmedicated bipo-

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lar patients in the depressed state. We hypothesized that PIP_2 levels would be abnormally increased in this mood state, suggesting state-related abnormalities in the phosphoinositide (PI) pathway in bipolar disorder individuals.

Nine unmedicated subjects meeting DSM-IV (Diagnostic and Statistical Manual, version IV) criteria for bipolar type I disorder were studied (six males, three females; mean age ± SD = 31.7 ± 4.4 years). They did not have any current medical problems, axis I psychiatric comorbidity, or significant history of substance abuse/dependence. All patients were depressed, and had been off all medications for at least 2 weeks, and off lithium treatment for at least 1 month. The DSM-IV diagnosis of bipolar I disorder was based on a Structured Clinical Interview for DSM-IV (SCID-IV) interview, and confirmed in a clinical evaluation conducted by an attending psychiatrist. Nineteen healthy controls (ten males, nine females; mean age ± SD = 34.2 ± 9.0 years) were studied. They did not have any past history of psychiatric problems, significant substance abuse/dependence, or any current medical problems. The SCID-IV interview, non-patient version, was used to rule out axis I psychiatric diagnosis in these healthy individuals. All subjects provided written informed consent. The protocol and consent forms were approved by the University of Pittsburgh Institutional Review Board.

Platelet membrane phosphoinositides were measured as previously reported [9]. A blood sample was obtained from a peripheral vein, in the time interval between 10:00 and 13:00 h. Subjects were asked to sit on a chair for at least 15 min before blood samples were collected. Blood samples (2 × 34 ml) were collected with a 21 gauge butterfly needle into plastic syringes containing 6 ml of acid citrate dextrose anticoagulant (Sigma Chemical Company, USA). The platelet membrane preparations were obtained, and stored at −80°C. Subsequently, platelet membrane suspensions were thawed at room temperature, and a biphasic lipid extraction was performed. The membrane phospholipids were separated using two-dimensional thin-layer chromatography on high performance plates, and quantitated by scanning laser densitometry. The levels of each phospholipid class were measured using an algorithmically calculated volume term (GelScan XL 2400 Software, version 2.1, Pharmacia LKB Biotechnology), based on the area of each phospholipid spot and the absorbances measured in that area. The phospholipid spots were outlined and quantitated with the GelScan XL 2400 software by a research assistant (C.SD), blindly to sample identity. The values were expressed as absorbance units times area (AU × mm^2), and the relative absorbances for each individual phosphoinositide were calculated as a percentage of the total phospholipid absorbance. The sensitivity of this method was previously determined to be <10 ng of lipid phosphorus [9].

Statistical analyses were performed with the software SPSS for Windows, version 8.0 (SPSS, Inc.). Individual unpaired *t*-tests were performed for each dependent variable for comparisons between bipolar patients and healthy individuals.

The depressed bipolar patients had significantly increased levels of platelet membrane PIP_2 compared to healthy individuals (0.67 ± 0.14 and 0.44 ± 0.17%, respectively, *t*-test = 3.71; d.f. = 26; $P = 0.001$). The data from individual subjects are illustrated in Fig. 1. No significant differences between bipolar patients and healthy controls were found in the levels of PIP (0.65 ± 0.17 and 0.58 ± 0.20%, respectively, *t*-test = 1.02; d.f. = 26; $P = 0.32$) or PI (5.92 ± 1.23 and 5.56 ± 1.45%, respectively, *t*-test = 0.68; d.f. = 26; $P = 0.51$).

These findings provide the first demonstration of increased PIP_2 levels in platelet membranes of bipolar disorder patients in the depressed state. In conjunction with previous results that demonstrated increased PIP_2 levels in the manic phase of the illness [2], they provide evidence that the phosphoinositide second messenger system may be a site of functional abnormality in bipolar disorder patients. In particular, our results suggest the possibility that a state-related hyperfunction of this pathway may be mechanistically involved in the pathophysiology of mood episodes in this severe mental disorder. This idea is further supported by our previous observations that administration of lithium, the prototype mood stabilizer, to bipolar patients results in lower platelet membrane PIP_2 levels [17,18].

In view of prior findings suggesting that lithium treatment reduces platelet membrane PIP_2 levels in bipolar patients [17,18], it seems likely that the elevation of PIP_2 reported in our studies could be a state-related abnormality. However, future studies that also examine bipolar patients in the euthymic state will be critical in order to further

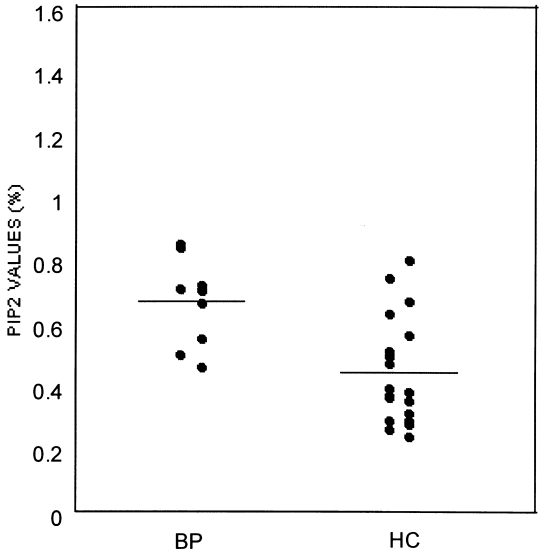

Fig. 1. The mean PIP_2 platelet membrane levels were significantly increased in untreated bipolar patients in the depressed state compared to healthy individuals (0.67 ± 0.14 and 0.44 ± 0.17%, respectively, *t*-test = 3.71; d.f. = 26; $P = 0.001$). BP, bipolar patients; HC, healthy controls.

examine this hypothesis. Due to the extensive cross-talk among the various intracellular signal transduction pathways (for review, see Manji et al. [10]), it is unclear what the ultimate physiological interpretation of increased PIP_2 levels might be. They may represent a state of hyperactivity in the cellular PI second messenger system. If so, these findings would be in line with reports of increased intracellular Ca^{2+} release in bipolar patients in various mood states [3], as well as findings of increased protein kinase C (PKC) activity in depressed and manic patients [5,19]. Studies that would concurrently examine different steps of these intracellular pathways in the same individuals will be needed to attempt to clarify the in vivo pathophysiological mechanisms involved in these disorders. Additionally, investigations in depressed unipolar patients will also be important to determine the specificity of these findings, particularly in light of recent findings from Pandey et al. [11] suggesting abnormalities in PKC-mediated mechanisms in depressed unipolar subjects.

A limitation present in our current approach originates from the fact that it is not known to which extent platelet membrane findings actually reflect brain abnormalities in these same pathways. However, prior work in the field of clinical neuropsychopharmacology suggests that platelets may be acceptable models for particular neuronal processes [4,13]. As new in vivo methods to directly examine the human brain neurochemistry become available, and make the examination of intracellular signal transduction processes feasible, it is expected that these promising lines of investigation will continue to advance, and eventually result in substantial progress to our understanding of the pathophysiological mechanisms involved in this severe mental disorder.

In conclusion, we found increased levels of PIP_2 in platelets of unmedicated depressed bipolar patients. These findings may represent a hyperactivity in the phosphoinositide second messenger system of relevance for the pathophysiology of bipolar disorder. Future studies should attempt to independently replicate these findings, and clarify whether these abnormalities are trait or state-related, and whether they correlate with therapeutic response or course of illness.

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