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 docosahexaenoic acid (DHA) in brain tissues from lithium-pretreated and 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.—Basselin, M., H-W. Kim, M. Chen, K. Ma, S. I. Rapoport, R. C. Murphy, and S. E. Farias. Lithium modifies brain arachidonic and docosahexaenoic metabolism in rat lipopolysaccharide model of neuroinflammation. J. Lipid Res. 2010. 51: 1049–1056.

Supplementary key words  eicosanoid • docosanoid • phospholipase A2

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Bipolar disorder, also known as manic-depressive illness, is 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 repair and recovery, it also contributes to neurodegeneration (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 mediators of neuroinflammation that could potentially exacerbate neuroinflammation or cause further brain damage. These mediators include the eicosanoids that are produced by different COX and lipoxygenase enzymes that regulate arachidonic acid (AA; 20:4n-6) metabolism (6, 7).

Abbreviations: AA, arachidonic acid; CSF, cerebrospinal fluid; COX, cyclooxygenase; aCSF, artificial cerebrospinal fluid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; ETE, eicosatetraenoic acid; HETE, hydroxyicosatetraenoic acid; LOX, lipoxygenase; LPS, lipopolysaccharide; 17-OH-DHA, 17-hydroxy-DHA; 5-oxo-ETE, 5-oxo-eicosatetraenoic acid; PLA2, phospholipase A2; cPLA2, Ca2+-dependent cytosolic PLA2; iPLA2, Ca2+-independent PLA2; sPLA2, Ca2+-dependent secretory PLA2; PGE2, prostaglandin E2; RP, reverse phase; TNF, tumor necrosis factor; TXB2, thromboxane B2.

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similar chemical structures were elucidated in tissues rich in DHA such as the brain (10–12). However, the terms resolvin (resolution phase interaction product) and docosatriene 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 17-hydroxy-DHA (17-OH-DHA) by a 15-lipoxygenase (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). In addition, the oxygenation of DHA to 17-OH-DHA can be mediated by nonenzymatic autoxidation (16).

Lithium has been used to treat bipolar disorder for over 50 years and remains the most common treatment for its manic phase (17, 18). 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 (19) and the prostaglandin E₂ (PGE₂) concentration (20). To study the 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 (21). A 6 day infusion of high-dose LPS (250 ng/h) increases activated microglia in the thalamus (22). A lower dose of LPS (100 ng/h) infused for 6 or 30 days produced amyloid deposition (23, 24). We reported that lithium downregulates brain activities of cytosolic AA-selective Ca²⁺-dependent PLA₂ (cPLA₂) and secretory PLA₂ (sPLA₂), turnover of AA in phospholipids, and concentrations of unesterified AA and PGE₂ and thromboxane B₂ (TXB₂) metabolites measured by ELISA or gas-liquid chromatography on high-energy microwaved brain tissue (23, 25). Feeding LiCl/kg (high LiCl) for 2 weeks (Harlan Telkaid, Madison, WI) or a therapeutically relevant LiCl diet for 36 days prior to LPS infusion did not change the brain unesterified DHA concentrations therapeutically relevant to bipolar disorder prevented many of these LPS-induced increments (25). The LPS infusion did not change the brain unesterified DHA concentration (23), DHA turnover in brain phospholipids (26), or activity of Ca₂⁺-independent PLA₂ (iPLA₂), which is selective for DHA (23, 27).

Reverse phase (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 (28). 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 (29, 30). Others and we have demonstrated that such radiation is essential for measuring accurate brain concentrations of unesterified fatty acids, eicosanoids, and anandamide (31). Indeed, during global ischemia caused by decapitation, concentrations of unesterified fatty acids are rapidly increased (29, 30, 32).

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 (29). 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) (25). Whole brain activities of cPLA₂-IV, iPLA₂-VI, sPLA₂, and 15-LOX-2 protein levels were measured. Briefly, we confirmed previous observations regarding the effect of lithium on AA and PGE₂ in a model of neuroinflammation with the LPS, suggesting a new beneficial mechanism of action of lithium in bipolar disorder as an antiinflammatory agent.

Total fatty acid concentrations in control and LiCl diets

To analyze each diet, total lipids were extracted (34) from random 0.7–0.8 g samples (n = 4). An aliquot of total lipid extract was methylated with 1% H₂SO₄-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 (21, 23, 25). Artificial cerebrospinal fluid (aCSF) or LPS (Sigma, Saint Louis,
Extraction and analysis of lipids

Brain lipids were extracted with 80% methanol and purified on a C18 column as described previously (29). Right and left microsomal cerebral hemispheres were homogenized separately in 4 mL of 80% methanol and d<sub>5</sub>-HETE, d<sub>5</sub>-AA, d<sub>5</sub>-TXB<sub>2</sub>, d<sub>5</sub>-PGE<sub>2</sub> (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 head-focused microwave irradiation (5.5 kW, 3.6 s; Cober Electronics, Stamford, CT). Brains were removed and stored at −80°C. In addition, six control and six lithium diet rats, which did not undergo surgery, were anesthetized with Nembutal<sup>®</sup> and subjected to head-focused microwave irradiation.

Brain-specific PLA<sub>2</sub> activities

Rats were anesthetized with Nembutal<sup>®</sup> and decapitated. Frozen half-hemispheres were homogenized in 3 vols 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<sub>2</sub>-IV and iPLA<sub>2</sub>-VI activities using the sensitive and specific method of Yang et al. (35) and for sPLA<sub>2</sub> activity using a sPLA<sub>2</sub> assay kit (Cayman Chemicals, 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<sub>2</sub> activities using SPSS 16.0. When LiCl × 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 clearly (36). A one-way ANOVA with Bonferroni’s posthoc test with correction for five 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 set as P < 0.05.

### RESULTS

#### Fatty acid composition of diets

The fatty acid concentrations (µmol/g diet) in the three diets are shown in Table 1. There was no significant difference among the three diets. The 5001 diet contained (as percent of total fatty acids): 25.2% saturated, 33.3% monounsaturated, 35.1% linoleic, 3.1% α-linolenic, 0.39% AA, 1.25% eicosapentaenoic acid, and 1.69% DHA, 0.06% EPA.

| Fatty Acid   | Control  | Low LiCl (µmol/g diet) | High LiCl (µmol/g diet) |
|-------------|----------|------------------------|-------------------------|
| 14:0        | 1.94 ± 0.24 | 1.80 ± 0.09 | 1.85 ± 0.17 |
| 14:1-9      | 0.06 ± 0.01 | 0.05 ± 0.00 | 0.05 ± 0.00 |
| 16:0        | 20.54 ± 11.6 | 26.28 ± 1.26 | 26.71 ± 2.18 |
| 16:1-9      | 3.05 ± 0.52 | 3.00 ± 0.16 | 2.83 ± 0.10 |
| 18:0        | 11.86 ± 1.97 | 11.74 ± 0.91 | 11.72 ± 0.50 |
| 18:1-9      | 4.08 ± 4.15 | 38.57 ± 2.93 | 39.08 ± 4.02 |
| 20:3n-6     | 46.45 ± 5.44 | 44.06 ± 2.53 | 44.84 ± 3.46 |
| 22:5n-6     | 4.07 ± 0.46 | 4.31 ± 1.03 | 3.88 ± 0.31 |
| 22:5n-3     | 0.28 ± 0.03 | 0.27 ± 0.01 | 0.26 ± 0.02 |
| 22:4n-6     | 0.52 ± 0.05 | 0.50 ± 0.03 | 0.49 ± 0.03 |
| 22:5n-3     | 1.65 ± 0.17 | 1.53 ± 0.12 | 1.56 ± 0.12 |
| 22:4n-6     | 0.29 ± 0.03 | 0.29 ± 0.07 | 0.27 ± 0.05 |
| 22:5n-6     | 0.11 ± 0.02 | 0.13 ± 0.02 | 0.12 ± 0.02 |
| 22:6n-3     | 0.31 ± 0.03 | 0.33 ± 0.04 | 0.33 ± 0.04 |
| 22:6n-3     | 2.15 ± 0.25 | 2.07 ± 0.14 | 2.09 ± 0.14 |

Total fatty acids: 132.16 ± 4.44, 133.10 ± 6.81, 134.17 ± 9.85.

Data are mean ± SD, n = 4.
a slight occasional effect of cannula implantation, likely due to minimal neuroinflammation around the cannula track (37). In a prior study, PGE$_2$ could not be detected in control microwaved rat brain in the absence of a cannula (29). 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 $\times$ LPS interaction ($P = 0.32$) with regard to body weight (data not shown). A Bonferroni posthoc 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 $\times$ LPS interaction for the AA concentration ($P < 0.001$). Subsequent one-way ANOVAs with Bonferroni posthoc 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 alter baseline AA concentration ($P = 0.70$) only in the control diet rats.

Eicosanoids

A low concentration of PGE$_2$ at the limit of detection was monitored by transition monitoring in the brain of control diet rats infused with aCSF. Higher concentrations were found in control diet rats infused with LPS (Table 2). The interaction between LiCl and LPS was significant ($P = 0.0001$) but no significant main effect of diet ($P = 0.67$) or diet $\times$ LPS interaction ($P = 0.32$) were found in control diet rats infused with aCSF. Neither LiCl nor LPS treatment significantly altered AA concentration (data not shown).

A low concentration of 5-oxo-ETE was present in the brain of control diet rats infused with aCSF. LiCl increased significantly 5-oxo-ETE ($P = 0.0001$) in control diet rats compared with aCSF (Table 2). The TXB$_2$ concentration is not reported, because it was below the limit of detection in each sample.

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 brain

|                | Control Diet | LiCl Diet | LiCl $\times$ LPS Interaction |
|----------------|--------------|-----------|-------------------------------|
|                | aCSF | Low LPS | High LPS | aCSF | Low LPS | High LPS | P                     |
| AA             | 3.99 ± 0.42 | 5.23 ± 0.59* | 5.51 ± 0.86*** | 5.08 ± 0.67 | 3.60 ± 0.48* | 4.16 ± 0.67 | <0.001 |
| DHA            | 12.26 ± 3.90 | 13.39 ± 1.86 | 14.7 ± 1.88 | 11.36 ± 5.02 | 11.34 ± 2.83 | 12.39 ± 1.83 | 0.846 0.109 0.403 |
| DPA            | 0.87 ± 0.30 | 0.91 ± 0.08 | 0.96 ± 0.13 | 1.16 ± 0.62 | 0.96 ± 0.67 | 0.89 ± 0.06 | 0.574 0.518 0.847 |
| PGE$_2$        | 0.13 ± 0.23 | 0.86 ± 0.32 | 2.41 ± 1.08*** | 0.19 ± 0.19 | 0.31 ± 0.19 | 0.25 ± 0.23 | <0.001 |
| 5-HETE         | 12.18 ± 4.80 | 12.25 ± 2.90 | 11.87 ± 6.83 | 23.62 ± 10.22 | 18.10 ± 9.25 | 24.20 ± 11.80 | 0.543 0.001 0.593 |
| 5-oxo-ETE      | 3.06 ± 1.07 | 2.04 ± 1.63 | 1.98 ± 1.81 | 8.29 ± 3.60 | 4.67 ± 2.07 | 9.95 ± 6.67 | 0.154 <0.001 0.119 |
| 12-HETE        | 9.18 ± 5.72 | 11.32 ± 7.40 | 8.58 ± 5.55 | 6.90 ± 2.00 | 7.14 ± 3.19 | 12.13 ± 5.62 | 0.185 0.567 0.569 |
| 15-HETE        | 11.87 ± 7.19 | 9.49 ± 2.67 | 7.47 ± 3.26 | 9.97 ± 4.59 | 11.82 ± 4.39 | 18.3 ± 6.73* | 0.020  |
| 17-OH DHA      | 0.41 ± 0.21 | 0.33 ± 0.06 | 0.33 ± 0.22 | 0.55 ± 0.28 | 0.61 ± 0.21 | 0.89 ± 0.38 | 0.747 0.001 0.388 |

Each value is a mean ± SD, $n = 5–6$, except for PGE$_2$, $n = 4$. Fatty acids are expressed in nmol/g brain, and eicosanoids and 17-OH-DHA in pmol/g brain.

* $P < 0.05$, *** $P < 0.001$. 

A two-way ANOVA on whole brain cPLA$_2$-IV and sPLA$_2$ activities and 15-LOX-2 protein levels showed significant diet $\times$ LPS interaction ($P = 0.0002$ and $P < 0.0001$, respectively (Table 3). A two-way ANOVA showed a significant main effect of LiCl on 5-HETE ($P = 0.001$) and 5-oxo-ETE ($P < 0.001$) and a significant diet $\times$ LPS interaction for 15-HETE ($P = 0.02$). LiCl increased significantly 5-HETE (mean = 21.70 pmol/g) by 1.8-fold compared with control diet (mean = 12.10 pmol/g; $P = 0.0006$). LiCl increased significantly 5-oxo-ETE (mean = 7.64 pmol/g) by 4.3-fold compared with the control diet (mean = 2.36 pmol/g; $P < 0.0001$). A one-way ANOVA with Bonferroni posthoc tests showed that LiCl increased 15-HETE in high-dose LPS-infused rats but had 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 monitoring with $m/z$ 245 $\rightarrow$ 181, was present in the brain of control diet rats infused with aCSF. The concentration was increased significantly (Table 2). A two-way ANOVA showed that treatment with LiCl significantly increased 17-OH-DHA ($P = 0.001$) in control diet rats compared with aCSF (mean = 13.92 pmol/g by 1.6-fold compared with control diet (mean = 8.41 pmol/g; $P < 0.0001$). A one-way ANOVA with Bonferroni posthoc tests showed that LiCl increased 17-OH-DHA in high-dose LPS-infused rats but had no significant effect at baseline. Neithe the high- nor low-dose LPS had a significant main effect on any of these concentrations.
Neuroinflammation and lithium

The major new finding of our study is that LiCl increased 17-OH-DHA formation in rat brain with aCSF and LPS infusion. 17-OH-DHA has been reported to have antiinflammatory 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 (38), and TNF-α release and 5-LOX protein expression in murine macrophages (39). 17-OH-DHA also is an agonist of the tran-

**TABLE 3. Effects of 6 day LPS infusion and 6 week feeding LiCl on brain PLA₂ activities**

|                      | Control Diet | LiCl Diet | LiCl × LPS Interaction | LiCl effect | LPS effect |
|----------------------|--------------|-----------|------------------------|-------------|-----------|
|                      | aCSF         | Low LPS   | High LPS               |             |           |
| cPLA₂-IV             | 4.03 ± 0.42  | 5.47 ± 0.04* | 9.99 ± 1.60***   | 3.64 ± 0.39 | 3.99 ± 0.18 | 5.99 ± 0.06***   |
| sPLA₂                | 1110 ± 186   | 1565 ± 133* | 1994 ± 307***      | 1368 ± 135  | 1016 ± 199  | 979 ± 167         |
| iPLA₂-VI             | 17.58 ± 2.89 | 18.87 ± 0.20 | 19.65 ± 1.54   | 20.84 ± 4.45 | 20.49 ± 3.69 | 19.73 ± 1.40 |

Each value is a mean ± SD, n = 4. Speciﬁc PLA₂ activities are expressed in pmol/mg protein/min. Data were compared using two-way ANOVA. When LiCl × LPS interactions were signiﬁcant, a one-way ANOVA with Bonferroni’s posttest with correction for ﬁve comparisons was performed. *P < 0.05 and ***P < 0.001.

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identified enzymes or pathways (16) 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 (33) or lysophospholipid acyl CoA transferase activity, making more unesterified AA available to the LOX pathways. Similarly, aspirin, ibuprofen, indomethacin, and valproate, which inhibit cyclooxygenase (COX) activity like lithium, have been reported to increase brain HETE concentrations (20, 44–47).

In this study, high-dose LPS infusion increased brain AA and PGE₂ concentrations and cPLA₂-IV and sPLA₂ activities without changing the brain DHA concentration or iPLA₂-VI activity, consistent with evidence that iPLA₂-VI is selective for DHA hydrolysis from phospholipid (27). Although the high-dose LPS significantly increased both cPLA₂-IV and sPLA₂ 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 cPLA₂ and sPLA₂ during high-dose LPS infusion, was converted rapidly to eicosanoids and/or reincorporated into brain phospholipids (48, 49). The LiCl diet prevented only the effect of sPLA₂ activity.

The results from this study are consistent with our ischemia study and other reports showing that the lithium increase of unesterified AA, 17-OH-DHA, 5-oxo-HETE, 5-HETE, and 17-OH-DHA was lower in high-energy microwaved than nonmicrowaved brain (29, 30, 32). PGE₂ was detected in only one of four brains from control microwaved brains. In the present study using an aCSF infusion model, we did not detect PGE₂ in control microwaved brains (29). Additionally, we did not observe a change in brain 5-oxo-PGE₂ was not degraded significantly by the microwaving procedure (29). These data suggest that little endogenous PGE₂ is produced under the absence of a brain insult and that the PGE₂ that we could detect in the two brains in this study likely was associated with cannula-related damage (37). In contrast to our earlier report regarding ischemia (29), we did not detect E₂/D₂ isoprostanes in any sample. TXB₂ was reported to be at the limit of detection in microwaved brain (29), 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 PGE₂ but not of DHA, as well as cPLA₂-IV activity, and that lithium attenuated these changes, confirms data obtained with different methods (23, 25). In this study, we confirmed an increased brain sPLA₂ activity by LPS infusion (23). 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 cPLA₂ and sPLA₂ activities. LiCl did not significantly alter the baseline brain unesterified AA concentration, consistent with lithium not changing baseline cPLA₂-IV and sPLA₂ activities. The absence of a LiCl effect on sPLA₂ agrees with a previous report (50), whereas cPLA₂-IV mRNA and protein were downregulated by LiCl in another study (51). Intravenous or intraperitoneal LPS in rodents has been reported to increase brain sPLA₂-IIA and sPLA₂-IIIE mRNA, respectively (52, 53). These data suggest that lithium acts differently in a "normal" unstimulated brain compared with an "inflammatory" brain. Lithium might modulate cPLA₂-IV and sPLA₂ upregulation in response to LPS by decreasing the intracellular Ca²⁺ released by glutamate acting at N-methyl-D-aspartic acid receptors (Ca²⁺ mediates translocation or phosphorylation of cPLA₂) or by reducing the level of phosphatidylinositol 4,5-bisphosphate, which anchors cPLA₂ to perinuclear and nuclear membranes (54).

This study also investigated possible effects of cannula implantation followed by a 6 day aCSF infusion. Except for a change in PGE₂, the procedure did not affect any measurement, consistent with the expected little or absence of an inflammatory reaction under the experimental conditions (37). Body weight loss was significantly by LPS infusion, more evident in the low dose. Weight loss has been reported to be induced by cerebroventricular injection (55). Interleukin-1β, interleukin-2, interleukin-6, and interleukin-10 have been reported to play a role in modulating AA metabolism (16, 18). In this study, LiCl increased levels of 15-HETE, 17-OH-DHA, 5-HETE, and 5-oxo-ETE in the brain of rats subjected to neuroinflammation. Supporting such a mechanism is epidemiological evidence that aspirin, which can increase

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**WITHDRAWN**

**June 29, 2018**

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**Fig. 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.
17(R)-OH-DHA by acetylating COX-2 (9, 59), when given chronically reduced untoward effects in (presumably) bipolar disorder patients on lithium therapy (60). 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-I dementia (61), amyotrophic lateral sclerosis (62), and Alzheimer’s disease (63) has been noted in recent limited clinical trials.

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