Molecular Psychiatry (2002) 7, 845–850
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www.nature.com/mp

ORIGINAL RESEARCH ARTICLE

Chronic lithium downregulates cyclooxygenase-2 activity and prostaglandin E_2 concentration in rat brain

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Rats treated with lithium chloride for 6 weeks have been reported to demonstrate reduced turnover of arachidonic acid (AA) in brain phospholipids, and decreases in mRNA and protein levels, and enzyme activity, of AA-selective cytosolic phospholipase A_2 ($cPLA_2$). We now report that chronic lithium administration to rats significantly reduced the brain protein level and enzyme activity of cyclooxygenase-2 (COX-2), without affecting COX-2 mRNA. Lithium also reduced the brain concentration of prostaglandin E_2 (PGE_2), a bioactive product of AA formed *via* the COX reaction. COX-1 and the Ca^{2+}-independent $iPLA_2$ (type VI) were unaffected by lithium. These and prior results indicate that lithium targets a part of the AA cascade that involves $cPLA_2$ and COX-2. This effect may contribute to lithium's therapeutic action in bipolar disorder.
Molecular Psychiatry (2002) **7,** 845–850. doi:10.1038/sj.mp.4001111

Keywords: lithium; arachidonic acid; cyclooxygenase; phospholipase A_2; prostaglandin; brain; rat; chronic; bipolar disorder

Introduction

Although lithium has been used for over 50 years to treat bipolar disorder,[1] the basis of its therapeutic effect remains unclear. Several molecular targets of lithium have been suggested. They involve guanine nucleotide binding protein, adenylyl cyclase, protein kinase C isoenzymes, the phosphoinositide cycle, and balance of neurotransmitter signaling.[2–4] Lithium also has been suggested to be neuroprotective, by increasing expression of the anti-apoptotic protein Bcl-2 and decreasing expression of the pro-apoptotic p53 and Bax *in vitro*.[5] On the other hand, the arachidonic acid (AA, 20:4 n-6) cascade, which plays a key role in brain signaling,[6–8] could represent a target of lithium and other mood-stabilizers. Indeed, previous reports suggest a role for PGE_1, a metabolite of dihomogamma-linolenic acid, in affective disorders,[9,10] but the exact mechanism by which the AA cascade is targeted has not been thoroughly investigated. This cascade involves phospholipase A_2 (PLA_2)-mediated release from phospholipids of the polyunsaturated fatty acid (PUFA), AA, and its conversion to bioactive eicosanoids.[11–13]

In this regard, we reported that chronically administered lithium in rats decreased by 80% the turnover rate of AA, regulated by PLA_2, within the stereospecifically numbered (*sn*)-2 position of brain phospholipids, without affecting palmitic or docosahexaenoic acid turnover.[14,15] Decreased AA turnover was accompanied by reduced activity of PLA_2 not caused by direct enzyme inhibition by lithium,[16] and by reduced mRNA and protein levels of the AA-selective cytosolic PLA_2 ($cPLA_2$, type IV).[17] These initial results suggested that lithium might target the AA cascade.

AA can be converted to prostaglandin H_2 (PGH_2), the common precursor for biologically active eicosanoids, by either cyclooxygenase (COX, prostaglandin-endoperoxide synthase)-1 or -2.[18] COX-1 is constitutively expressed and is thought to produce eicosanoids for normal physiological function, whereas COX-2 is induced in pathological conditions, often in response to proinflammatory agents.[19] COX-2 is the predominant isoform in brain and spinal cord, where it is considered involved in synaptic signaling,[20] cerebral blood flow,[21] and behavior.[22]

The aim of this study was to determine whether chronic oral administration of LiCl has a downstream effect on the AA cascade. We therefore measured brain mRNA, protein, and enzyme activity levels of COX enzymes, as well as the concentration of prostaglandin E_2 (PGE_2). While PGE_2 is not the only prostaglandin present in brain, it does represent a major product of the cyclooxygenase reaction and plays a key role in sleep regulation, which is altered in bipolar disorder.[23] We also measured the protein level of the intracellular Ca^{2+}-independent $iPLA_2$ (type VI), to see if lithium affected brain phospholipases generally or $cPLA_2$ specifically.

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Received 3 December 2001; revised 17 January 2002; accepted 7 February 2002

Materials and methods

Lithium administration

The study conformed to the *Guideline for the Care and Use of Laboratory Animals* (NIH Publication No. 80–23). Adult male Fischer-344 rats (200–250 g) were fed *ad lib* Purina rat chow containing 1.70 g LiCl per kg for 4 weeks followed by chow containing 2.55 g LiCl per kg for 2 weeks.[14] Control rats were given lithium-free chow under parallel conditions. Rats used for PGE_2 measurements were killed with sodium pentobarbital (50 mg kg^{-1} i.p.), then subjected to head-focused microwave irradiation (5.5 kW, 3.4 s, Cober Electronics, Stanford, CT, USA). Rats used for RNA, protein or enzyme analysis were killed by carbon dioxide inhalation, and then decapitated. Brains were rapidly excised, frozen in –50°C 2-methylbutane, and stored at –80°C until use.

Measurement of lithium concentration in brain and plasma

Brain and plasma concentrations of lithium were quantified using a graphite furnace Zeeman 5100 atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT, USA), at a wavelength of 670.8 nm. Frontal cortex samples (50 mg) were digested overnight in 0.5 ml concentrated HNO_3 at 60°C and then diluted to 5 ml total volume with 0.2% HNO_3. Plasma samples were initially diluted 3-fold with 0.2% HNO_3. Brain and plasma samples were further diluted as necessary to keep the lithium concentration below the highest standard.

RNA isolation and RT-PCR

Total RNA was isolated with a Qiagen RNeasy Maxy kit (Qiagen, Valencia, CA, USA). Two μg of total RNA were reverse transcribed using a RETROscript kit (Ambion, Austin, TX, USA). Half of each RNA sample was incubated similarly in the absence of reverse transcriptase to test for genomic DNA contamination. PCR amplification was performed using specific oligonucleotide primers for COX-1 (forward: 5′-CCTTC TCCAACGTGAGCTACTA-3′, reverse: 5′-GTGGAGAA GAGCATCAGACC-3′, 486 bp),[24] and COX-2 (forward: 5′-ACTTGCTCACTTTGTTGAGT3′; reverse: 5′-TTGA TTAGTACTGTAGGGTT-3′, 581 bp).[25] Specific G3PDH primers (983 bp, Clontech, Palo Alto, CA, USA) were used as an internal control to normalize the sample amounts. After an initial 5-min denaturation at 95°C, the DNA was amplified for 30 cycles of 20 s denaturation at 94°C, primer annealing at 55°C for 30 s, and extension at 72°C for 40 s, with a final extension at 72°C for 5 min. Agarose gels (1.2%) were stained with ethidium bromide and the bands were quantified by AlphaEase Stand Alone software (Alpha Innotech, San Leandro, CA, USA). Integrated densities were normalized to G3PDH values to yield a semi-quantitative assessment of individual transcript levels. Preliminary experiments confirmed that the PCR conditions and the image analysis system were in the linear range of detection.

Western blot analysis

Brains were homogenized and Western blot analysis carried out as previously reported,[17] using primary antibodies for COX-1 (polyclonal, 1:1000), COX-2 (polyclonal, 1:2000), $iPLA_2$, type VI (monoclonal, 1:2000) (Cayman Chemicals, Ann Arbor, MI, USA), or actin (1:10 000, Santa Cruz Biotechnology). For COX-1, COX-2, and actin a secondary antibody conjugated with horseradish peroxidase (HRP, 1:1000, 1:5000, and 1:10 000, respectively, Bio-Rad, Hercules, CA, USA) was used. For $iPLA_2$ a biotinylated secondary antibody (1:2000) followed by HRP (1:1000, Vector, Burlingame, CA, USA) was used. Immunoblots were visualized on X-ray film by chemiluminescence reaction (Pierce, Rockford, IL, USA), and image analysis was performed on optical density-calibrated images by AlphaEase Stand Alone software (Alpha Innotech).

PGE_2 enzyme immunoassay

Levels of PGE_2 were determined in microwaved brain extracts. Brains were weighed, then extracted in 18 volumes of hexane: 2-propanol (3:2, by volume) using a glass Tenbroeck homogenizer. The prostaglandins were purified from the lipid extract using a C_{18} Sep-Pak cartridge (Waters, Milford, MA, USA) by the method of Powell.[26] The concentration of PGE_2 was determined using an enzyme-linked immunosorbent assay (ELISA) (Oxford Biomedical, Oxford, MI, USA).

Measurement of COX activity

COX activity was determined by the method of Taniguchi *et al*[27] with modifications. One half of a brain was homogenized in 3 ml of lysate buffer (10 mM Tris-HCl, pH 7.8, containing 1% Nonidet P-40, 0.15 M NaCl, and 1 mM EDTA), then chilled on ice for 30 min and centrifuged at 4000 rpm for 25 min. The supernatant was diluted 1:10 with lysate buffer. To 500 μl of the diluted sample was added 60 μl of lysate buffer containing 10 mM phenol, 18.2 mM l-epinephrine, 4.6 mM glutathione, and 9.3 μM hematin. To determine whether LiCl directly inhibited COX activity, the reaction was carried out on brain homogenates from control animals in the presence or absence of 1 mM LiCl. The mixture was chilled on ice for 10 min, then 60 μl of lysate buffer containing 1 mM AA was added, and the mixture was incubated at 37°C for 10 min. The reaction was terminated by adding 250 μl of 1 M HCl. PGE_2 was extracted by ethyl acetate[27] and determined using a PGE_2 immunoassay kit (Cayman). A sample not allowed to react with AA was prepared and assayed in the same manner, and used for blank determination. The intra- and inter-assay variability for this kit was $\leq$ 10%. Cross-reactivity with PGE_1 was 18.7% and with PGE_3 was 43%.

Statistical analysis

Results are expressed as means ± SEM. Statistical analysis was performed using unpaired Student's *t*-tests and significance was taken as $P \leq 0.05$.

Results

Time course of lithium concentration in plasma and brain
Lithium was not detected in plasma or brain of control rats fed a lithium-free diet. Lithium concentration, expressed as the mean ± SEM of four independent samples, was 0.79 ± 0.07 mM in the brain and 0.74 ± 0.03 mM in the plasma of rats fed lithium for 6 weeks. The brain lithium concentration became equivalent to the plasma concentration after 14 days of daily oral administration, giving an approximate half-life of 1 week to reach a steady state in brain.[28]

Lithium downregulates COX-2 but not COX-1 protein
No statistically significant difference was observed by RT-PCR in brain mRNA levels of COX-1 or COX-2, normalized to the G3PDH mRNA level, between lithium-treated and control rats ($n = 10$) (Figure 1a, b). In Western blots, the COX-2 antibody detected a prominent band at about 72 kDa and the COX-1 antibody at about 70 kDa (Figure 2a). In the lithium-treated group, the COX-2 protein level was decreased by 31.4 ± 5.1% ($P < 0.01$, $n = 12$) compared to the control level (Figure 2b). Thus, chronic lithium downregulated COX-2 post-transcriptionally. In contrast, the COX-1 protein level was not changed by chronic lithium. There also was no decrease in the protein level of the Ca^{2+}-independent $iPLA_2$ (type VI) (Figure 3), suggesting that chronic lithium specifically affects $cPLA_2$,[17] as well as COX-2 protein (Figure 2).

Lithium decreases brain PGE_2 concentration and COX enzyme activity
To see if the observed reduction of COX-2 protein was accompanied by a decrease in PGE_2, an AA metabolite produced by COX-2, we measured PGE_2 in brains of control and lithium-treated rats. The brain PGE_2 con-

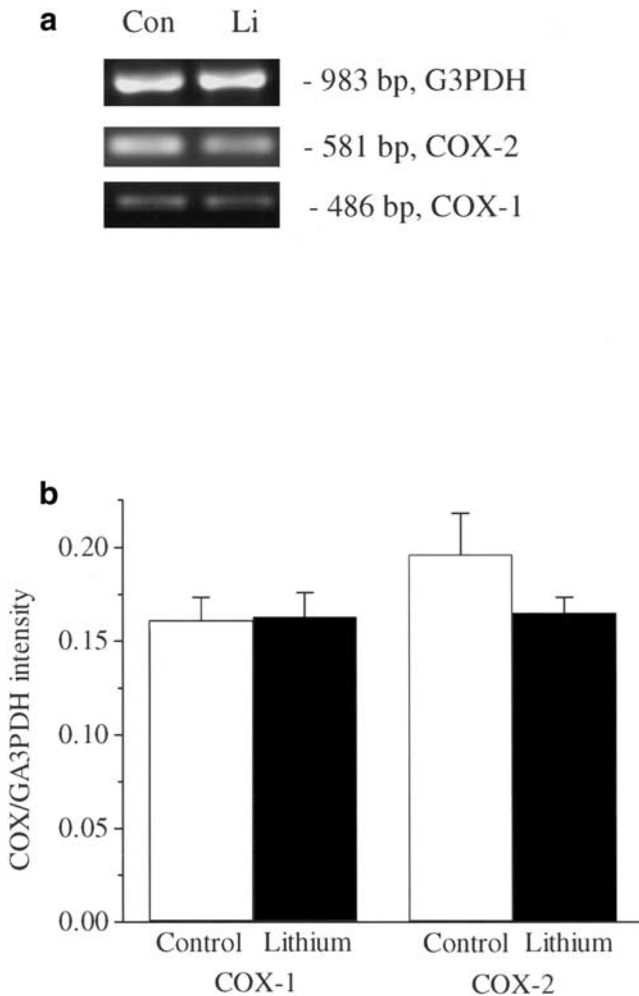

Figure 1 Brain COX-1 and COX-2 mRNA expression following chronic lithium. (a) Representative gel illustrating COX-1, COX-2, and G3PDH mRNA expression in rat brain, assessed by RT-PCR, after lithium administration for 6 weeks compared to controls. (b) COX-1 and COX-2/G3PDH ratios in brain of controls and lithium-treated rats ($n = 10$).

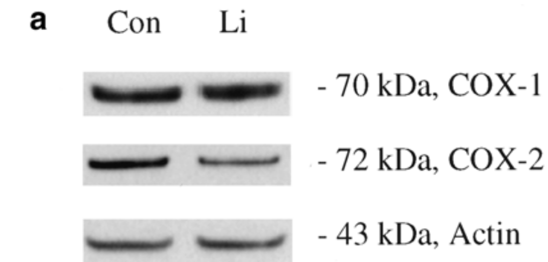

Figure 2 Brain COX-1 and COX-2 protein levels following chronic lithium. (a) Representative immunoblots of COX-1, COX-2 and actin. (b) Relative optical density (OD) of COX-1 and COX-2 to actin. Values are means ± SEM ($n = 12$). ** $P < 0.01$.

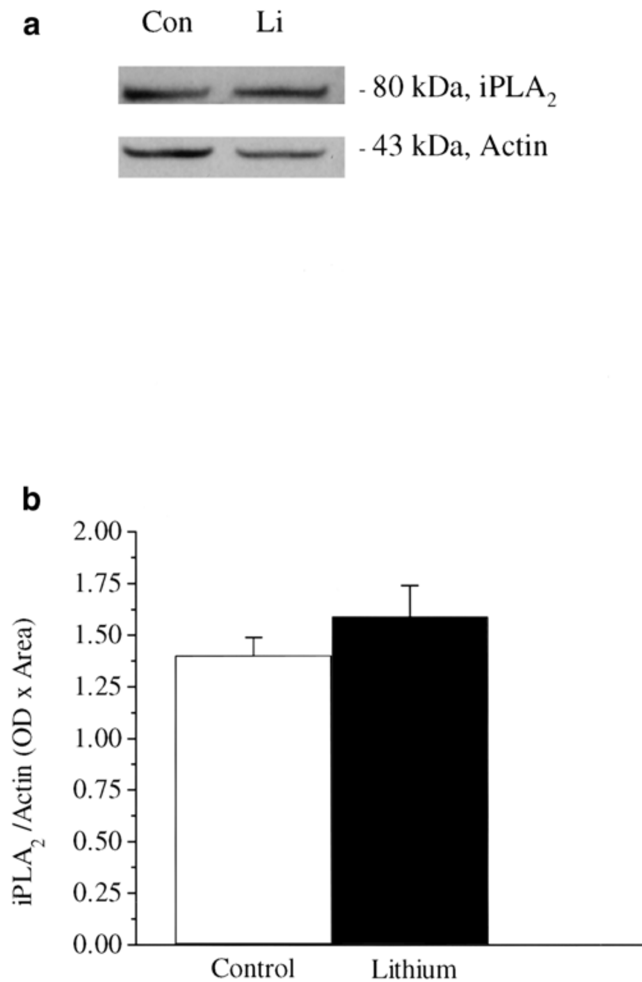

Figure 3 Brain $iPLA_2$ protein level following chronic lithium. (a) Representative immunoblots of $iPLA_2$ (type VI) and actin. (b) Relative optical density (OD) of $iPLA_2$ (type VI) to actin. Values are presented as means ± SEM ($n = 6$).

centration was significantly decreased by 49% in the lithium-treated rats compared to controls (Table 1). COX enzyme activity was decreased significantly in brain tissue of lithium-treated compared to control rats (91.9 ± 5.6 *vs* 182.2 ± 6.0 pg PGE_2 min^{-1} g^{-1} cytosolic protein, $n = 3$, $P < 0.01$) (Figure 4). Preincubation of control samples with 1 mM LiCl did not change COX activity (201.7 ± 11.1 pg min^{-1} g^{-1} protein, $n = 3$) (Figure 4), indicating that lithium did not directly inhibit COX-2, but reduced its activity by a post-transcriptional or post-translational mechanism.

Table 1 Brain prostaglandin E_2 levels in control and chronic lithium-treated rats

Group	*PGE_2 (ng g^{-1} wet brain)*
Control ($n = 5$)	19.8 ± 2.7
Lithium ($n = 5$)	10.1 ± 1.3*

Data are expressed as mean ± SEM. *$P < 0.05$.

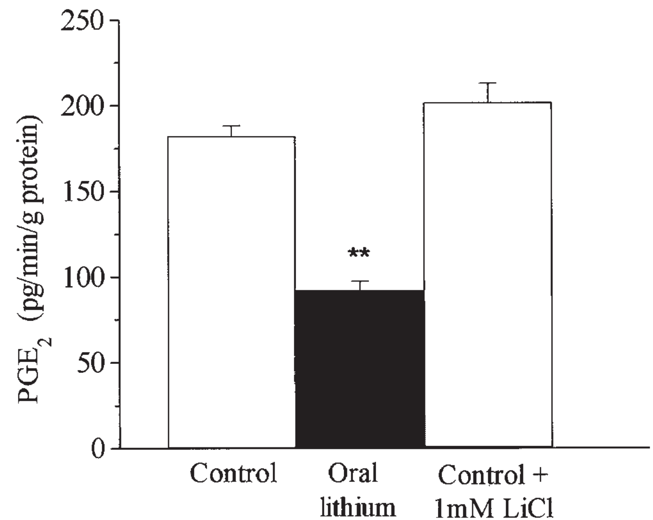

Figure 4 Cyclooxygenase activity following chronic lithium or incubation with LiCl *in vitro*. Brain cytosolic fraction from control and lithium-treated rats was assayed for COX activity. In a separate set of experiments, control cytosolic fraction was incubated with 1 mM LiCl (therapeutic concentration), and then assayed for COX activity to test for direct inhibition. COX activity is expressed as pg PGE_2 min^{-1} g^{-1} cytosolic protein. Data are means ± SEM of three independent samples for each group. **$P < 0.01$.

Discussion

This study supports our hypothesis that the brain AA cascade is a target for lithium. In addition to reducing AA turnover and $cPLA_2$ expression as previously reported,[14–17] we now show that chronic lithium reduces COX-2 protein and its enzymatic activity but not its mRNA level in rat brain. Chronic lithium also reduces the basal brain concentration of PGE_2, a bioactive metabolite of AA produced *via* COX, thus downregulating an important downstream step in the AA cascade. Lithium's effects on the cascade appear to be selective, as brain $iPLA_2$ and COX-1 protein levels were unchanged by chronic lithium, and the mRNA level for $iPLA_2$ is reported to be unaffected.[17]

The selective effect of lithium on $cPLA_2$ and COX-2 may be related to the functional coupling of these enzymes. Studies in isolated cells indicate that labeled AA taken up from the medium cannot be converted to eicosanoids by COX unless it is first esterified into phospholipids and then released by PLA_2.[29] Both $cPLA_2$ and COX-2 are localized at the nuclear envelope and perinuclear area, allowing them to act in a coordinated fashion,[30] and their genes are adjacent to each other on chromosome 1.[31]

The hypothesis that lithium targets $cPLA_2$ and COX-2 function is consistent with evidence in rats that lithium also reduces the brain concentration of arachidonoyl-CoA, the precursor pool for AA reincorporation into phospholipids.[14] Furthermore, valproic acid, also a mood-stabilizer, reduces AA turnover in brain phospholipids as well as the plasma AA concentration in unanesthetized rats.[32] Valproic acid also is reported to reduce levels of lipoxygenase and COX bioactive pro-

ducts in rat platelets.[33] One way to test whether lithium's targeting of the AA cascade is relevant to its therapeutic effect would be to treat bipolar patients with a COX-2 inhibitor.[34] In this regard, aspirin, a non-selective COX inhibitor, is reported to have a beneficial mood-modulating effect when used for antithrombosis.[35]

Reduced AA turnover due to chronic lithium[14] may promote downregulation of COX-2 activity by a post-transcriptional or post-translational mechanism, or lithium may affect transcription or translation of COX-2 in another way. COX-2 mRNA has 'AUUUA' motifs in its 3′-untranslated region (3′-UTR)[36] that confer post-transcriptional control of expression by acting as mRNA instability determinant or as translation inhibitory element.[37] The 3′-UTR of many 'unstable' messages carries motifs that may regulate translational efficiency by reversibly binding to cytosolic or nuclear factors.[38] Chronic lithium treatment, either directly or indirectly, may affect these RNA–protein interactions. Lithium's post-transcriptional effect on COX-2 could also be mediated by reduced formation of the PGE_2 that mitigates COX-2 mRNA decay and inhibition of protein translation, normally mediated by the 3′-UTR region of COX-2 mRNA.[39]

There is limited evidence to date to link abnormal AA signaling to bipolar disorder. It has been suggested that stimulation of prostaglandin synthesis by prolactin or other hormones can contribute to mood disorders.[40] Furthermore, an allelic association has been reported between bipolar disorder in some families and pancreatic PLA_2,[41,42] implying a role for AA. Bipolar patients may have a genetic predisposition to an abnormal circadian rhythm and sleep-wake cycle,[43–46] suggesting a role for PGD_2 and PGE_2, which are said to be involved in sleep-wake regulation. Although PGD_2 is a key eicosanoid in brain signaling and in sleep-wake regulation, its physiological action is opposite to that of PGE_2. Indeed, when PGD synthase, the enzyme that produces PGD_2 in the brain, was inhibited by the intracerebroventricular infusion of its selective inhibitors, the amount of sleep decreased in both a time- and dose-dependent manner.[45] PGE_2, on the contrary, promotes wakefulness.[46] Since mania in bipolar subjects is accompanied by sleep reduction,[23] we focused on PGE_2 because its reduction by a pharmacological agent could normalize the sleep/wake cycle and thereby stabilize mood. This does not rule out a possible alteration in other AA metabolites, such as thromboxanes, leukotrienes, and prostacyclins.

It has been suggested that clinically relevant lithium concentrations can inhibit the synthesis of PGE_1, a metabolite of free dihomogamma-linolenic acid and that, since PGE_1 blocks mobilization of AA, a lack of PGE_1 could be associated with AA mobilization and an excess of the 2 series PGs.[47] However, our results differ from this suggestion, as they show that AA turnover and PGE_2 are decreased by lithium. It also has been reported that lithium modulates the effects of prolactin and vasopressin on prostaglandin biosynthesis, without interfering with basal prostaglandin production.[40] Our results indicate that, in addition to regulating hormone-mediated prostaglandin synthesis,[40] lithium can affect the basal production of PGE_2.

Inhibition of part of the AA cascade by lithium would be consistent with a 'functional' excess of n-6 (eg, AA) compared with n-3 PUFAs (eg, docosahexaenoic acid) contributing to bipolar disorder. Supporting this interpretation is evidence that dietary n-3 PUFA supplementation was beneficial in patients with bipolar disorder,[48] and that seafood consumption, a measure of n-3 PUFA intake, correlated with a lower prevalence of bipolar disorder in a cross-national epidemiological study.[49] The ratio of n-3 to n-6 PUFAs can modulate a number of functionally relevant cellular processes, including PUFA elongation and desaturation, PUFA distribution among complex lipids, and conversion of AA to prostaglandins by COX-2, the latter being inhibited by docosahexaenoic acid.[50–52]

In summary, we have shown that chronic lithium, in addition to reducing AA turnover in rat brain phospholipids, downregulates AA conversion to PGE_2 by COX-2. This effect could contribute to lithium's therapeutic action in bipolar disorder. Although care must be paid in extrapolating data from rodents to a complex human psychiatric disorder, if COX-2 and PGE_2 were found to be increased in postmortem brain or in cerebrospinal fluid from bipolar patients, COX-2 inhibitors could represent a new therapeutic approach for the treatment of this disease.

Acknowledgements

The authors wish to thank Professor Harvey Herschman for his critical review of this manuscript and Jane Bell for technical help.

References

1 Cade JFJ. Lithium salts in the treatment of psychotic excitement. *Med J Austral* 1949; **36**: 349–352.
2 Manji HK, Potter WZ, Lenox RH. Signal transduction pathways. Molecular targets for lithium's action. *Arch Gen Psychiatry* 1995; **52**: 531–543.
3 Jope RS. Anti-bipolar therapy: mechanism of action of lithium. *Mol Psychiatry* 1999; **4**: 117–128.
4 Phiel CJ, Klein PS. Molecular targets of lithium action. *Annu Rev Pharmacol Toxicol* 2001; **41**: 789–813.
5 Chen RW, Chuang DM. Long term lithium treatment suppresses p53 and Bax expression but increases Bcl-2 expression. A prominent role in neuroprotection against excitotoxicity. *J Biol Chem* 1999; **274**: 6039–6042.
6 Axelrod J. Receptor-mediated activation of phospholipase A_2 and arachidonic acid release in signal transduction. *Biochem Soc Trans* 1990; **18**: 503–507.
7 Reddy ST, Herschman HR. Prostaglandin synthase-1 and prostaglandin synthase-2 are coupled to distinct phospholipases for the generation of prostaglandin D_2 in activated mast cells. *J Biol Chem* 1997; **272**: 3231–3237.
8 Fitzpatrick FA, Soberman R. Regulated formation of eicosanoids. *J Clin Invest* 2001; **107**: 1347–1351.
9 Horrobin DF, Manku MS. Possible role of prostaglandin E_1 in the affective disorders and in alcoholism. *Br Med J* 1980; **280**: 1363–1366.
10 Karmazyn M, Manku MS, Horrobin DF. Changes of vascular reactivity induced by low vasopressin concentrations: interactions with cortisol and lithium and possible involvement of prostaglandins. *Endocrinology* 1978; **102**: 1230–1236.

11 Farooqui AA, Horrocks LA, Farooqui T. Glycerophospholipids in brain: their metabolism, incorporation into membranes, functions, and involvement in neurological disorders. *Chem Phys Lipids* 2000; **106**: 1–29.
12 Brash AR. Arachidonic acid as a bioactive molecule. *J Clin Invest* 2001; **107**: 1339–1345.
13 Wolfe LS, Horrocks LA. Eicosanoids. In: Siegel GJ, Agranoff BW, Albers RW, Molinoff PB (eds). *Basic Neurochemistry*, 5th edn. Raven Press: New York, 1994, pp 475–490.
14 Chang MC, Grange E, Rabin O, Bell JM, Rapoport SI. Lithium decreases turnover of arachidonate in several phospholipids. *Neurosci Lett* 1996; **220**: 171–174.
15 Chang MC, Bell JM, Purdon AD, Chikhale EG, Grange E. Dynamics of docosahexaenoic acid metabolism in the central nervous system: lack of effect of chronic lithium treatment. *Neurochem Res* 1999; **24**: 399–406.
16 Chang MC, Jones CR. Chronic lithium treatment decreases brain phospholipase A_2 activity. *Neurochem Res* 1998; **23**: 887–892.
17 Rintala J, Seemann R, Chandrasekaran K, Rosenberger TA, Chang L, Contreras MA *et al*. 85 kDa cytosolic phospholipase A_2 is a target for chronic lithium in rat brain. *Neuroreport* 1999; **10**: 3887–3890.
18 Herschman HR. Prostaglandin synthase 2. *Biochim Biophys Acta* 1996; **1299**: 125–140.
19 Smith WL, Garavito DL, DeWitt DL. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. *J Biol Chem* 1996; **271**: 33157–33160.
20 Kaufmann WE, Worley PF, Pegg J, Bremer M, Isakson P. COX-2, a synaptically induced enzyme, is expressed by excitatory neurons at postsynaptic sites in rat cerebral cortex. *Proc Natl Acad Sci USA* 1996; **93**: 2317–2321.
21 Niwa K, Araki E, Morham SG, Ross ME, Iadecola C. Cyclooxygenase-2 contributes to functional hyperemia in whisker-barrel cortex. *J Neurosci* 2000; **20**: 763–770.
22 Yamamoto T, Nozaki-Taguchi N. Analysis of the effects of cyclooxygenase (COX)-1 and COX-2 in spinal nociceptive transmission using indomethacin, a non-selective COX inhibitor, and NS-398, a COX-2 selective inhibitor. *Brain Res* 1996; **739**: 104–110.
23 Frank E, Swartz HA, Kupfer DJ. Interpersonal and social rhythm therapy: managing the chaos of bipolar disorder. *Biol Psychiatry* 2000; **48**: 430–432.
24 Martinez FE, Harabor A, Amankwah EK, Hart DA, Belik J. Urethane suppresses rat lung inducible cyclooxygenase and nitric oxide synthase mRNA levels. *Inflamm Res* 2000; **49**: 727–731.
25 Feng L, Xia Y, Garcia GE, Hwang D, Wilson CB. Involvement of reactive oxygen intermediates in cyclooxygenase-2 expression induced by interleukin-1, tumor necrosis factor-alpha, and lipopolysaccharide. *J Clin Invest* 1995; **95**: 1669–1675.
26 Powell WS. Reversed-phase high-pressure liquid chromatography of arachidonic acid metabolites formed by cyclooxygenase and lipoxygenases. *Anal Biochem* 1985; **148**: 59–69.
27 Taniguchi Y, Yokoyama K, Inui K, Deguchi Y, Furukawa K, Noda K. Inhibition of brain cyclooxygenase-2 activity and the antipyretic action of nimesulide. *Eur J Pharmacol* 1997; **330**: 221–229.
28 Bosetti F, Seemann R, Bell JM, Zahorchak R, Friedman E, Rapoport SI *et al*. Analysis of gene expression with cDNA microarrays in rat brain after 7 and 42 days of oral lithium administration. *Brain Res Bull* 2002; **57**: 205–209.
29 Furth EE, Hurtubise V, Schott MA, Laposata M. The effect of endogenous essential and nonessential fatty acids on the uptake and subsequent agonist-induced release of arachidonate. *J Biol Chem* 1989; **264**: 18494–18501.
30 Morita L, Schindler M, Regier MK, Otto JC, Hori T, DeWitt DL *et al*. Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2. *J Biol Chem* 1995; **270**: 10902–10908.
31 Tay A, Simon JS, Squire J, Hamel K, Jacob HJ, Skorecki K. Cytosolic phospholipase A_2 gene in human and rat: chromosomal localization and polymorphic markers. *Genomics* 1995; **26**: 138–141.
32 Chang MC, Contreras MA, Rosenberger TA, Rintala JJ, Bell JM, Rapoport SI. Chronic valproate treatment decreases the in vivo turnover of arachidonic acid in brain phospholipids: a possible common effect of mood stabilizers. *J Neurochem* 2001; **77**: 796–803.
33 Szupera Z, Mezei Z, Kis B, Gecse A, Vecsei L, Telegdy G. The effects of valproate on the arachidonic acid metabolism of rat brain microvessels and of platelets. *Eur J Pharmacol* 2000; **387**: 205–210.
34 Verburg KM, Maziasz TJ, Weiner E, Loose L, Geis GS, Isakson PC. COX-2 specific inhibitors: definition of a new therapeutic concept. *Am J Ther* 2001; **8**: 49–64.
35 Ketterer MW, Brymer J, Rhoads K, Kraft P, Lovallo WR. Is aspirin, as used for antithrombosis, an emotion-modulating agent? *J Psychosom Res* 1996; **40**: 53–58.
36 Kujubu DA, Fletcher BS, Varnum BC, Lim RW, Herschman HR. TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. *J Biol Chem* 1991; **266**: 12866–12872.
37 Dixon DA, Kaplan CD, McIntyre TM, Zimmerman GA, Prescott SM. Post-transcriptional control of cyclooxygenase-2 gene expression. The role of the 3′ untranslated region. *J Biol Chem* 2000; **275**: 11750–11757.
38 Hla T, Neilson K. Human cyclooxygenase-2 cDNA. *Proc Natl Acad Sci USA* 1992; **89**: 7384–7388.
39 Faour WH, He Y, He QW, de Ladurantaye M, Quintero M, Mancini A *et al*. Prostaglandin E_2 regulates the level and stability of cyclooxygenase-2 mRNA through activation of p38 mitogen activated protein kinase in interleukin-1β-treated human synovial fibroblasts. *J Biol Chem* 2001; **34**: 31720–31731.
40 Horrobin DF, Mtabaji JP, Manku MS, Karmazyn M. Lithium as a regulator of hormone-stimulated prostaglandin synthesis. In: Johnson FN, Johnson S (eds). *Lithium in Medical Practice*. University Park Press: Baltimore MD, 1978, pp 243–246.
41 Dawson E, Gill M, Curtis D, Castle D, Hunt N, Murray R *et al*. Genetic association between alleles of pancreatic phospholipase A_2 gene and bipolar affective disorder. *Psychiatr Genet* 1995; **5**: 177–180.
42 Jacobsen NJ, Franks EK, Owen MJ, Craddock NJ. Mutational analysis of phospholipase A2A: a positional candidate susceptibility gene for bipolar disorder. *Mol Psychiatry* 1999; **4**: 274–279.
43 Hayaishi O. Prostaglandin D2 and sleep—a molecular genetic approach. *J Sleep Res* 1999; **8**: 60–64.
44 Yoshida Y, Matsumura H, Nakajima T, Mandai M, Urakami T, Kuroda K *et al*. Prostaglandin E (EP) receptor subtypes and sleep: promotion by EP4 and inhibition by EP1/EP2. *Neuroreport* 2000; **11**: 2127–2131.
45 Hayaishi O. Molecular mechanisms of sleep-wake regulation: a role of prostaglandin D2. *Philos Trans R Soc Lond B Biol Sci* 2000; **355**: 275–280.
46 Matsumura H, Honda K, Choi WS, Inoue S, Sakai T, Hayaishi O. Evidence that brain prostaglandin E2 is involved in physiological sleep-wake regulation in rats. *Proc Natl Acad Sci USA* 1989; **86**: 5666–5669.
47 Horrobin DF, Lieb J. A biochemical basis for the actions of lithium on behaviour and on immunity: relapsing and remitting disorders of inflammation and immunity such as multiple sclerosis or recurrent herpes as manic-depression of the immune system. *Med Hypotheses* 1981; **7**: 891–905.
48 Stoll AL, Severus WE, Freeman MP, Rueter S, Zboyan HA, Diamond E *et al*. Omega 3 fatty acids in bipolar disorder: a preliminary double-blind, placebo-controlled trial. *Arch Gen Psychiatry* 1999; **56**: 407–412.
49 Noaghiul S, Hibbeln JR, Weissman MM. Cross-national comparisons of seafood consumption and rates of bipolar disorder. *Am J Psychiatry* (in press).
50 Corey EJ, Shih C, Cashman JR. Docosahexaenoic acid is a strong inhibitor of prostaglandin but not leukotriene biosynthesis. *Proc Natl Acad Sci USA* 1983; **80**: 3581–3584.
51 Rubin D, Laposata M. Cellular interactions between n-6 and n-3 fatty acids: a mass analysis of fatty acid elongation/desaturation, distribution among complex lipids, and conversion to eicosanoids. *J Lipid Res* 1992; **33**: 1431–1440.
52 Seung Kim HF, Weeber EJ, Sweatt JD, Stoll AL, Marengell LB. Inhibitory effects of omega-3 fatty acids on protein kinase C activity *in vitro*. *Mol Psychiatry* 2001; **6**: 246–248.