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 LC/MS/MS 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 with artificial cerebrospinal fluid increased brain unesterified AA and prostaglandin E_2 concentrations and activities of AA-selective Ca^{2+}-dependent cytosolic phospholipase A_2 (cPLA$_2$)-IV and Ca^{2+}-dependent secretory sPLA$_2$. LiCl feeding prevented these increments. Lithium had a significant main effect by increasing brain concentrations of lipoxygenase-derived AA metabolites, 5- hydroxyeicosatetraenoic acid (HETE), 5-oxo-eicosatetranoic acid, and 17-hydroxy-DHA by 1.8-, 4.3- and 1.9-fold compared with control diet. Lithium also increased 15-HETE in high-dose LPS-infused rats. Ca^{2+}-independent iPLA$_2$-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 A_2

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 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 (AA; 20:4n-6) metabolism (6, 7).

Mediators of neuroinflammation can be bioactive lipids derived from AA and docosahexaenoic acid (DHA; 22:6n-3). During the neuroinflammatory response, phospholipase A_2 (PLA_2) 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 PLA_2 from glycerophospholipids can be metabolized to

This work was supported by the Intramural Research Program of the National Institute on Aging, the National Institutes of Health (M.B., H-W.K, M.C., K.M., and S.I.R.), and by LIPID MAPS Large Scale Collaborative grant number GM-069338 from the National Institutes of Health (R.C.M. and S.E.F.). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health or other granting agencies.

Manuscript received 19 November 2009 and in revised form 22 December 2009.

Published, JLR Papers in Press, December 22, 2009
DOI 10.1194/jlr.M002469

Abbreviations: AA, arachidonic acid; CSF, cerebrospinal fluid; COX, cyclooxygenase; aCSF, artificial cerebrospinal fluid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; ETE, eicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid; LOX, lipoxygenase; LPS, lipopolysaccharide; 17-OH-DHA, 17*S*-hydroxy-DHA; 5-oxo-ETE, 5-oxo-eicosatetraenoic acid; PLA_2, phospholipase A_2; cPLA$_2$, Ca^{2+}-dependent cytosolic PLA_2; iPLA$_2$, Ca^{2+}-independent PLA_2; sPLA$_2$, Ca^{2+}-dependent secretory PLA_2; PGE_2, prostaglandin E_2; RP, reverse phase; TNF, tumor necrosis factor; TXB_2, thromboxane B_2.

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docosanoids, including resolvins, docosatrienes, and neuroprotectins. 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 product) and docosatriene were introduced, because they displayed potent antiinflammatory 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*S*-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 E_2 (PGE_2) 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 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 (23, 24). 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 PLA_2 ($cPLA_2$) and secretory PLA_2 ($sPLA_2$), 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 (23, 25). Feeding LiCl to rats for 6 weeks to produce plasma and brain lithium 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 Ca^{2+}-independent PLA_2 ($iPLA_2$), 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 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 antiinflammatory mediators known as resolvins, was increased in LiCl-fed rats infused with artificial cerebrospinal fluid (aCSF) and LPS, suggesting a new beneficial mechanism of action of lithium in bipolar disorder as an antiinflammatory agent.

MATERIALS AND METHODS

Animals

All procedures were performed under a protocol (no. 06-026) approved by the Animal Care and Use Committee of Eunice Kennedy Shriver National Institute of Child Health and Human Development, in accordance with the National Institutes of Health 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 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) (25). This regimen produces plasma and brain lithium concentrations of about 0.7 mM, therapeutically relevant to bipolar disorder (19, 33). Control rats were fed lithium-free Purina 5001 chow 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 (34) from random 0.7–0.8 g samples (n = 4). An aliquot of total lipid extract was methylated with 1% H_2SO_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 (21, 23, 25). 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. Postoperative care included triple antibiotic ointment applied to the wound and 5 ml of sterile 0.9% NaCl (sc) to prevent dehydration during recovery. Following 6 days of LPS or aCSF infusion, starting after a rat had been on a control or lithium diet for 36 days, rats were anesthetized with Nembutal® (40 mg/kg, ip) 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® 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 (29). Right and left microwaved cerebral hemispheres were homogenized separately in 4 ml of 80% methanol and d_8-5-HETE, d_8-AA, d_5-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 $d8$-AA; m/z 332 → 288 for $d5$-DHA; m/z 373 → 173 for $d4$-TXB_2; m/z 355 → 275 for $d4$-PGE_2; m/z 327 → 116 for $d8$-5-HETE. Quantitation was performed via standard isotope dilution (29).

Brain-specific PLA_2 activities

Rats were anesthetized with Nembutal® 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$_2$-IV and iPLA$_2$-VI activities using the sensitive and specific method of Yang et al. (35) 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 × 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 $P \leq 0.05$.

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 1.62% DHA.

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 one of four brains of control diet and in one of four brains of lithium diet rats infused with aCSF. These findings show

TABLE 1. Diet fatty acid composition

Fatty Acid	Control	Low LiCl (μmol/g diet)	High LiCl
14:0	1.94 ± 0.24	1.80 ± 0.09	1.85 ± 0.17
14:1n-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
16:1n-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
18:1n-9	40.89 ± 4.15	38.57 ± 2.93	39.08 ± 4.02
18:2n-6	46.45 ± 5.44	44.06 ± 2.53	44.84 ± 3.46
18:3n-3	4.07 ± 0.46	4.31 ± 1.03	3.88 ± 0.31
20:3n-6	0.28 ± 0.03	0.27 ± 0.01	0.26 ± 0.02
20:4n-6	0.52 ± 0.05	0.50 ± 0.03	0.49 ± 0.03
20:5n-3	1.65 ± 0.17	1.55 ± 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
22:5n-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	132.16 ± 4.44	133.10 ± 6.81	134.17 ± 9.85
Total n-6	47.66 ± 5.55	45.24 ± 2.62	45.98 ± 3.49
Total n-3	8.18 ± 0.89	8.26 ± 1.08	7.85 ± 0.60
Total saturated	34.34 ± 9.84	39.83 ± 1.29	40.28 ± 2.05
Total monounsaturated	44.00 ± 4.62	41.62 ± 3.09	41.95 ± 3.99

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 × 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 × 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 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 one of four brains from control as well as from 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 × LPS interaction for the PGE_2 concentration ($P < 0.001$). Subsequent one-way ANOVAs with Bonferroni posthoc 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, because 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 × 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 m/z 343→245, was present in the brain of control diet rats and that its concentration was increased by the LiCl diet (**Fig. 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.

PLA_2 activities and 15-LOX-2 protein

A two-way ANOVA on whole brain $cPLA_2$-IV and $sPLA_2$ specific activities showed significant diet × LPS interactions, at $P = 0.0002$ and $P < 0.0001$, respectively (**Table 3**). Subsequent one-way ANOVAs with Bonferroni posthoc tests showed that both doses of LPS compared with aCSF significantly increased brain $cPLA_2$-IV activity by 36% and

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 × LPS In teract ion	LiCl effect	LPS effect
	aCSF	Low LPS	High LPS	aCSF	Low LPS	High LPS	*P*	*P*	*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	14.71 ± 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 ± 8.89	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	11.52 ± 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 ± 5.26	9.97 ± 4.59	11.82 ± 4.39	18.54 ± 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.

When LiCl × LPS interactions were significant, a one-way ANOVA with Bonferroni's posttest with correction for five comparisons was performed. *$P < 0.05$, ***$P < 0.001$.

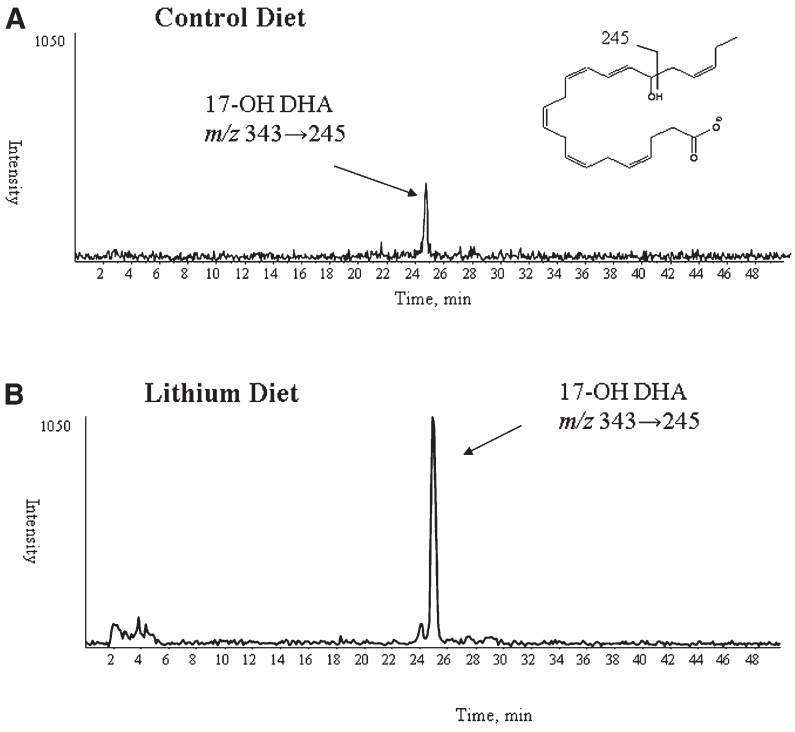

Fig. 1. 17-OH-DHA levels in high LPS-infused brains of rats subjected to control (A) and LiCl (B) diets analyzed by LC/MS/MS.

148%, respectively, and brain $sPLA_2$ activity by 41% and 80%, respectively. The LiCl diet prevented the significant increment of $cPLA_2$-IV activity following low- but not high-dose LPS, as well as the significant increments in $sPLA_2$ activity caused by low- and high-dose LPS. Neither the LiCl diet nor LPS infusion significantly affected whole brain $iPLA_2$-VI activity.

Brain cytosolic 15-LOX-2 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 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 transcription factor, peroxisome proliferator-activated receptor γ, which is believed to act in an antiinflammatory manner (40).

The mechanism underlying the 17-OH-DHA elevation is uncertain. Because LiCl did not increase the concentration of its precursor, unesterified DHA, or $iPLA_2$-VI activity, consistent with prior data (20, 33), 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 (41, 42)], as reported for unesterified AA (23), 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, because 15-HETE is generated from AA by the action of 15-LOX. Although cytosolic 15-LOX-2 protein level was not significantly increased in the LPS-infused rats fed LiCl, we cannot rule out posttranslational upregulation of 15-LOX activity, which has been reported (43). 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

TABLE 3. Effects of 6 day LPS infusion and 6 week feeding LiCl on brain PLA_2 activities

	Control Diet			LiCl Diet			LiCl × LPS Interaction	LiCl effect	LPS effect
	aCSF	Low LPS	High LPS	aCSF	Low LPS	High LPS	*P*	*P*	*P*
$cPLA_2$-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***	**0.0002**		
$sPLA_2$	1110 ± 186	1565 ± 133*	1994 ± 307***	1368 ± 135	1016 ± 199	979 ± 167	**<0.0001**		
$iPLA_2$-VI	17.58 ± 2.89	18.87 ± 0.20	19.85 ± 1.54	20.84 ± 4.45	20.49 ± 3.69	19.83 ± 1.40	0.5099	0.1696	0.8951

Each value is a mean ± SD, n = 4. Specific PLA_2 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 ***$P < 0.001$.

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 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 (27). 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 (48, 49). 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 nonmicrowaved brain (29, 30, 32). PGE_2 was detected in only one of four brains 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) (29). Additionally, we showed that intracerebrally injected d_4-PGE_2 was not degraded substantially by the microwaving procedure (29). 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 (37). In contrast to our earlier report regarding ischemia (29), we did not detect E_2/D_2 isoprostanes in any sample. TXB_2 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 PGE_2 but not of DHA, as well as $cPLA_2$-IV activity, and that lithium attenuated these changes, confirms data obtained with different methods (23, 25). In this study, we confirmed an increased brain $sPLA_2$ 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 $cPLA_2$ 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 (50), whereas $cPLA_2$-IV mRNA and protein were downregulated by LiCl in another study (51). Intravenous or intraperitoneal LPS in rodents has been reported to increase brain $sPLA_2$-IIA and $sPLA_2$-IIE mRNA, respectively (52, 53). 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 (54).

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 (37). 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 (21, 22) and with peripheral LPS injection (55). The proinflammatory cytokines TNF-α, interleukin-1β, and interleukin-6 have been suggested to play a role in weight loss (55). Peripheral LPS produces sleepiness and inactivity (56), both of which were more evident in the high-dose LPS-infused rats.

In summary (**Fig. 2**), finding that LiCl prefeeding upregulated the brain concentration of 17-OH-DHA provides a new possible mechanism for lithium's reported neuroprotective action (57), in addition to downregulating the AA cascade (25, 58). Supporting such a mechanism is epidemiological evidence that aspirin, which can increase

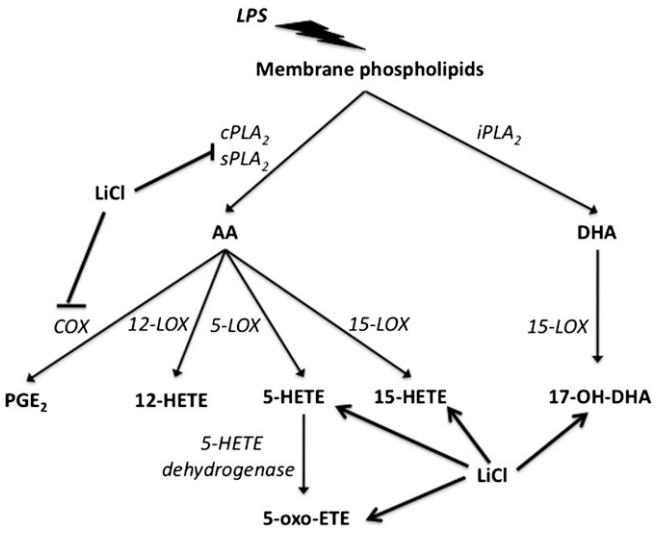

Fig. 2. LPS infusion increases brain concentration of unesterified AA via $cPLA_2$ and $sPLA_2$ and PGE_2 via COX without altering DHA release via $iPLA_2$, and LiCl blocks these increases. In addition, LiCl increases levels of 15-HETE, 17-OH-DHA, 5-HETE, and 5-oxo-ETE in the brain of rats subjected 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-1 dementia (61), amyotrophic lateral sclerosis (62), and Alzheimer's disease (63) has been noted in recent limited clinical trials.

REFERENCES

1. Belmaker, R. H. 2004. Bipolar disorder. *N. Engl. J. Med.* **351:** 476–486.
2. Benazzi, F. 2003. Frequency of bipolar spectrum in 111 private practice depression outpatients. *Eur. Arch. Psychiatry Clin. Neurosci.* **253:** 203–208.
3. Moore, A. H., and M. K. O'Banion. 2002. Neuroinflammation and anti-inflammatory therapy for Alzheimer's disease. *Adv. Drug Deliv. Rev.* **54:** 1627–1656.
4. Esposito, G., G. Giovacchini, J. S. Liow, A. K. Bhattacharjee, D. Greenstein, M. Schapiro, M. Hallett, P. Herscovitch, W. C. Eckelman, R. E. Carson, et al. 2008. Imaging neuroinflammation in Alzheimer's Disease with radiolabeled arachidonic acid and PET. *J. Nucl. Med.* **49:** 1414–1421.
5. Minghetti, L. 2005. Role of inflammation in neurodegenerative diseases. *Curr. Opin. Neurol.* **18:** 315–321.
6. Rao, J. S., G. J. Harry, S. I. Rapoport, and H. W. Kim. 2009. Increased excitotoxicity and neuroinflammatory markers in postmortem frontal cortex from bipolar disorder patients. *Mol. Psychiatry*. Epub ahead of print. June 2, 2009; doi: 10.1038/mp.2009.47.
7. Kim, H. Y., S. I. Rapoport, and J. S. Rao. Altered arachidonic acid cascade enzymes in postmortem brain from bipolar disorder patients. *Mol. Psychiatry*. Epub ahead of print. December 29, 2009; doi: 10.1038/mp.2009.137.
8. Phillis, J. W., L. A. Horrocks, and A. A. Farooqui. 2006. Cyclooxygenases, lipoxygenases, and epoxygenases in CNS: their role and involvement in neurological disorders. *Brain Res. Brain Res. Rev.* **52:** 201–243.
9. Serhan, C. N., S. Hong, K. Gronert, S. P. Colgan, P. R. Devchand, G. Mirick, and R. L. Moussignac. 2002. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J. Exp. Med.* **196:** 1025–1037.
10. Hong, S., K. Gronert, P. R. Devchand, R. L. Moussignac, and C. N. Serhan. 2003. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation. *J. Biol. Chem.* **278:** 14677–14687.
11. Marcheselli, V. L., S. Hong, W. J. Lukiw, X. H. Tian, K. Gronert, A. Musto, M. Hardy, J. M. Gimenez, N. Chiang, C. N. Serhan, and N. G. Bazan. 2003. Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression. *J. Biol. Chem.* **278:** 43807–43817.
12. Hong, S., E. Tjonahen, E. L. Morgan, Y. Lu, C. N. Serhan, and A. F. Rowley. 2005. Rainbow trout (*Oncorhynchus mykiss*) brain cells biosynthesize novel docosahexaenoic acid-derived resolvins and protectins-Mediator lipidomic analysis. *Prostaglandins Other Lipid Mediat.* **78:** 107–116.
13. Serhan, C. N. 2007. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu. Rev. Immunol.* **25:** 101–137.
14. Dangi, B., M. Obeng, J. M. Nauroth, M. Teymourlouei, M. Needham, K. Raman, and L. M. Arterburn. 2009. Biogenic synthesis, purification, and chemical characterization of anti-inflammatory resolvins derived from docosapentaenoic acid (DPAn-6). *J. Biol. Chem.* **284:** 14744–14759.
15. Butovich, I. A., M. Hamberg, and O. Rådmark. 2005. Novel oxylipins formed from docosahexaenoic acid by potato lipoxygenase–10(S)-hydroxydocosahexaenoic acid and 10,20-dihydroxydocosahexaenoic acid. *Lipids.* **40:** 249–257.
16. Gleissman, H., R. Yang, K. Martinod, M. Lindskog, C. N. Serhan, J. I. Johnsen, and P. Kogner. 2010. Docosahexaenoic acid metabolome in neural tumors: identification of cytotoxic intermediates. *FASEB J.* **24:** 906–915.
17. Cade, J. F. J. 1949. Lithium salts in the treatment of psychotic excitement. *Med. J. Aust.* **36:** 349–352.
18. Harwood, A. J., and G. Agam. 2003. Search for a common mechanism of mood stabilizers. *Biochem. Pharmacol.* **66:** 179–189.
19. Chang, M. C. J., E. Grange, O. Rabin, J. M. Bell, D. D. Allen, and S. I. Rapoport. 1996. Lithium decreases turnover of arachidonate in several brain phospholipids. *Neurosci. Lett.* **220:** 171–174. [Erratum. 1997. Neurosci. Lett. 1931, 1222:1141].
20. Bosetti, F., J. Rintala, R. Seemann, T. A. Rosenberger, M. A. Contreras, S. I. Rapoport, and M. C. Chang. 2002. Chronic lithium downregulates cyclooxygenase-2 activity and prostaglandin E_2 concentration in rat brain. *Mol. Psychiatry.* **7:** 845–850.
21. Hauss-Wegrzyniak, B., L. Lukovic, M. Bigaud, and M. E. Stoeckel. 1998. Brain inflammatory response induced by intracerebroventricular infusion of lipopolysaccharide: an immunohistochemical study. *Brain Res.* **794:** 211–224.
22. Marriott, L. K., K. R. McGann-Gramling, B. Hauss-Wegrzyniak, L. C. Sheldahl, R. A. Shapiro, D. M. Dorsa, and G. L. Wenk. 2007. Brain infusion of lipopolysaccharide increases uterine growth as a function of estrogen replacement regimen: suppression of uterine estrogen receptor-alpha by constant, but not pulsed, estrogen replacement. *Endocrinology.* **148:** 232–240.
23. Rosenberger, T. A., N. E. Villacreses, J. T. Hovda, F. Bosetti, G. Weerasinghe, R. N. Wine, G. J. Harry, and S. I. Rapoport. 2004. Rat brain arachidonic acid metabolism is increased by a 6-day intracerebral ventricular infusion of bacterial lipopolysaccharide. *J. Neurochem.* **88:** 1168–1178. [Erratum. 2004. J. Neurochem. 1190, 1255.]
24. Richardson, R. L., E. M. Kim, T. Gardiner, and E. O'Hare. 2005. Chronic intracerebroventricular infusion of lipopolysaccharide: effects of ibuprofen treatment and behavioural and histopathological correlates. *Behav. Pharmacol.* **16:** 531–541.
25. Basselin, M., N. E. Villacreses, H. J. Lee, J. M. Bell, and S. I. Rapoport. 2007. Chronic lithium administration attenuates up-regulated brain arachidonic acid metabolism in a rat model of neuroinflammation. *J. Neurochem.* **102:** 761–772.
26. Rosenberger, T. A., N. E. Villacreses, M. T. Weis, and S. I. Rapoport. 2010. Rat brain docosahexaenoic acid metabolism is not altered by a 6-day intracerebral ventricular infusion of bacterial lipopolysaccharide. *Neurochemistry Int.* **56:** 501–507.
27. Strokin, M., M. Sergeeva, and G. Reiser. 2003. Docosahexaenoic acid and arachidonic acid release in rat brain astrocytes is mediated by two separate isoforms of phospholipase A_2 and is differently regulated by cyclic AMP and Ca^{2+}. *Br. J. Pharmacol.* **139:** 1014–1022.
28. Murphy, R. C., R. M. Barkley, K. Zemski Berry, J. Hankin, K. Harrison, C. Johnson, J. Krank, A. McAnoy, C. Uhlson, and S. Zarini. 2005. Electrospray ionization and tandem mass spectrometry of eicosanoids. *Anal. Biochem.* **346:** 1–42.
29. Farias, S. E., M. Basselin, L. Chang, K. A. Heidenreich, S. I. Rapoport, and R. C. Murphy. 2008. Formation of eicosanoids, E_2/D_2-isoprostanes and docosanoids following decapitation-induced Ischemia, measured in high-energy microwaved rat brain. *J. Lipid Res.* **49:** 1990–2000.
30. Murphy, E. J. 2010. Brain fixation for analysis of brain lipid-mediators of signal transduction and brain eicosanoids requires head-focused microwave irradiation: an historical perspective. *Prostaglandins Other Lipid Mediat.* **91:** 63–67.
31. Bazinet, R. P., H. J. Lee, C. C. Felder, A. C. Porter, S. I. Rapoport, and T. A. Rosenberger. 2005. Rapid high-energy microwave fixation is required to determine the anandamide (N-arachidonoylethanolamine) concentration of rat brain. *Neurochem. Res.* **30:** 597–601.
32. Deutsch, J., S. I. Rapoport, and A. D. Purdon. 1997. Relation between free fatty acid and acyl-CoA concentrations in rat brain following decapitation. *Neurochem. Res.* **22:** 759–765.
33. Chang, M. C. J., J. M. Bell, A. D. Purdon, E. G. Chikhale, and E. Grange. 1999. Dynamics of docosahexaenoic acid metabolism in the central nervous system: lack of effect of chronic lithium treatment. *Neurochem. Res.* **24:** 399–406.
34. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.

35. Yang, H. C., M. Mosior, C. A. Johnson, Y. Chen, and E. A. Dennis. 1999. Group-specific assays that distinguish between the four major types of mammalian phospholipase A_2. *Anal. Biochem.* **269:** 278–288.
36. Tabachnick, B. G., and L. S. Fidell. 2001. Computer-assisted research design and analysis. Allyn and Bacon, Boston.
37. Williams, L. R., H. L. Vahlsing, T. Lindamood, S. Varon, F. H. Gage, and M. Manthorpe. 1987. A small-gauge cannula device for continuous infusion of exogenous agents into the brain. *Exp. Neurol.* **95:** 743–754.
38. Ziboh, V. A., C. C. Miller, and Y. Cho. 2000. Metabolism of polyunsaturated fatty acids by skin epidermal enzymes: generation of anti-inflammatory and antiproliferative metabolites. *Am. J. Clin. Nutr.* **71:** 361S–366S.
39. González-Périz, A., A. Planagumà, K. Gronert, R. Miquel, M. López-Parra, E. Titos, R. Horrillo, N. Ferré, R. Deulofeu, V. Arroyo, et al. 2006. Docosahexaenoic acid (DHA) blunts liver injury by conversion to protective lipid mediators: protectin D1 and 17S-hydroxy-DHA. *FASEB J.* **20:** 2537–2539.
40. Vanden Berghe, W., L. Vermeulen, P. Delerive, K. De Bosscher, B. Staels, and G. Haegeman. 2003. A paradigm for gene regulation: inflammation, NF-κB and PPAR. *Adv. Exp. Med. Biol.* **544:** 181–196.
41. De Smedt-Peyrusse, V., F. Sargueil, A. Moranis, H. Harizi, S. Mongrand, and S. Laye. 2008. Docosahexaenoic acid prevents lipopolysaccharide-induced cytokine production in microglial cells by inhibiting lipopolysaccharide receptor presentation but not its membrane subdomain localization. *J. Neurochem.* **105:** 296–307.
42. Ong, W. Y., J. F. Yeo, S. F. Ling, and A. A. Farooqui. 2005. Distribution of calcium-independent phospholipase A_2 ($iPLA_2$) in monkey brain. *J. Neurocytol.* **34:** 447–458.
43. Kuhn, H., and V. B. O'Donnell. 2006. Inflammation and immune regulation by 12/15-lipoxygenases. *Prog. Lipid Res.* **45:** 334–356.
44. Edwin, S. S., R. J. Romero, H. Munoz, D. W. Branch, and M. D. Mitchell. 1996. 5-Hydroxyeicosatetraenoic acid and human parturition. *Prostaglandins.* **51:** 403–412.
45. Vanderhoek, J. Y., and J. M. Bailey. 1984. Activation of a 15-lipoxygenase/leukotriene pathway in human polymorphonuclear leukocytes by the anti-inflammatory agent ibuprofen. *J. Biol. Chem.* **259:** 6752–6756.
46. Szupera, Z., Z. Mezei, B. Kis, A. Gecse, L. Vecsei, and G. Telegdy. 2000. The effects of valproate on the arachidonic acid metabolism of rat brain microvessels and of platelets. *Eur. J. Pharmacol.* **387:** 205–210.
47. Sawazaki, S., N. Salem, Jr., and H. Y. Kim. 1994. Lipoxygenation of docosahexaenoic acid by the rat pineal body. *J. Neurochem.* **62:** 2437–2447.
48. Rapoport, S. I. 2003. In vivo approaches to quantifying and imaging brain arachidonic and docosahexaenoic acid metabolism. *J. Pediatr.* **143:** S26–S34.
49. Robinson, P. J., J. Noronha, J. J. DeGeorge, L. M. Freed, T. Nariai, and S. I. Rapoport. 1992. A quantitative method for measuring regional in vivo fatty-acid incorporation into and turnover within brain phospholipids: review and critical analysis. *Brain Res. Brain Res. Rev.* **17:** 187–214.
50. Weerasinghe, G. R., S. I. Rapoport, and F. Bosetti. 2004. The effect of chronic lithium on arachidonic acid release and metabolism in rat brain does not involve secretory phospholipase A_2 or lipoxygenase/cytochrome P450 pathways. *Brain Res. Bull.* **63:** 485–489.
51. Rintala, J., R. Seemann, K. Chandrasekaran, T. A. Rosenberger, L. Chang, M. A. Contreras, S. I. Rapoport, and M. C. J. Chang. 1999. 85 kDa cytosolic phospholipase A_2 is a target for chronic lithium in rat brain. *Neuroreport.* **10:** 3887–3890.
52. Murakami, M., K. Yoshihara, S. Shimbara, G. Lambeau, A. Singer, M. H. Gelb, M. Sawada, N. Inagaki, H. Nagai, and I. Kudo. 2002. Arachidonate release and eicosanoid generation by group IIE phospholipase A_2. *Biochem. Biophys. Res. Commun.* **292:** 689–696.
53. Oka, S., and H. Arita. 1991. Inflammatory factors stimulate expression of group II phospholipase A_2 in rat cultured astrocytes. Two distinct pathways of the gene expression. *J. Biol. Chem.* **266:** 9956–9960.
54. Burke, J. E., and E. A. Dennis. 2009. Phospholipase A_2 structure/function, mechanism and signaling. *J. Lipid Res.* **50 (Suppl.):** S237–S242.
55. Elander, L., L. Engström, M. Hallbeck, and A. Blomqvist. 2007. IL-1beta and LPS induce anorexia by distinct mechanisms differentially dependent on microsomal prostaglandin E synthase-1. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292:** R258–R267.
56. Holmes, J. E., and N. E. Miller. 1963. Effects of bacterial endotoxin on water intake, food intake, and body temperature in the Albino rat. *J. Exp. Med.* **118:** 649–658.
57. Rowe, M. K., and D. M. Chuang. 2004. Lithium neuroprotection: molecular mechanisms and clinical implications. *Expert Rev. Mol. Med.* **6:** 1–18.
58. Rapoport, S. I., M. Basselin, H-W. Kim, and J. S. Rao. 2009. Bipolar disorder and mechanism of action of mood stabilizers. *Brain Res. Brain Res. Rev.* **61:** 185–209.
59. Meade, E. A., W. L. Smith, and D. L. DeWitt. 1993. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. *J. Biol. Chem.* **268:** 6610–6614.
60. Stolk, P., P. C. Souverein, I. Wilting, H. G. Leufkens, D. F. Klein, S. I. Rapoport, and E. R. Heerdink. 2010. Is aspirin useful in patients on lithium? A pharmacoepidemiological study related to bipolar disorder. *Prostaglandins Leukot. Essent. Fatty Acids.* **82:** 9–14.
61. Letendre, S. L., S. P. Woods, R. J. Ellis, J. H. Atkinson, E. Masliah, G. van den Brande, J. Durelle, I. Grant, and I. Everall. 2006. Lithium improves HIV-associated neurocognitive impairment. *AIDS.* **20:** 1885–1888.
62. Fornai, F., P. Longone, L. Cafaro, O. Kastsiuchenka, M. Ferrucci, M. L. Manca, G. Lazzeri, A. Spalloni, N. Bellio, P. Lenzi, et al. 2008. Lithium delays progression of amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. USA.* **105:** 2052–2057.
63. Leyhe, T., G. W. Eschweiler, E. Stransky, T. Gasser, P. Annas, H. Basun, and C. Laske. 2009. Increase of BDNF serum concentration in lithium treated patients with early Alzheimer's disease. *J. Alzheimers Dis.* **16:** 649–656.