Lithium modifies brain arachidonic and docosahexaenoic metabolism in rat lipopolysaccharide model of neuroinflammation

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Abstract

Neuroinflammation, caused by 6 days of intracerebroventricular infusion of a low dose of lipopolysaccharide (LPS, 0.5 ng/h), stimulates brain arachidonic acid (AA) metabolism in rats, but 6 weeks of lithium pretreatment reduces this effect. To further understand this action of lithium, we measured concentrations of eicosanoids and docosanoids generated from AA and docosahexaenoic acid (DHA), respectively, in high-energy microwaved rat brain, using liquid chromatography-tandem mass spectrometry and two doses of LPS. In rats fed a lithium-free diet, low (0.5 ng/h) or high (250 ng/h) dose LPS compared to artificial cerebrospinal fluid increased brain unesterified AA and prostaglandin E₂ concentrations, and activities of AA-selective cytosolic cPLA₂-IV and secretory sPLA₂. LiCl feeding prevented these increments. Lithium had a significant main effect by increasing brain concentrations of lipoygenase-derived AA metabolites, 5-hydroxyeicosatetraenoic acid (HETE), 5-oxo-eicosatetraenoic acid, and 17-hydroxy-DHA by 1.8-, 4.3- and 1.9-fold compared to control diet. Lithium also increased 15-HETE in high dose LPS-infused rats. Ca²⁺-independent iPLA₂-VI activity, and unesterified DHA and docosapentaenoic acid (22:5n-3) concentrations were unaffected by LPS or lithium. This study demonstrates, for the first time, that lithium can increase brain 17-hydroxy-DHA formation, indicating a new and potentially important therapeutic action of lithium.

Keywords: arachidonic, docosahexaenoic, eicosanoid, docosanoid, phospholipase A₂, lithium, lipopolysaccharide, brain

Running title: Neuroinflammation and lithium
**Abbreviations:** AA, arachidonic acid; aCSF, artificial CSF; COX, cyclooxygenase; CSF, cerebrospinal fluid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; ETE, eicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid; LPS, lipopolysaccharide; LOX, lipoxygenase; PLA₂, phospholipase A₂; cPLA₂, Ca\(^{2+}\)-dependent cytosolic PLA₂; iPLA₂, Ca\(^{2+}\)-independent PLA₂; sPLA₂, Ca\(^{2+}\)-dependent secretory PLA₂; PGE\(_2\), prostaglandin E\(_2\); RP-HPLC/MS/MS, reverse phase-HPLC coupled with mass spectrometry; TNF, tumor necrosis factor; TXB\(_2\), thromboxane B\(_2\)
Introduction

Bipolar disorder also known as manic-depressive illness is a psychiatric condition characterized by drastic mood shifts ranging from severe depression to mania (1). Bipolar disorder represents a major mental illness worldwide, causing devastating medical, social and economic consequences for patients and their families (2). Neuroinflammation is a host defense mechanism associated with neutralization of an insult and restoration of normal structure, and function of brain. Although neuroinflammation serves as a neuroprotective mechanism associated with repair and recovery, it also contributes to brain dysfunction (3). Recently, neuroinflammation has emerged as a key player in many human psychiatric and degenerative diseases, including Alzheimer’s disease, AIDS dementia, and bipolar disorder (4-6). Postmortem frontal cortex from bipolar disorder patients shows increased levels of neuroinflammatory markers such as interleukin-1β and its receptor, glial fibrillary acidic protein, and CD11b, as well as upregulated expression of enzymes that regulate arachidonic acid metabolism (AA, 20:4n-6) (6, 7).

Mediators of neuroinflammation can be bioactive lipids derived from AA and docosahexaenoic acid (DHA, 22:6n-3). During the neuroinflammatory response, phospholipase A₂ (PLA₂) enzymes are activated, resulting in AA release from neuronal membrane glycerophospholipids and generation of lipid mediators, including prostaglandins, leukotrienes and thromboxanes (8). DHA released by PLA₂ from glycerophospholipids can be metabolized to docosanoids, including resolvins, docosatrienes and neuroprotectin. These novel oxygenated products of DHA were identified in resolving inflammatory exudates (9), and similar chemical structures were elucidated in tissues rich in DHA such as the brain (10-12). Hence, the terms resolvin
(resolution phase interaction products) and docosatrienes were introduced because they displayed potent anti-inflammatory and immunoregulatory properties. The enzymatic conversion of DHA to docosanoids has not been fully characterized, but appears to involve an initial conversion of DHA to 17S-hydroxy-DHA (17-OH-DHA) by a 15-lipoxygenase (15-LOX)-like enzyme and further conversion to resolvins D via epoxide intermediates (13). So far, only isolated soybean and potato 15-LOX and porcine 12-LOX have been shown to convert DHA to 17-OH-DHA in vitro (10, 14, 15).

Lithium has been used to treat bipolar disorder for over 50 years, and remains the most common treatment for its manic phase (16, 17). While lithium's mechanism of action is not agreed on, recent animal studies suggest that lithium downregulates the brain AA cascade by decreasing AA turnover within brain phospholipids (18) and the prostaglandin E2 (PGE2) concentration (19). To study effects of lithium on the brain AA and DHA cascades during neuroinflammation, we used an animal model of neuroinflammation. In rats, neuroinflammation can be produced by chronic infusion of bacterial lipopolysaccharide (LPS) into the fourth cerebral ventricle (20). A 6-day infusion of high dose LPS (250 ng/h) increases activated microglia in the thalamus (21). A lower LPS dose (0.5 or 1 ng/h) infused for 6 or 30 days produces behavioral deficits, induces amyloid deposits, and activates microglia and astrocytes (22, 23). We reported that a 6-day infusion of the low dose also increases markers of the brain AA metabolic cascade: activities of cytosolic AA-selective Ca\(^{2+}\)-dependent phospholipase A\(_2\) (cPLA\(_2\)) and secretory sPLA\(_2\), turnover of AA in phospholipids, and concentrations of unesterified AA and its PGE\(_2\) and thromboxane B\(_2\) (TXB\(_2\)) metabolites measured by ELISA or gas-liquid chromatography on high-energy microwaved brain tissue (22, 24). Feeding LiCl to
rats for 6 weeks, so as to produce plasma and brain lithium concentrations therapeutically relevant to bipolar disorder, prevented many of these LPS-induced increments (24). The LPS infusion did not change the brain unesterified DHA concentration (22), DHA turnover in brain phospholipids (Rosenberger T.A. and Rapoport, S. I., unpublished observations), or activity of Ca²⁺-independent iPLA₂, which is selective for DHA (22, 25).

Reverse phase-HPLC coupled with mass spectrometry (RP-HPLC/MS/MS) has emerged as one of the most specific and sensitive approaches used in the analysis of lipid mediators in biological samples (26). This method has been validated for quantifying concentrations of unesterified fatty acids and their metabolites in rodent brains that have been subjected to high-energy head-focused microwaving to stop lipid metabolism and limit postmortem alterations (27, 28). Others and we have demonstrated that such radiation is essential for measuring accurate brain concentrations of unesterified fatty acids, eicosanoids and anandamide (29). Indeed, during global ischemia caused by decapitation, concentrations of unesterified fatty acids are rapidly increased (27, 28, 30).

The goal of this study was to further investigate the interaction between chronic lithium and neuroinflammation, by measuring concentrations of unesterified polyunsaturated fatty acids and some of their metabolites in high-energy microwaved brain of rats fed LiCl chronically, using RP-HPLC/MS/MS as described in our ischemia study (27). We quantified concentrations of unesterified AA, DHA, docosapentaenoic acid (DPA, 22:5n-3), 17-OH-DHA, PGE₂, TXB₂, 5-, 12- and 15- hydroxyeicosatetraenoic acids (HETEs), and 5-oxo-eicosatetraenoic acid (5-oxo-ETE), in brains from rats subjected to 6 days of intracerebroventricular infusion with a high (250 ng/h) or low (0.5
ng/h) dose of LPS. The rats had been fed a control lithium-free or a therapeutically relevant LiCl diet for 36 days prior to LPS infusion (total diet duration 42 days) (24). Whole brain activities of cPLA₂-IV, iPLA₂-VI and sPLA₂, and 15-LOX protein levels, also were measured. Briefly, we confirmed previous observations regarding the effect of lithium on AA and PGE₂ in a model of neuroinflammation with the RP-HPLC/MS/MS technique, and extended the list of analyzed metabolites, including 5-, 12- and 15-HETE, and 17-OH-DHA. We also found that the brain concentration of 17-OH-DHA, the precursor of several anti-inflammatory mediators known as resolvins, was increased in LiCl-fed rats infused with LPS, suggesting a new beneficial mechanism of action of lithium in bipolar disorder as an anti-inflammatory agent.

Materials and methods

Animals

All procedures were performed under a protocol (#06-026) approved by the Animal Care Committee of the National Institute of Child Health and Human Development, in accordance with NIH guidelines on the care and use of laboratory animals. Two-month-old male Fischer F344 rats (Taconic Farms, Rockville, MD) were housed in a facility with a 12/12 light dark cycle. One group of rats was fed ad libitum Purina 5001 chow diet containing 1.70 g LiCl/kg (low LiCl) for 4 weeks, followed by chow containing 2.55 g LiCl/kg (high LiCl) for 2 weeks (Harlan Telkad, Madison, WI) (24). This regimen produces plasma and brain lithium concentrations of about 0.7 mM, therapeutically relevant to bipolar disorder (18, 31). Control rats were fed lithium-free Purina 5001 chow...
diet for 6 weeks. Water and NaCl solution (0.45 M) were available *ad libitum* to both groups.

**Total fatty acid concentrations in control and LiCl diets**

To analyze each diet, total lipids were extracted (32) from 0.7-0.8 g samples. An aliquot of total lipid extract was methylated with 1% H$_2$SO$_4$-methanol for 3 h at 70°C. Fatty acid methyl esters were then separated and quantified by gas-liquid chromatography. Before the sample was methylated, di-17:0 choline glycerophospholipid was added as an internal standard.

**Surgery**

Rats were anesthetized and an indwelling cerebroventricular cannula was fixed in place as previously described (20, 22, 24). Artificial cerebrospinal fluid (aCSF) or LPS (Sigma, Saint Louis, MO; *Escherichia coli*, serotype 055:B5) at a low dose (1 µg/ml at 0.5 ng/h) or a high dose (0.5 mg/ml at 250 ng/h) was infused into the fourth ventricle through the cannula via an osmotic pump (Alzet®, Model 2002, Cupertino, CA). Before surgery, the prefilled pump was placed in sterile 0.9% NaCl at 37°C overnight to start immediate pumping. Post-operative care included triple antibiotic ointment applied to the wound, and 5 ml of sterile 0.9% NaCl (s.c.) to prevent dehydration during recovery. Following 6 days of LPS or aCSF infusion, starting had been on a control or lithium diet for 36 days, rats were anesthetized with Nembutal® (40 mg/kg, i.p.) and subjected to head-focused microwave irradiation (5.5 kW, 3.6 s; Cober Electronics, Stamford CT). Brains were removed and stored at -80°C. In addition, 6 control and 6 lithium diet rats, which did not
undergo surgery, were anesthetized with Nembutal® and subjected to head-focused microwave irradiation.

**Extraction and analysis of lipids**

Brain lipids were extracted with 80% methanol and purified on a C18 column as described previously (27). Right and left microwaved cerebral hemispheres were homogenized separately in 4 ml of 80% methanol and $ds$-5-HETE, $ds$-AA, $ds$-DHA, $d_4$-TXB$_2$, $d_4$-PGE$_2$ (Cayman Chemicals, Ann Arbor, MI) as internal standards. Tissue debris was removed by centrifugation and the supernatant was loaded onto a Strata C18-E cartridge (Phenomenex, Torrance, CA). The eluate was taken to dryness and reconstituted in 70 µl of HPLC solvent A (8.3 mM acetic acid, pH 5.7) + 20 µl of solvent B (acetonitrile-MeOH, 65:35, v/v). A 35-µl aliquot of each sample was injected into a HPLC system and subjected to RP-HPLC, and eluted at a flow rate of 50 µl/min, with a linear gradient from 25% to 100% of mobile phase B. Solvent B was increased from 25% to 85% in 24 min, to 100% in 26 min, and held at 100% for a further 12 min. The HPLC effluent was directly connected to the electrospray source of a triple quadrupole mass spectrometer. Analytes were detected in negative ion mode using multiple reaction monitoring of the specific transitions, $m/z$ 303 → 205 for AA; $m/z$ 327 → 283 for DHA; $m/z$ 329 → 285 for DPA; $m/z$ 369 → 169 for TXB$_2$; $m/z$ 351 → 271 for PGE$_2$; $m/z$ 319 → 115 for 5-HETE; $m/z$ 317 → 113 for 5-oxo-ETE; $m/z$ 319 → 179 for 12-HETE; $m/z$ 319 → 219 for 15-HETE; $m/z$ 343 → 245 for (±)17-OH-DHA; $m/z$ 311 → 267 for $ds$-AA; $m/z$ 332 → 288 for $ds$-DHA; $m/z$ 373 → 173 for $ds$-TXB$_2$; $m/z$ 355 → 275 for $ds$-PGE$_2$; $m/z$ 327 → 116 for $ds$-5-HETE. Quantitation was performed via standard isotope dilution
Brain specific PLA$_2$ activities

Rats were anesthetized with Nembutal® and decapitated. Frozen half-hemispheres were homogenized in 3 volumes of ice-cold buffer containing 10 mM HEPES, pH 7.5, 1 mM EDTA, 0.34 M sucrose and protease inhibitor cocktail tablet (Roche Diagnostics, Mannheim, Germany). The homogenates were centrifuged at 100,000 g for 1 h at 4°C. Supernatants corresponding to the cytosolic fractions were assayed for cPLA$_2$-IV and iPLA$_2$-VI activities using the sensitive and specific method of Yang et al. (33), and for sPLA$_2$ activity using a sPLA$_2$ assay kit (Cayman Chemical, Ann Arbor, MI).

Western blot analysis

Proteins (50 µg) from the cytosolic fractions were separated on 4-20% SDS-PAGE (Bio-Rad, Hercules, CA), blotted onto a polyvinylidene difluoride membrane (Bio-Rad), and then immunoblotted with the goat anti-15-LOX-2 polyclonal antibody (1:1000) (Santa Cruz, Santa Cruz, CA). Blotted proteins were quantified using Alpha Innotech Software (Alpha Innotech, San Leandro, CA) and were normalized to β-actin (Sigma).

Statistical analysis

A two-way ANOVA, comparing diet (LiCl vs. control) with infusion (LPS vs. aCSF) was performed for body weight loss, brain lipids and PLA$_2$ activities using SPSS 16.0. When LiCl x LPS interactions were statistically insignificant, probabilities of main effects of LiCl and LPS were reported. When interactions were statistically significant, these
probabilities were not reported because they cannot be interpreted (34). A one-way ANOVA with Bonferroni’s post-hoc test with correction for 5 comparisons (effect of low and high LPS in control and LiCl fed rats, and aCSF effect in LiCl compared with control diet rats) was performed. Data are reported as means (left and right) ± SD with statistical significance taken as \( p \leq 0.05 \).

Results

Fatty acid composition of diets

(Table 1)

The fatty acid concentrations (\( \mu \text{mol/g diet} \)) in the three diets are shown in Table 1. There was no significant difference between the three diets in fatty acid composition. The analysis showed that the 5001 diet contained (as percent of total fatty acids): 25.2% saturated, 33.3% monounsaturated, 35.1% linoleic, 3.1% \( \alpha \)-linolenic, 0.39% AA, 1.25% eicosapentaenoic and 1.62% docosahexaenoic acid.

Effect of cannula implantation

Initial experiments investigated the effects, if any, of implanting the cannula and infusing aCSF on brain concentrations of unesterified fatty acids, eicosanoids and 17-OH-DHA. Except for PGE\(_2\), the concentration of none of these substances was altered by the cannula implant plus aCSF infusion (data not shown). A very low concentration of PGE\(_2\) (at the limit of detection) was detected in 1 of 4 brains of control diet and in 1 of 4 brains of lithium diet rats infused with aCSF. These findings show a slight occasional effect of cannula implantation, likely due to minimal neuroinflammation around the cannula track (35), whereas in a prior study PGE\(_2\) could not be detected in control microwaved rat brain.
in the absence of a cannula (27). Low dose LPS- or aCSF-infused rats with indwelling catheters appeared behaviorally normal after 24 h, whereas high dose LPS-infused rats were lethargic and docile throughout the 6-day infusion period.

Weight and other effects
A two-way ANOVA showed a significant main effect of LPS infusion (p < 0.0001) but no significant main effect of diet (p = 0.67) or diet x LPS interaction (p = 0.32) with regard to body weight (data not shown). A Bonferroni post-hoc test indicated that high dose LPS significantly decreased body weight in both groups by 20% (p < 0.001) whereas low dose LPS had a significant effect (7% reduction) only in the control diet rats.

Unesterified fatty acids
Brain concentrations of unesterified AA, DHA and DPA are summarized in Table 2. A two-way ANOVA showed a significant diet x LPS interaction for the AA concentration (p < 0.001). Subsequent one-way ANOVAs with Bonferroni post-hoc tests showed that both the low and high doses of LPS compared with aCSF significantly increased brain AA by 31% and 38%, respectively. The LiCl diet prevented the significant increments with both LPS doses. LiCl did not significantly alter the baseline AA concentration (after aCSF infusion). Neither LiCl nor LPS infusion modified DHA or DPA concentrations significantly.

Eicosanoids
A low concentration of PGE$_2$ at the limit of detection was detected in 1 of 4 brains from control and lithium diet rats infused with aCSF. Higher concentrations were found in control diet rats infused with LPS (Table 2). A two-way ANOVA showed a significant diet x LPS interaction for the PGE$_2$ concentration (p < 0.001). Subsequent one-way ANOVAs with Bonferroni post-hoc tests showed that high dose LPS significantly increased brain PGE$_2$ by 18.5-fold, and that the LiCl diet prevented this increase (Table 2). The TXB$_2$ concentration is not reported since it was below the limit of detection in each sample.

Treatment effects on concentrations of 5-HETE, 5-oxo-ETE, 12-HETE and 15-HETE also are summarized in Table 2. A two-way ANOVA showed significant main effects of LiCl on 5-HETE (p = 0.001) and 5-oxo-ETE (p < 0.001), and a significant diet x LPS interaction for 15-HETE (p = 0.02). LiCl increased significantly 5-HETE (mean = 21.70) by 1.8-fold compared to control diet (mean = 12.10), p = 0.0006. LiCl increased significantly 5-oxo-ETE (mean = 7.64) by 4.3-fold compared to control diet (mean = 2.36), p<0.0001. A one-way ANOVA with Bonferroni post-hoc tests showed that LiCl increased 15-HETE in high dose LPS-infused rats but has no significant effect at baseline. Neither the high nor low dose LPS had a significant main effect on any of these concentrations.

**17-OH-DHA**

LC/MS/MS analysis revealed that 17-OH-DHA, monitored by transition m/z 343→245, was present in the brain of control diet rats, and that its concentration was increased by the LiCl diet (Figure 1). A two-way ANOVA showed that the LiCl diet had a significant
main effect (p = 0.001), in increasing the concentration of 17-OH-DHA by 1.9-fold (LiCl mean = 0.67 vs. control diet mean = 0.36, p = 0.002) (Table 2). The interaction between LiCl and LPS was insignificant, and LPS had no main effect.

**PLA₂ activities and 15-LOX protein**

A two-way ANOVA on whole brain cPLA₂-IV and sPLA₂ specific activities showed significant diet x LPS interactions, at p = 0.0002 and p < 0.0001, respectively (Table 3). Subsequent one-way ANOVAs with Bonferroni post-hoc tests showed that both doses of LPS compared with aCSF significantly increased brain cPLA₂-IV activity by 36% and 148%, respectively, and brain sPLA₂ activity by 41% and 80%, respectively. The LiCl diet prevented the significant increment of cPLA₂-IV activity following low but not high dose LPS, as well as the significant increments in sPLA₂ activity caused by low and high dose LPS. Neither the LiCl diet nor LPS infusion significantly affected whole brain iPLA₂-V activity.

Brain cytosolic 15-LOX protein levels were not significantly altered by LPS infusion in LiCl-treated rats (n = 4, p > 0.05) (data not shown).

**Discussion**

The major new finding of our study is that LiCl increased 17-OH-DHA formation in rat LPS models of neuroinflammation. 17-OH-DHA has been reported to have anti-inflammatory actions. For example, 17-OH-DHA inhibited tumor necrosis factor-α (TNF-α)-induced interleukin-1β gene expression in human microglial cells (10), human neutrophil 5-LOX (36), and TNF-α release and 5-LOX protein expression in murine
macrophages (37). 17-OH-DHA also is an agonist of the transcription factor, peroxisome proliferator-activated receptor γ, which is believed to act in an anti-inflammatory manner (38).

The mechanism underlying the 17-OH-DHA elevation is uncertain. Since LiCl did not increase its precursor unesterified DHA concentration nor iPLA$_2$-VI activity in whole brain, consistent with prior data (19, 31), the increment may have arisen from enhanced 15-LOX activity. On the other hand, unesterified DHA likely is partitioned in different brain compartments (it is found in neurons and glia (39, 40)), as reported for unesterified AA (22), one of which may be the precursor to 17-OH-DHA. Increased 15-LOX activity is suggested by the increased 15-HETE in the rats fed the LiCl diet during high LPS exposure, since 15-HETE is generated from AA by the action of 15-LOX. Although whole brain cytosolic 15-LOX protein level was not significantly increased in the LPS-infused rats fed LiCl, we cannot rule out post-translational upregulation of 15-LOX activity, which has been reported (41). Increasing the number of animals in future experiments, and measuring membrane 15-LOX protein and activity might be helpful. Whether 15-LOX or other yet to be identified enzymes are involved in 17-OH-DHA formation following lithium remains to be elucidated.

The LiCl diet increased brain 5-HETE and 5-oxo-ETE without affecting 12-HETE, whereas neither low nor high dose LPS affected these metabolites. One possible explanation for this observation is that lithium affects AA remodeling within phospholipids by reducing AA-CoA formation (31) or lysophospholipid acyl CoA transferase activity, making more unesterified AA available to the LOX pathways. Similarly, aspirin, ibuprofen, indomethacin, and valproate, which inhibit COX activity
like lithium, have been reported to increase brain HETE concentrations (19, 42-45).

In this study, high dose LPS infusion also increased brain AA and PGE$_2$ concentrations and cPLA$_2$-IV and sPLA$_2$ activities, without changing the brain DHA concentration or iPLA$_2$-VI activity, consistent with evidence that iPLA$_2$-VI is selective for DHA hydrolysis from phospholipid (25). Although the high dose LPS significantly increased both cPLA$_2$-IV and sPLA$_2$ activities more than did the low dose, we did not observe a dose-dependent response to LPS in the brain unesterified AA concentration. These data suggest that AA, released by cPLA$_2$ and sPLA$_2$ during high dose LPS infusion, was converted rapidly to eicosanoids and/or reincorporated into brain phospholipids (46, 47). Pretreatment with the LiCl diet prevented only the effect on sPLA$_2$ activity.

The results from this study are consistent with our ischemia study and other reports showing that concentrations of unesterified AA, 17-OH-DHA, 5- and 12- HETEs, and 5-oxo-HETE, measured by RP-HPLC/MS/MS, are much lower in high-energy microwaved than non-microwaved brain (27, 28, 30). PGE$_2$ formation was detected only in 1 of 4 samples in a very low amount in brain from control diet rats infused with aCSF. In our previous study using another rodent diet we could not detect PGE$_2$ in control microwaved brain (without a cannula) (27). Additionally, we showed that intracerebrally-injected $d_4$-PGE$_2$ was not degraded substantially by the microwaving procedure (27). These observations indicate that little endogenous PGE$_2$ is produced in the absence of a brain insult, and that the PGE$_2$ that we could detect in the two brains in this study likely was associated with cannula-related damage (35). In contrast to our earlier report regarding ischemia (27), we did not detect E$_2$/D$_2$ isoprostanes in any sample. TXB$_2$ also
was reported to be at the limit of detection in microwaved brain (27), as was the case in the present study.

This study showing that low dose LPS compared with aCSF infusion in control diet rats significantly increased brain concentrations of AA and PGE$_2$ but not of DHA, as well as whole brain cPLA$_2$-IV activity compared, and that lithium attenuated these changes, confirms our prior data obtained with different methods (22, 24). In this study, we confirmed an increased brain sPLA$_2$ by LPS infusion (22). Dampening by lithium of elevated AA concentrations caused by low or high dose LPS is consistent with lithium also dampening the LPS-induced increases in cPLA$_2$ and sPLA$_2$ activities. LiCl did not significantly alter the baseline brain unesterified AA concentration, consistent with lithium not changing baseline cPLA$_2$-IV and sPLA$_2$ activities. The absence of a LiCl effect on sPLA$_2$ agrees with a previous report (48), whereas cPLA$_2$-IV mRNA and protein were downregulated by LiCl in another study (49). Intravenous or intraperitoneal LPS in rodents has been reported to increase brain sPLA$_2$-IIA and sPLA$_2$-IIE mRNA, respectively (50, 51). These data suggest that lithium acts differently in a “normal” unstressed brain compared with an “inflammatory” brain. Lithium might modulate cPLA$_2$-IV and sPLA$_2$ upregulation in response to LPS by decreasing the intracellular Ca$^{2+}$ released by glutamate acting at N-methyl-D-aspartic acid receptors (Ca$^{2+}$ mediates translocation or phosphorylation of cPLA$_2$) or by reducing the level of phosphatidylinositol 4,5-bisphosphate, which anchors cPLA$_2$ to perinuclear and nuclear membranes (52).

This study also investigated possible effects of cannula implantation followed by a 6-day aCSF infusion. Except for a change in PGE$_2$, the procedure did not affect any
measurement, consistent with the reported little or absence of an inflammatory reaction under the experimental conditions (35). Body weight was reduced significantly by LPS infusion, more so by the high than the low dose. Weight loss has been noted with high dose intracerebroventricular LPS (20, 21), and with peripheral LPS injection (53). Its exact cause is not agreed on, but pro-inflammatory cytokines TNF-α, interleukin-1β and interleukin-6 have been suggested to play a role in weight loss (53). Peripheral LPS produces sleepiness and inactivity (54), both of which were more evident in the high dose LPS-infused rats.

In summary (Figure 2), finding that LiCl prefeeding upregulated the brain concentration of 17-OH-DHA in the presence of LPS-induced neuroinflammation provides a new possible mechanism for lithium's reported neuroprotective action (55), in addition to downregulating the AA cascade (24, 56). Supporting such a mechanism is epidemiological evidence that aspirin, which can increase 17(R)-OH-DHA by acetylating COX-2 (9, 57), when given chronically reduced untoward effects in (presumably) bipolar disorder patients on lithium therapy (58). Neuroinflammation also has been associated with an upregulated AA cascade in bipolar disorder (6, 7). Lithium’s ability to suppress this cascade while stimulating 17-OH-DHA formation may contribute to its efficacy in bipolar disorder and other neuroinflammatory diseases (4, 5). Efficacy of lithium treatment in HIV-1 dementia (59), amyotrophic lateral sclerosis (60) and Alzheimer’s disease (61) has been noted in recent limited clinical trials.

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Figure Legends

Figure 1
17-OH-DHA levels in high LPS-infused brains of rats subjected to control (A) and LiCl (B) diets analyzed by LC/MS/MS.

Figure 2
LPS infusion increases brain concentration of unesterified AA via cPLA₂ and sPLA₂ and PGE₂ via COX without altering DHA release via iPLA₂, and LiCl blocks these increases. In addition LiCl increases levels of 15-HETE, 17-OH-DHA, 5-HETE and 5-oxo-ETE in the brain of rats subjected to neuroinflammation. AA, arachidonic acid; COX, cyclooxygenase; DHA, docosahexaenoic acid; ETE, eicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid; LPS, lipopolysaccharide; LOX, lipoxygenase; PLA₂, phospholipase A₂; cPLA₂, cytosolic PLA₂; iPLA₂, Ca²⁺-independent PLA₂; sPLA₂, secretory PLA₂; PGE₂, prostaglandin E₂.
A) Control Diet

17-OH DHA
m/z 343→245

B) Lithium Diet

17-OH DHA
m/z 343→245

Figure 1
Figure 2
<table>
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<tr>
<th>Fatty Acid</th>
<th>Control</th>
<th>Low LiCl (μmol/g diet)</th>
<th>High LiCl</th>
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<tr>
<td>14:0</td>
<td>1.94 ± 0.24</td>
<td>1.80 ± 0.09</td>
<td>1.85 ± 0.17</td>
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<td>0.05 ± 0.00</td>
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<td>20.54 ± 11.6</td>
<td>26.28 ± 1.26</td>
<td>26.71 ± 2.18</td>
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<tr>
<td>16:1n-9</td>
<td>3.05 ± 0.52</td>
<td>3.00 ± 0.16</td>
<td>2.83 ± 0.10</td>
</tr>
<tr>
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<td>11.86 ± 1.97</td>
<td>11.74 ± 0.91</td>
<td>11.72 ± 0.50</td>
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<tr>
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<td>38.57 ± 2.93</td>
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<td>18:3n-3</td>
<td>4.07 ± 0.46</td>
<td>4.31 ± 1.03</td>
<td>3.88 ± 0.31</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>0.28 ± 0.03</td>
<td>0.27 ± 0.01</td>
<td>0.26 ± 0.02</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>0.52 ± 0.05</td>
<td>0.50 ± 0.03</td>
<td>0.49 ± 0.03</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>1.65 ± 0.17</td>
<td>1.55 ± 0.12</td>
<td>1.56 ± 0.12</td>
</tr>
<tr>
<td>22:4n-6</td>
<td>0.29 ± 0.03</td>
<td>0.29 ± 0.07</td>
<td>0.27 ± 0.05</td>
</tr>
<tr>
<td>22:5n-6</td>
<td>0.11 ± 0.02</td>
<td>0.13 ± 0.02</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>0.31 ± 0.03</td>
<td>0.33 ± 0.04</td>
<td>0.33 ± 0.04</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>2.15 ± 0.25</td>
<td>2.07 ± 0.14</td>
<td>2.09 ± 0.14</td>
</tr>
<tr>
<td>Total</td>
<td>132.16 ± 4.44</td>
<td>133.10 ± 6.81</td>
<td>134.17 ± 9.85</td>
</tr>
<tr>
<td>Total n-6</td>
<td>47.66 ± 5.55</td>
<td>45.24 ± 2.62</td>
<td>45.98 ± 3.49</td>
</tr>
<tr>
<td>Total n-3</td>
<td>8.18 ± 0.89</td>
<td>8.26 ± 1.08</td>
<td>7.85 ± 0.60</td>
</tr>
<tr>
<td>Total saturated</td>
<td>34.34 ± 9.84</td>
<td>39.83 ± 1.29</td>
<td>40.28 ± 2.05</td>
</tr>
<tr>
<td>Total monounsaturated</td>
<td>44.00 ± 4.62</td>
<td>41.62 ± 3.09</td>
<td>41.95 ± 3.99</td>
</tr>
</tbody>
</table>

Data are mean ± SD (n=4).
Table 2 Effects of 6-day LPS infusion and 6-week LiCl diet on concentrations of unesterified fatty acids, HETEs and 17-OH-DHA in rat brains

<table>
<thead>
<tr>
<th></th>
<th>Control diet</th>
<th>LiCl diet</th>
<th>LiCl x LPS interaction</th>
<th>p-value</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aCSF</td>
<td>low LPS</td>
<td>high LPS</td>
<td>aCSF</td>
<td>low LPS</td>
<td>High LPS</td>
</tr>
<tr>
<td>AA</td>
<td>3.99 ± 0.42</td>
<td>5.23 ± 0.59*</td>
<td>5.51 ± 0.86***</td>
<td>5.08 ± 0.67</td>
<td>3.60 ± 0.48*</td>
<td>4.16 ± 0.67</td>
</tr>
<tr>
<td>DHA</td>
<td>12.26 ± 3.90</td>
<td>13.39 ± 1.86</td>
<td>14.71 ± 1.88</td>
<td>11.36 ± 5.02</td>
<td>11.34 ± 2.83</td>
<td>12.39 ± 1.83</td>
</tr>
<tr>
<td>DPA</td>
<td>0.87 ± 0.30</td>
<td>0.91 ± 0.08</td>
<td>0.96 ± 0.13</td>
<td>1.16 ± 0.62</td>
<td>0.96 ± 0.67</td>
<td>0.89 ± 0.06</td>
</tr>
<tr>
<td>PGE₂</td>
<td>0.13 ± 0.23</td>
<td>0.86 ± 0.32</td>
<td>2.41 ± 1.08***</td>
<td>0.19 ± 0.19</td>
<td>0.31 ± 0.19</td>
<td>0.25 ± 0.23</td>
</tr>
<tr>
<td>5-HETE</td>
<td>12.18 ± 4.80</td>
<td>12.25 ± 2.90</td>
<td>11.87 ± 6.83</td>
<td>23.62 ± 10.22</td>
<td>18.10 ± 9.25</td>
<td>24.20 ± 8.89</td>
</tr>
<tr>
<td>5-oxo-ETE</td>
<td>3.06 ± 1.07</td>
<td>2.04 ± 1.63</td>
<td>1.98 ± 1.81</td>
<td>8.29 ± 3.60</td>
<td>4.67 ± 2.07</td>
<td>9.95 ± 6.67</td>
</tr>
<tr>
<td>12-HETE</td>
<td>9.18 ± 5.72</td>
<td>11.52 ± 7.40</td>
<td>8.58 ± 5.55</td>
<td>6.90 ± 2.00</td>
<td>7.14 ± 3.19</td>
<td>12.13 ± 5.62</td>
</tr>
<tr>
<td>15-HETE</td>
<td>11.87 ± 7.19</td>
<td>9.49 ± 2.67</td>
<td>7.47 ± 5.26</td>
<td>9.97 ± 4.59</td>
<td>11.82 ± 4.39</td>
<td>18.54 ± 6.73*</td>
</tr>
<tr>
<td>17-OH DHA</td>
<td>0.41 ± 0.21</td>
<td>0.33 ± 0.06</td>
<td>0.33 ± 0.22</td>
<td>0.55 ± 0.28</td>
<td>0.61 ± 0.21</td>
<td>0.89 ± 0.38</td>
</tr>
</tbody>
</table>

Each value is a mean ± SD (n=5-6) except for PGE₂ (n=4). Fatty acids are expressed in nmol/g brain, and eicosanoids and 17-OH-DHA in pmol/g brain.

When LiCl x LPS interaction were significant, a one-way ANOVA with Bonferroni’s post-test with correction for 5 comparisons was performed. *p < 0.05, ***p < 0.001.
Table 3  Effects of 6-day LPS infusion and 6-week feeding LiCl on brain PLA<sub>2</sub> activities

<table>
<thead>
<tr>
<th></th>
<th>Control diet</th>
<th>LiCl diet</th>
<th>LiCl x LPS</th>
<th>LiCl</th>
<th>LPS interaction</th>
<th>effect</th>
<th>effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aCSF</td>
<td>low LPS</td>
<td>high LPS</td>
<td>aCSF</td>
<td>low LPS</td>
<td>High LPS</td>
<td>p-value</td>
</tr>
<tr>
<td>cPLA&lt;sub&gt;2&lt;/sub&gt;-IV</td>
<td>4.03 ± 0.42</td>
<td>5.47 ± 0.04*</td>
<td>9.99 ± 1.60***</td>
<td>3.64 ± 0.39</td>
<td>3.99 ± 0.18</td>
<td>5.99 ± 0.06***</td>
<td>0.0002</td>
</tr>
<tr>
<td>sPLA&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1110 ± 186</td>
<td>1565 ± 133*</td>
<td>1994 ± 307***</td>
<td>1368 ± 135</td>
<td>1016 ± 199</td>
<td>979 ± 167</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>iPLA&lt;sub&gt;2&lt;/sub&gt;-VI</td>
<td>17.58 ± 2.89</td>
<td>18.87 ± 0.20</td>
<td>19.85 ± 1.54</td>
<td>20.84 ± 4.45</td>
<td>20.49 ± 3.69</td>
<td>19.83 ± 1.40</td>
<td>0.569</td>
</tr>
</tbody>
</table>

Each value is a mean ± SD (n = 4). Specific PLA<sub>2</sub> activities are expressed in pmol/mg protein/min. Data were compared using two-way ANOVA. When LiCl x LPS interaction were significant, a one-way ANOVA with Bonferroni’s post-test with correction for 5 comparisons was performed. *p < 0.05 and ***p < 0.001.