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Altered Arachidonic Acid Cascade Enzymes in Postmortem Brain from Bipolar Disorder Patients

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Abstract

Mood stabilizers that are approved for treating bipolar disorder (BD), when given chronically to rats, decrease expression of markers of the brain arachidonic metabolic cascade, and reduce excitotoxicity and neuroinflammation-induced upregulation of these markers. These observations, plus evidence for neuroinflammation and excitotoxicity in BD, suggest that AA cascade markers are upregulated in the BD brain. To test this hypothesis, these markers were measured in postmortem frontal cortex from 10 BD patients and 10 age-matched controls. Mean protein and mRNA levels of AA-selective cytosolic phospholipase A_2 IVA (cPLA$_2$), secretory (s)PLA$_2$ IIA, cyclooxygenase (COX)-2, and membrane prostaglandin E synthase (mPGES) were significantly elevated in the BD cortex. Levels of COX-1 and cytosolic PGES (cPGES) were significantly reduced in BD cortex relative to controls, whereas levels of Ca^{2+}-independent iPLA$_2$VIA, 5-, 12-, and 15-lipoxygenase, thromboxane synthase and cytochrome p450 epoxygenase protein and mRNA levels were not significantly different. These results confirm that the brain AA cascade is disturbed in BD, and that certain enzymes associated with AA release from membrane phospholipid and with its downstream metabolism are upregulated. Since mood stabilizers downregulate many of these brain enzymes in animal models, their clinical efficacy may depend on suppressing a pathologically upregulated cascade in BD. An upregulated brain AA cascade should be considered as a target for future drug development and for neuroimaging in BD.

Keywords

PLA$_2$; inflammation; mood stabilizers; COX; PGES; excitotoxicity

Introduction

Bipolar disorder (BD) is characterized by recurrent depressive and manic episodes. It afflicts about 1.5% of the United States population (1), increases the risk of suicide by approximately 5–17 fold (2), and has multiple risk alleles consistent with a polygenic inheritance (3). Recent studies suggest progressive brain atrophy and neuronal loss in BD patients, with increased brain levels of proinflammatory cytokines, and evidence of increased glutamatergic function and excitotoxicity (4–6). Some of these features are also found in psychiatric and neurodegenerative diseases including schizophrenia (SZ) and

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Conflict of interest
The authors declare no conflict of interest.

Alzheimer disease (AD). However, patients with BD have many more features that overlap with those of SZ patients (7,8), than with AD patients (9,10), such as an early onset, genetic association and drug therapy.

Inflammation and excitotoxicity can activate many brain signaling pathways, including the arachidonic acid (AA, 20:4n-6) metabolic cascade (11–13). For example, activation of the cytokine interleukin (IL)-1 receptor cascade can increase expression of AA metabolizing enzymes, including AA-selective cytosolic phospholipase A_2 ($cPLA_2$) (14-16), secretory $sPLA_2$ (16), and cyclooxygenase (COX)-2 (17), as well as transcription factors that regulate gene transcription of these enzymes, particularly activator protein (AP)-2 and/or nuclear kappa B (NF-κB). With regard to excitotoxicity, rats chronically administered a subconvulsant dose of NMDAshowed an increase in brain AA turnover, protein and mRNA levels of $cPLA_2$ IVA, AP-2 DNA binding activity, AP-2α and AP-2β protein, and cytokine levels (13,18).

AA is a nutritionally essential polyunsaturated fatty acid (PUFA) found mainly in the stereospecifically numbered (*sn*)-2 position of membrane phospholipids, from which it can be hydrolyzed by $cPLA_2$ or $sPLA_2$ (19). A portion of the AA released can be metabolized into bioactive prostaglandin H_2 (PGH_2) by COX-1 or COX-2, to cytoprotective epoxyeicosatrienoic acids by cytochrome p450 epoxygenase, or to cytotoxic leukotrienes by lipoxygenase subtypes 5,12 and 15 (20). Bioactive PGH_2 is converted to prostaglandin E_2 (PGE_2) by membrane prostaglandin synthase-1 (mPGES-1) or cytosolic prostaglandin synthase (cPGES). PGH_2 can also be converted to thromboxane A_2 (TXA_2) by thromboxane synthase (TXS) (21) (Figure 1). Of the two COX isoenzymes, COX-1 is constitutively expressed, whereas COX-2 is inducible (22,23). cPGES uses PGH_2 produced by COX-1, whereas mPGES-1 uses COX-2-derived endoperoxide (24). AA and its metabolites can modulate signal transduction, transcription, neuronal activity, apoptosis, and many other processes within the brain (25–27).

Lithium, valproic acid, carbamazepine and lamotrigine are approved by the FDA as “mood stabilizers” for treating BD. Each of these agents, when given chronically to rats to produce a therapeutically relevant plasma concentration, downregulate parts of the brain AA cascade, including AA turnover in brain phospholipids (lithium, valproic acid, carbamazepine), $cPLA_2$ IVA and its transcription factor AP-2 (lithium and carbamazepine), acyl-CoA synthetase (valproic acid), COX-1 (valproate), COX-2 (all four), and NF-κB (valproate) (28–33). Chronic lithium and carbamazepine also prevent elevations of brain AA cascade markers in rat models of neuroinflammation and excitotoxicity (34,35).

In view of the evidence linking excitotoxicity and neuroinflammation to BD (see above) (11), and the inhibition rat brain AA metabolism by mood stabilizers, we hypothesized that the AA cascade is upregulated in the BD brain. To test this hypothesis, protein and mRNA levels of AA cascade enzymes (see above) were compared between postmortem frontal cortex from 10 BD patients and 10 unaffected controls. We also compared expression of Ca^{2+}-independent $iPLA_2$, which is selective for docosahexaenoic acid (DHA, 22:6n-3) in membrane phospholipid (36), and of neuron-specific enolase (NSE), a marker of postmortem tissue integrity in the absence of acute injury (37,38). The frontal cortex (Brodmann area 9) was chosen for this study because functional and structural abnormalities have been reported in this region in BD patients (5), and because relevant data on this region have been published previously (11,38). Preliminary data on the subjects have been published in abstract form (39).

Materials and Methods

Post-mortem brain samples

The protocol was approved by the Institutional Review Board of McLean Hospital, and by the Office of Human Subjects Research (OHSR) of the NIH (# 4380). Frozen postmortem human frontal cortex from 10 BD patients and 10 age-matched controls was provided by the Harvard Brain Tissue Resource Center (McLean Hospital, Belmont, MA) under PHS grant number R24MH068855 to JS Rao. Age (years, control: 43 ± 3.5 vs. BD: 49 ± 7.2), postmortem interval (hours, control: 27 ± 1.5 vs. BD: 21 ± 3.0), and brain pH (control: 6.6 ± 0.16 vs. BD: 6.7 ± 0.09) did not differ significantly between the two groups, whereas the BD patients were exposed to various psychotropic medications as reported previously (Table 1) (38).

Preparation of cytosolic and membrane fraction

Cytosolic and membrane extracts were prepared from postmortem frontal cortex of BD and control subjects as previously reported (40). Frontal cortex tissue was homogenized in a homogenizing buffer containing 20 mM Tris-HCl (pH 7.4), 2 mM EGTA, 5 mM EDTA,1.5 mM pepstatin, 2 mM leupeptin, 0.5 mM phenylmethylsulfonyl fluoride, 0.2 U/ml aprotinin, and 2 mM dithiothreitol, using a Teflon homogenizer. The homogenate was centrifuged at 100,000*g* for 60 minat 4°C. The resulting supernatant-1 (S1) was the cytosolic fraction, andthe pellet was resuspended in the homogenizing buffer containing 0.2% (w/v) Triton X-100. The suspension was kept at 4°C for 60min with occasional stirring and then centrifuged at 100,000*g* for 60 min at 4°C. The resulting supernatant-2 (S2) was the membrane fraction. Protein concentrations in membrane and cytosolic fractions were determined with Bio-Rad Protein Reagent (Bio-Rad, Hercules, CA). The membrane and cytosolic fractions were confirmed using the specific markers, cadherin and tubulin, respectively.

Western blot analysis

Proteins (50 μg) from the cytoplasmic and membrane extracts were separated on 4–20% SDS-polyacrylamide gels (PAGE) (Bio-Rad). Following electrophoresis, the proteins were transferred to a PVDF membrane (Bio-Rad). Cytoplasmic protein blots were incubated overnight in Tris-buffered-Saline buffer, containing 5% nonfat dried milk and 0.1% Tween-20, with specific primary antibodies (1:200 dilution) for the group IVA cPLA$_2$, group IIA sPLA$_2$, group VIA iPLA$_2$, COX-1 (1:1000), COX-2 (1:500), cytochrome P450 epoxygenase, TXS, 5-, 12-, and 15-LOX (Cell Signaling, Beverly, MA) and NSE (1:10,000) (Abcam, Cambridge, MA). mPGES-1 was determined using a specific (1:200) primary antibody (Abcam). Cytoplasmic and membrane protein blots were incubated with appropriate HRP-conjugated secondary antibodies (Bio-Rad) and visualized (Kodak, Rochester, NY). Optical densities of immunoblot bands were measured using Alpha Innotech Software (Alpha Innotech, San Leandro, CA) and were normalized to β–actin (Sigma-Aldrich, St. Louis, MO) to correct for unequal loading. All experiments were carried out twice with 10 controls and 10 post-mortem brain samples from BD patients. Values were expressed as percent of control.

Total RNA isolation and real time RT-PCR

Total RNA was isolated from the frontal cortex using an RNeasy mini kit (Qiagen, Valencia, CA). RNA integrity number (RIN) was measured using Bioanalyzer (Agilent 2100 Bioanalyzer, Santa Clara, CA). RIN values for control and BD were 6.9 ± 0.4 and 7.1 ± 0.5, respectively (Mean + SEM). Complementary DNA (cDNA) was prepared from total RNA using a high-capacity cDNA Archive kit (Applied Biosystems, Foster City, CA). mRNA

levels of $cPLA_{2,}$ $sPLA_{2,}$ $iPLA_{2,}$ COX-1, COX-2, mPGES-1, cPGES, LOX-5, 12, 15, TXS, cytochrome P450 epoxygenase and NSE were measured by quantitative RT-PCR, using an ABI PRISM 7000 sequence detection system (Applied Biosystems). Specific primers and probes for $cPLA_{2,}$ $sPLA_{2,}$ $iPLA_{2,}$ COX-1, COX-2, mPGES-1, cPGES, LOX-5, 12, 15, TXS, and cytochrome P450 epoxygenase were purchased from TaqMan^R gene expression assays (Applied Biosystems), and consisted of a 20X mix of unlabeled PCR primers and Taqman minor groove binder (MGB) probe (FAM dye-labeled). The fold-change in gene expression was determined by the $\Delta\Delta C_T$ method (41). Data were expressed as the relative level of the target gene ($cPLA_{2,}$ $sPLA_{2,}$ $iPLA_{2,}$ COX-1, COX-2, mPGES-1, cPGES, LOX-5, 12, 15, TXS, cytochrome P450 epoxygenase and NSE) in the post-mortem BD patients normalized to the endogenous control (β-globulin) and relative to the control (calibrator), as previously described (42). All experiments were carried out twice in triplicates with 10 controls and 10 post-mortem brain samples from BD patients. The data were expressed as relative expression of control.

Statistical Analysis

The data are presented as mean ± SEM. Statistical significance of means was calculated using a two-tailed unpaired *t*-test. Power analysis was carried out according to Mitulsky (1995). We have set α, the threshold for significance for two-tailed distribution, to 0.05 and β, the power index to 20%. Pearson correlations were made between age, post-mortem interval and pH of the frontal cortex, and mRNA levels of $cPLAB_{2B}$, $sPLAB_{2B}$, $iPLAB_{2B}$, COX-1, COX-2, mPGES-1 and cPGES in post-mortem brain from controls and BD patients combined. A subgroup statistical comparison was performed on control, all BD subjects and BD subjects that were on lithium medication using Bonferroni's multiple comparison test, to assess the effects of lithium on the molecular markers analyzed. A separate Bonferroni's multiple comparison test was made between control, all BD and BD subjects who died by suicide, to determine whether suicide was a factor affecting gene and protein expression. Statistical significance was set at $p < 0.05$.

Results

Upregulated protein and mRNA levels of $cPLA_2$, $sPLA_2$ and COX-2

Mean protein levels of $cPLA_2$ IVA and $sPLA_2$ IIA were increased significantly ($p < 0.01$), by 87% and 92% respectively (Fig. 2A and 2B), in BD compared with control frontal cortex, whereas the mean $iPLA_2$ protein level did not differ significantly between the groups (Fig. 2C). Mean mRNA levels of $cPLA_2$ and $sPLA_2$ were increased significantly in BD compared with control brain by three-fold ($p < 0.001$) and six-fold ($p < 0.01$), respectively (Figs. 2D and E), but $iPLA_2$ mRNA was not significantly different (Fig. 2F). COX-2 protein and mRNA levels were increased significantly by 82% (Fig. 3A, $p < 0.01$) and 3.4-fold (Fig. 3B, $p < 0.01$), respectively, whereas COX-1 protein and mRNA were significantly decreased in the BD cortex by 40% ($p < 0.01$, Fig. 3C) and 0.6 fold ($p < 0.05$, Fig. 3D), respectively.

Increased protein and mRNA levels of mPGES-1

Statistically significant increases were found in mPGES-1 protein (by 71%, $p < 0.01$, Fig. 4A) and mRNA (by 3.6 fold, $p < 0.01$, Fig. 4C) in samples from BD patients relative to controls. cPGES was significantly decreased with regard to the levels of its protein (by 54%, $p < 0.01$, Fig. 4B) and mRNA (by 0.76 fold, $p < 0.01$, Fig. 4C). There was no significant difference in either the protein (Fig. 5A, B and C) or mRNA (Fig. 5D) level for LOX 5, 12, 15, TXS (Fig. 6A and D), or cytochrome P450 (Fig. 6B and E) between groups.

Mean protein and mRNA levels of NSE did not differ significantly between BD and control brains (Fig. 6C and E).

Power analysis and correlations with brain variables

Power analysis revealed that a sample size of 10 in each group is sufficient to detect a difference of 20% between the two groups, based on our estimated mean and SD values (as described in the material and methods). Pearson correlations between variables (age, PMI and pH) and the mRNA levels from across all 20 brain samples (control and BD patients combined) were not statistically significant (Table 2).

Bonferroni's multiple comparison tests showed a significant decrease in AA cascade markers (protein and mRNA) between controls and BD subjects, and controls and BD subjects on lithium medication ($p < 0.05$). However, no significant changes in AA cascade markers were observed between all BD subjects and the subgroup of BD subjects treated with lithium medication. Similarly, both BD subjects and BD subjects who committed suicide showed reduced AA cascade markers (protein and mRNA) relative to controls ($p < 0.05$). No significant differences were found between all BD subjects and the subgroup of BD subjects who committed suicide, in AA cascade markers levels.

Discussion

In this study, mean protein and mRNA levels of $cPLA_2$ IVA, $sPLA_2$ IIA, COX-2, and mPGES were significantly elevated in postmortem frontal cortex of BD patients compared with controls. Protein and mRNA levels of COX-1 and of cPGES were significantly reduced, whereas protein and mRNA levels of $iPLA_2$, 5-, 12-, and 15-lipoxygenase, thromboxane synthase, cytochrome p450 epoxygenase were not significantly altered. These results are consistent with the hypothesis that the brain AA cascade is disturbed in BD. The hypothesis is based on the observation that each of the four mood stabilizers approved for treating BD, when given chronically to rats, downregulate AA turnover in brain phospholipids and other markers of brain AA metabolism, and on evidence of neuroinflammation and excitotoxicity associated with disease progression in BD, including brain atrophy and cell loss, cognitive decline and symptom worsening (5,43–46), in BD.

An upregulated AA cascade may contribute to disease progression in BD in many ways (47). For example, excess unesterified AA and lysophospholipids formed following AA hydrolysis can induce apoptosis by damaging mitochondria (48), activating caspases-3 and -9, releasing cytochrome C (49), decreasing expression of brain derived neurotrophic factor (BDNF) (50), and reducing neuronal viability (51).

The increased expression of $cPLA_2$ IVA, $sPLA_2$ IIA and COX-2 in the BD brain may be related to underlying excitotoxicity and/or neuroinflammation. An elevated brain glutamate/glutamine ratio, increased glutamate concentration, and decreased levels of the NMDA receptor subunits NR1, NR2A and NR3A, have been reported in the BD brain (41,42,52,53). In this regard, studies have demonstrated that chronic subconvulsive NMDA administration to rats reduced brain levels of NR1 and NR3A, increased AA turnover in brain membrane phospholipids and increased protein and mRNA levels of $cPLA_2$ IVA and $sPLA_2$ IIA in the brain (42). Increased Ca^{2+} entry into a cell *via* the glutamatergic NMDA receptor may directly activate Ca^{2+}-dependent AA-selective $cPLA_2$ to release AA from membrane phospholipids (34,54); chronic lithium, carbamazepine or valproate can inhibit this process (34,35).

Neuroinflammation has been reported to activate AA cascade markers. For instance, the exposure of rat astrocytes to bacterial lipopolysaccharide (LPS) was reported to increase $cPLA_2$ transcription via an AP-2 and NF-κB dependent manner (41,55). A rat model of inflammation, caused by chronic LPS infusion into the cerebroventricular system showed an increase AA incorporation and turnover within brain phospholipids, elevated concentrations

of unesterified AA, PGE_2 and other AA metabolites, and increased $cPLA_2$ and $sPLA_2$ activities (35,56). LPS infusion was also shown to increase IL-1β, TNFα, and beta-amyloid precursor protein in activated microglia and astrocytes, resulting in degeneration of hippocampal CA3 pyramidal neurons, and altered behavior (56–58). Cytokines formed during inflammation have been reported to activate both $cPLA_2$ and $sPLA_2$ at astrocytic cytokine receptors (19,59–61).

Excitotoxicity and neuroinflammation have been associated with upregulation of mPGES-1, which is functionally coupled to COX-2 (24,62). Coupling is consistent with our finding of increased expression of both mPGES-1 and COX-2 in the BD frontal cortex. On the other hand, cPGES is coupled to COX-1, and the expression of both these enzymes was significantly reduced in the BD brain. This is consistent with evidence showing that products of COX-1 are selectively metabolized by cPGES (24,62). Decreased expression of COX-1 and cPGES might be a compensatory response for increased expression of COX-2 and mPGES.

Consistent with an elevated AA metabolism in BD, studies have reported increased hydrolysis of serum phospholipids (63–65) and increased levels of AA-derived prostaglandins in saliva (66), cerebrospinal fluid (67) and serum (64) from BD patients. An increase in AA cascade markers, including $cPLA_2$ IVA, $sPLA_2$ IIA and COX-2 protein and mRNA were elevated in frontal cortex of n-3 PUFA deprived rats (42), which were shown to exhibit BD-like behavioral symptoms (68). Expression of BDNF and cyclic AMP response element binding (CREB) protein was also reduced in the n-3 PUFA deprived animals (42).

The absence of a significant difference in $iPLA_2$ expression in the frontal cortex between BD patients and controls is consistent with unaltered $iPLA_2$ activity in BD serum (69,70). $iPLA_2$ is thought to hydrolyze DHA from membrane phospholipids (71), and its expression was not elevated in rat brain following either chronic NMDA administration or cerebroventricular LPS infusion (35,42,56). There was no significant difference in other AA and prostaglandin metabolism enzymes, such as P450 expoxygenease, 5-, 12-, and 15-LOX, as well as TXS, between BD and control frontal cortex. These results suggest that increased AA signaling is channeled into prostanoid synthesis, and is selective only to parts of the brain AA cascade.

In parallel with BD, studies in schizophrenic patients have indicated an increase in brain calcium dependent and independent PLA_2 activity, as well as PLA_2 IVA protein level in red blood cells (72,73). Similar AA cascade changes also have been reported in postmortem brains from Alzheimer disease (AD) patients, where excitotoxicity and neuroinflammation are considered to play a role (74,75). In AD post mortem brain tissue, $cPLA_2$ (76), $sPLA_2$ (59), and COX-2 expression (77) are upregulated. Reduced $cPLA_2$ expression ameliorated cognitive deficits in a mouse model of Alzheimer disease (78). Thus, the changes noted here may not be specific to BD, but may be generally related to excitotoxic and inflammatory processes that occur in multiple chronic and progressive neurodegenerative and neuropsychiatric disorders, including Alzheimer disease, Parkinson disease, schizophrenia and unipolar depression (52,69,79).

Many but not all of the differences between the BD and control brain were in an opposite direction to brain changes in rats chronically administered mood stabilizers. For example, chronic lithium and carbamazepine was shown to decrease mRNA and protein levels of $cPLA_2$ IVA in rat brain while this enzyme's expression was upregulated in the BD brain. $sPLA_2$ expression also was upregulated in the BD brain; chronic lithium did not reduce $sPLA_2$ expression in the normal rat brain (18), but prevented the upregulation that was

caused by cerebroventricular LPS infusion (Basselin et al., unpublished results). Increased expression of $sPLA_2$ IIA in BD is consistent with reports of increased risk associated with alleles for pancreatic PLA_2 (80,81) and for the $sPLA_2$ receptor in BD (3). However, COX-1 was reduced in the BD brain as well as in the brain of rats given chronic valproate (32), whereas COX-2 was elevated in the BD brain but reduced by lithium, carbamazepine, valproate and lamotrigine (18). Opposite changes in AA cascade markers in the BD brain compared with the brain of rats treated with mood stabilizers may be the basis, in part, for their efficacy in BD.

Levels of mRNA in either BD or control brains did not correlate significantly with postmortem interval, brain pH, or subject age, and mean values of these parameters did not differ significantly between the two groups. Nevertheless, the BD patients were exposed to a variety of drugs not taken by the control subjects, which may have affected the results, since antipsychotics and mood stabilizers can have neurotoxic effects when given chronically (82,83). No statistical differences were found in all AA cascade genes studied in the present study when the BD subjects were compared to the subgroup of BD subjects treated with lithium. Also, no statistical significance was found when the BD subjects were compared to BD subjects that died by suicide. This suggests that lithium or suicide do not have profound effects on the studied AA cascade markers.

The limitation of the present study is non-availability of medical diagnosis at the time of death, whether patients are in manic or depressive conditions. However, since several BD patients died by suicide, they may have been in a depressed phase of their illness. Future studies should examine AA cascade markers in brains from patients with schizophrenia (to control for comparable drug exposure), or with unipolar (primary major) depression or Alzheimer disease to test for disease specificity (84)

In conclusion, many markers of the AA cascade were significantly upregulated in postmortem frontal cortex from BD patients. These changes may reflect neuroinflammation and excitotoxicity, associated with cell death or drug exposure, or may be intrinsic to the disease independent of these pathological processes. Some of these AA cascade markers were downregulated in rat brain by chronically administered mood stabilizers, which may account for their efficacy in BD. Accordingly, new agents that are shown to downregulate the brain AA cascade in animal models could be considered for treating BD.

The results suggest that brain AA metabolism is elevated in BD, and this could be tested directly with the help of positron emission tomography and [1-^{11}AA] as a radioligand (85). If correct, an increased AA image would be a biological marker of disease progression and could be used to evaluate therapeutic efficacy. Increased brain AA metabolism has been imaged in patients with Alzheimer disease using positron emission tomography (86), in which $cPLA_2$, $sPLA_2$ and COX-2 were also found to be elevated in post-mortem tissue (59,76,77).

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Abbreviations

AA Arachidonic acid

AP-2	activator protein-2
BD	bipolar disorder
BDNF	brain derived neurotrophic factor
cPLA$_2$	cytosolic phospholipase A$_2$
COX	cyclooxygenase
DHA	docosahexaenoic acid
iPLA$_2$	calcium-independent phospholipase A$_2$
LPS	lipopolysaccharide
NF-κB	nuclear factor kappa B
sPLA$_2$	secretory phospholipase A$_2$
NMDA	N-methyl-D-aspartate
PGE$_2$	prostaglandin E$_2$
TXS	thromboxane synthase
LOX	lipoxygenase
NSE	neuron-specific enolase

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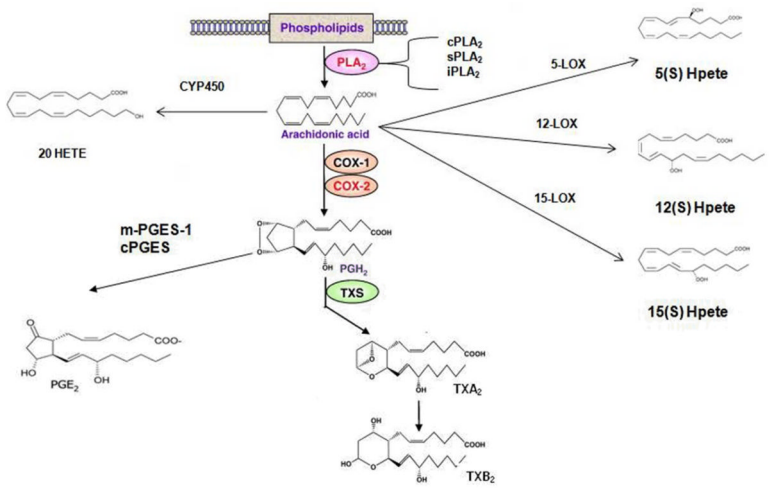

Figure 1.
Schematic diagram of arachidonic acid cascade.

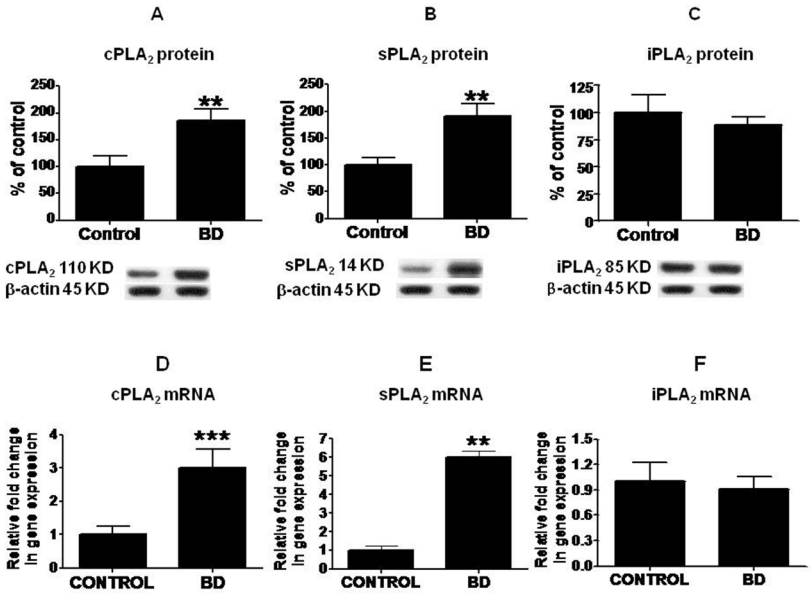

Figure 2. Protein and mRNA levels of PLA_2 enzymes

Mean $cPLA_2$ (A), $sPLA_2$ (B) and $iPLA_2$ (C) protein (with representative immunoblots) as percent of control in frontal cortex, from control (n = 10) and BD (n = 10) subjects. Data are optical densities relative to that of β-actin. Mean mRNA as percent of control of $cPLA_2$ (D), $sPLA_2$ (E) and $iPLA_2$ (F) in frontal cortex from control (n = 10) and BD (n =10) subjects, measured using RT-PCR. Data are normalized to the endogenous control (β-globulin) and expressed relative to the control (calibrator), using the $\Delta\Delta C_T$ method. Mean ± SEM, ** $p < 0.01$, ***$p < 0.001$.

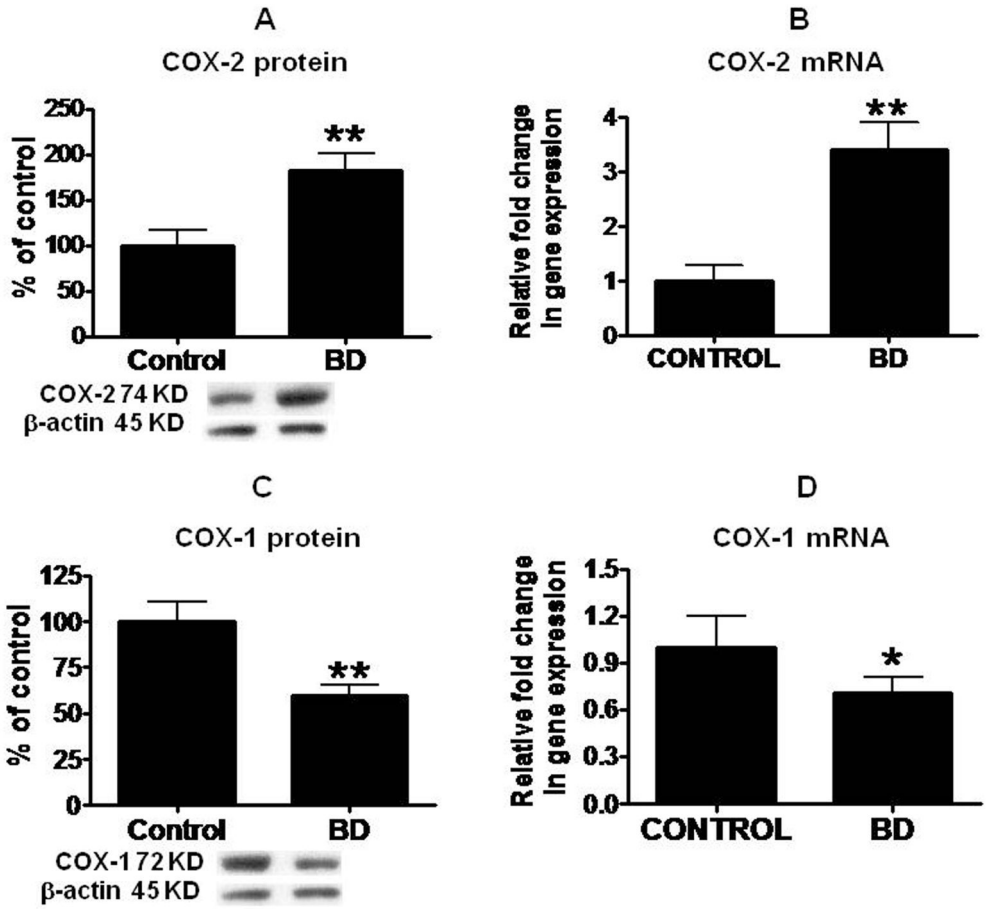

Figure 3. Protein and mRNA levels of COX enzymes

Mean COX-2 (A) and COX-1 (C) protein (with representative immunoblots) as percent of control in frontal cortex, from control (n = 10) and BD (n = 10) subjects. Data are optical densities relative to that of β-actin. COX-2 (B) and COX-1 (D) mRNA levels in the frontal cortex from controls (n = 10) and BD patients (n = 10), measured using RT-PCR. Data are normalized to the endogenous control (β-globulin) and expressed relative to the control (calibrator), using the $\Delta\Delta C_T$ method. Mean ± SEM, * $p < 0.05$, **$p < 0.01$.

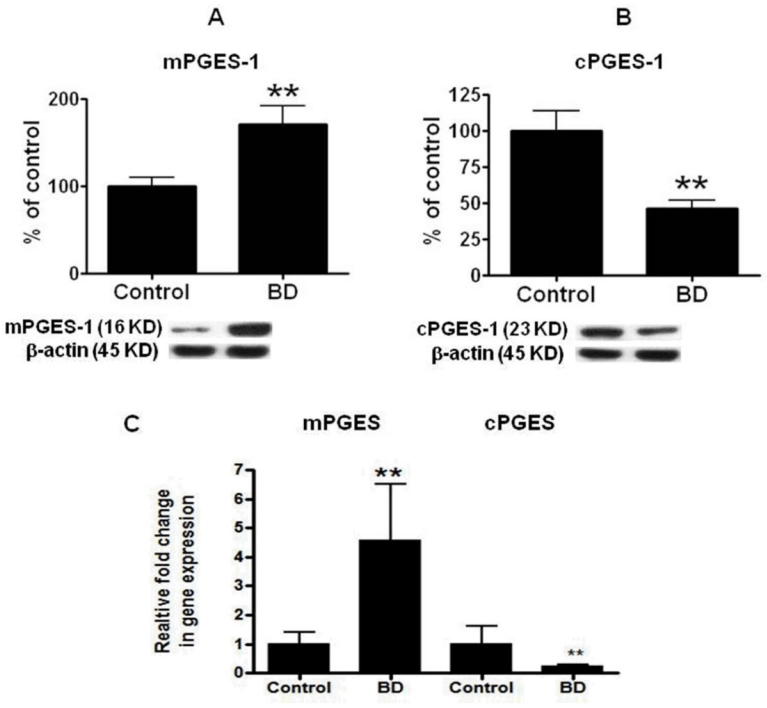

Figure 4. Protein and mRNA levels of PGES enzymes

Mean mPGES-1 (A) and cPGES- 2 (B) protein (with representative immunoblots) in control (n = 10) and BD (n = 10) frontal cortex. Data are optical densities of PGES protein to β-actin, expressed as percent of control. mRNA levels of mPGES-1 and cPGES-2 (C) in postmortem control (n = 10) and BD (n =10) frontal cortex, measured using RT-PCR. Data are levels of PGES in the BD patients normalized to the endogenous control (β-globulin) and relative to control level (calibrator), using the $\Delta\Delta C_T$ method. Mean ± SEM, ** $p < 0.01$.

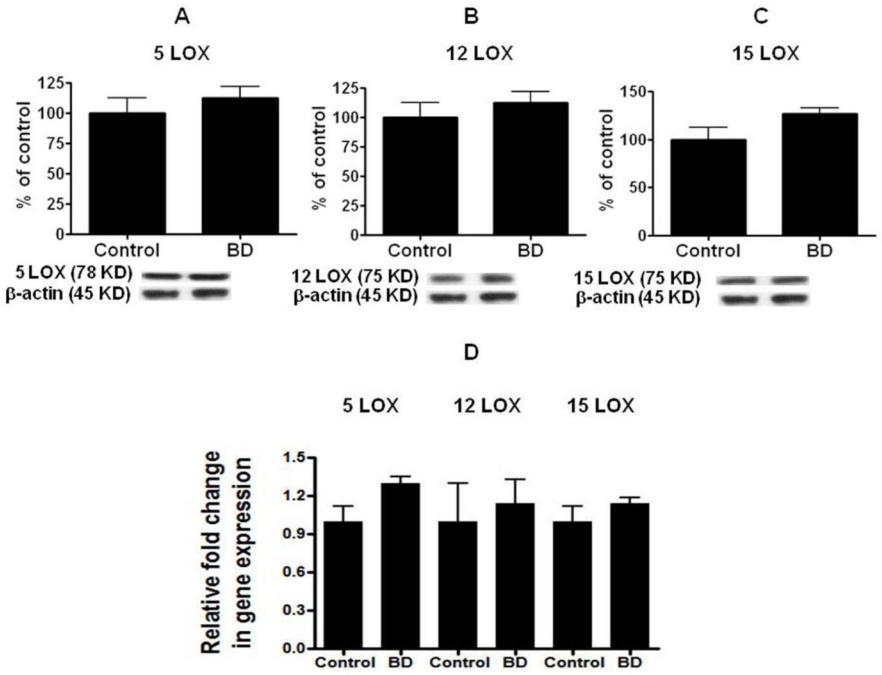

Figure 5. Protein and mRNA levels of lipoxygenases

Mean 5 LOX (A), 12 LOX (B) and 15 LOX (C) protein levels (with representative immunoblots) in frontal cortex from control (n = 10) and BD (n = 10) subjects. Bar graphs are ratios of optical densities of LOXs to that of β-actin, expressed as percent of control. LOX mRNA (D) in postmortem frontal cortex from the control (n = 10) and BD (n = 10) subjects, measured using RT-PCR. Data are levels of LOXs in BD normalized to the endogenous control (β-globulin) and relative to the control (calibrator), using the $\Delta\Delta C_T$ method. Mean ± SEM.

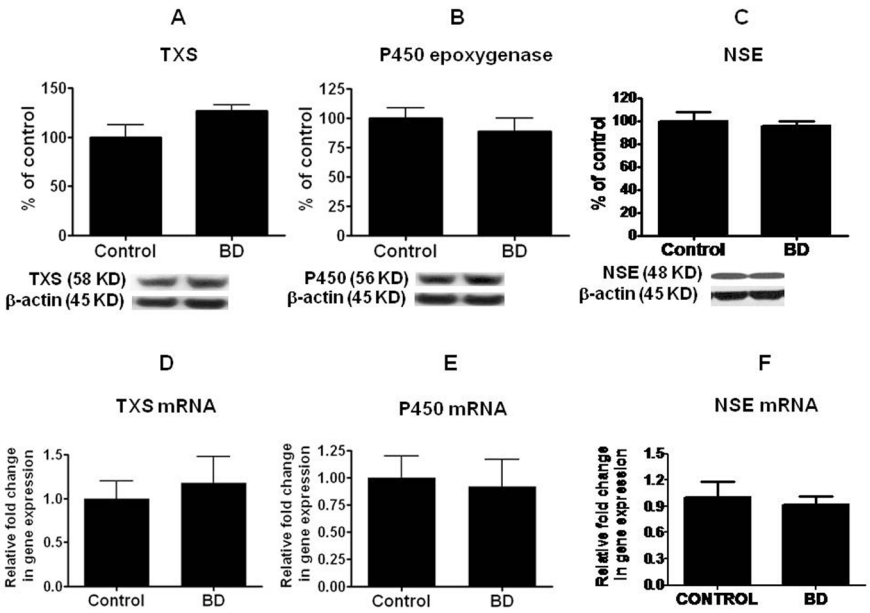

Figure 6. Protein and mRNA levels of thromboxane synthase, P450 epoxygenase and neuron specific enolase

Mean TXS (A), P450 epoxygenase (B) and neuronal specific enolase (NSE) (C) protein in postmortem frontal cortex from control and BD subjects. Bar graph is ratio of optical density of each protein to that of β-actin, expressed as percent of control. TXS (D), P450 epoxygenase (E) and NSE mRNA (F) in postmortem frontal cortex from control (n = 10) and BD (n = 10) subjects, measured using RT-PCR. Data are level in the BD brain normalized to the endogenous control (β-globulin) and relative to control (calibrator), using the $\Delta\Delta C_T$ method. Mean ± SEM.

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Table 1

Characteristics of control and bipolar disorder subjects

Group	Age, (yr)	Sex	PMI, (h)	Cause of death	Medications
Control	32	F	29	Cardiopulmonary attack	Antibiotics
Control	46	M	30	Cardiopulmonary attack	Insulin
Control	54	M	24	Cardiopulmonary attack	Insulin
Control	36	M	21	Electrocution	Vitamins
Control	41	M	30	Cardiopulmonary attack	None
Control	49	M	27	Cardiopulmonary attack	Vitamins
Control	35	M	20	Cardiac arrest	Not available
Control	35	M	26	unknown	Not available
Control	45	M	24	unknown	Not available
Control	25	M	15	Myocardial Infarction	Not available
BD	29	M	20	Suicide	Paxil
BD	74	M	7	Pneumonia	Neurontin
BD	51	F	35	Ischemic heart disease	Ambien
BD	47	F	16	Major system failure	Lithium carbonate
BD	40	M	30	Suicide	Risperidone
BD	75	M	20	Myocardial infarction	Prozac, Avandia
BD	90	F	19	Ventricular tachycardia	Lithium carbonate,
BD	27	M	20	Suicide	Lithium carbonate
BD	25	F	11	Suicide	Not available
BD	35	M	42	Suicide	Lithium

PMI, postmortem interval; F, Female; M, Male

Table 2

Probabilities and Pearson correlation r squared between brain mRNA levels and subject age, postmortem interval and brain pH.

N=20		**cPLA$_2$**	**sPLA$_2$**	**iPLA$_2$**	**COX-1**	**COX-2**	**mPGES**	**cPGES**
Age, (yr)	P	0.45	0.16	0.23	0.15	0.32	0.82	0.81
	r^2	0.03	0.10	0.07	0.10	0.05	0.00	0.00
PMI, (hr)	P	0.62	0.96	0.19	0.69	0.45	0.13	0.31
	r^2	0.01	0.00	0.09	0.00	0.03	0.12	0.05
pH	P	0.46	0.82	0.62	0.11	0.77	0.82	0.53
	r^2	0.04	0.24	0.30	0.37	0.098	0.08	0.04

PMI, postmortem interval