

Decrease in the AP-2 DNA-Binding Activity and in the Protein Expression of AP-2 α and AP-2 β in Frontal Cortex of Rats Treated with Lithium for 6 Weeks

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Lithium chloride (LiCl), when fed to rats for 6 weeks, has been reported to decrease brain mRNA, protein, and activity levels of arachidonic acid (AA)-selective cytosolic phospholipase A₂ (cPLA₂), without affecting secretory sPLA₂ or Ca²⁺-independent iPLA₂. We investigated whether transcription factors known to regulate cPLA₂ gene expression are modulated by chronic lithium treatment. Male Fischer-344 rats were fed a LiCl-containing diet for 6 weeks to produce a therapeutically relevant brain lithium concentration. Control animals were fed a LiCl-free diet. Using a gelshift assay, we found that LiCl significantly decreased activating protein 2 (AP-2)-binding activity, and protein levels of the AP-2 α and AP-2 β but not of the AP-2 γ subunits in the frontal cortex. Activating protein 1 (AP-1)-binding activity was increased, whereas glucocorticoid response element, polyoma enhancer activator 3, and nuclear factor kappa B DNA-binding activities were not changed significantly. Since both cPLA₂ and AP-2 can be activated by protein kinase C (PKC), we examined the frontal cortex protein levels of PKC α and PKC ϵ , as well as AA-dependent PKC activity. The protein levels of PKC α and PKC ϵ were decreased significantly, as was AA-dependent PKC activity, in the lithium-treated compared to control rats. Our results suggest that the reported decrease in brain gene expression of cPLA₂ by chronic lithium may be mediated by reduced AP-2 transcriptional activity, and that decreased expression of PKC α and PKC ϵ contributes to lowering the AP-2 activity.

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INTRODUCTION

Lithium is commonly used to treat bipolar disorder, but its precise mechanism of action is not clear. Evidence that lithium's mood-stabilizing effect requires weeks to develop (Jope and Williams, 1994; Manji *et al*, 1999) suggests alterations at the gene expression level (Hyman and Nestler, 1996), possibly mediated by the activation or inactivation of signaling cascades. It is well established that chronic, but not acute, treatment with lithium results in induction and altered expression of many genes in cells (Asghari *et al*, 1998) and in the intact rat brain (Bosetti *et al*, 2002b). Thus, it is possible that there is a chronic alteration in the steady-state activity of a given gene induced by prolonged lithium exposure. Another reason for delayed gene expression during lithium feeding to intact animals is the approximately 1-week half-life for lithium to reach a

steady-state brain concentration, due to its slow penetration across the blood–brain barrier (Bosetti *et al*, 2002b). In this regard, lithium has been reported to target G-proteins (Li *et al*, 1993; Miki *et al*, 2001; Wang and Friedman, 1999), cyclic adenosine monophosphate (cAMP) (Mork, 1993; Mork and Geisler, 1995), protein kinase A (Mori *et al*, 1998), protein kinase C (PKC) (Manji *et al*, 1993, 1999, 1996; Soares *et al*, 2000) and its substrate MARCKS (Lenox *et al*, 1992; Watson and Lenox, 1996), glycogen synthase kinase-3 beta (GSK-3 β) (De Sarno *et al*, 2002), and activating protein 1 (AP-1) transcription factor (Ozaki and Chuang, 1997; Yuan *et al*, 1999). We reported that 6 weeks of lithium administration to rats, so as to produce therapeutically relevant plasma and brain lithium concentrations, resulted in reduced arachidonic acid (AA) turnover in brain phospholipids (Chang *et al*, 1996). In addition, chronic lithium reduced brain cytosolic phospholipase A₂ (cPLA₂) activity (Chang and Jones, 1998), protein and mRNA expression, and downregulated COX-2-mediated AA metabolism to prostaglandin E₂ (Bosetti *et al*, 2002a; Rintala *et al*, 1999). The effect on cPLA₂ was selective, as secretory sPLA₂ and Ca²⁺-independent iPLA₂ were unaltered (Weerasinghe *et al*, 2004). Activation of cPLA₂ is mediated by Ca²⁺ and phosphorylation (Nemenoff *et al*, 1993).

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Protein kinase C (PKC) comprises a large family of phospholipid-dependent protein-serine/threonine kinase proteins, has 12 known subspecies in mammalian tissues, and can be activated by free Ca²⁺, phospholipids, or, in brain extracts, by AA (Koide *et al*, 1992). PKC also plays a role in the stimulus-mediated activation of cPLA₂ (Xu *et al*, 2002), and lithium has been reported to influence PKC distribution and translocation in platelets of bipolar disorder patients (Friedman *et al*, 1993). Postmortem studies have also reported increased membrane-associated PKC and increased translocation of PKC isoforms from cytosol to membrane in response to phorbol 12-myristate 13-acetate in frontal cortex of brains from bipolar disorder compared with control subjects (Wang and Friedman, 1996). In the rat brain, chronic lithium treatment reduced cytosolic PKC α and PKC δ and increased membranal PKC ζ immunoreactivities, without altering total PKC activity (Wang *et al*, 2001). Chronic lithium also suppressed the translocation of PKC α and of other PKC isoforms in rat brain (Hahn and Friedman, 1999).

In this study, we further characterized the mechanism of downregulation of brain cPLA₂ expression and activity by chronic lithium, by examining brain transcription factors recognized on the promoter region of the cPLA₂ gene: AP-1, activation protein 2 (AP-2), glucocorticoid response element (GRE), polyoma enhancer activator 3 (PEA3), and nuclear factor kappa B (NF- κ B) (Morri *et al*, 1994). A decreased DNA-binding activity of one or more of these factors might be responsible for the reported decrease in the brain cPLA₂ mRNA level by lithium (Rintala *et al*, 1999). Since cPLA₂ can be phosphorylated and activated by PKC, we also determined protein levels of PKC α and PKC ε as well as AA-dependent PKC activity, in frontal cortex of control and lithium-treated rats. We studied the frontal cortex because of evidence of decreased gray matter volume, and reduced neuronal and glial densities, in the frontal cortex of bipolar disorder patients (Lopez-Larson *et al*, 2002; Lyoo *et al*, 2004; Rajkowska, 2002).

MATERIALS AND METHODS

Animals

The study was approved by the National Institute of Child Health and Human Development (NICHD) Animal Care and Use Committee, in accordance with NIH guidelines on the care and use of laboratory animals. Eight adult male Fischer F-344 rats (200–250 g) were given LiCl at 1.70 g/kg in their chow (Teklad, Madison, WI, USA) for 4 weeks, followed by chow (Teklad) containing LiCl at 2.55 g/kg for another 2 weeks (Chang *et al*, 1996).

To prevent hyponatremia, water and NaCl solution (450 mM) were made available to all animals. This feeding regimen produces therapeutically relevant plasma and brain lithium concentrations of \sim 0.8 mM (Bosetti *et al*, 2002b). Control rats received a lithium-free diet for the same period. Rats were killed by an overdose of sodium pentobarbital (100 mg/kg, i.p.). The brains were rapidly excised and the frontal cortex was dissected, frozen in 2-methylbutane at -50°C and stored at -80°C until use.

Preparation of Cytosolic and Nuclear Extracts from Rat Frontal Cortex

Cytoplasmic and nuclear extracts were prepared from frontal cortex of control and lithium-treated rats, as described (Lahiri, 1998). Briefly, tissue was homogenized in 10 mM HEPES, pH 7.9, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 10 mM KCl buffer with a cocktail of protease inhibitors (Roche, Indianapolis, IN) using a Teflon-glass homogenizer. After adding 0.5% NP-40, five additional strokes of homogenization were performed. The suspension was incubated for 10 min on ice, and then centrifuged at 13 000 g for 1 min at 4 $^{\circ}\text{C}$. The supernatant contained mostly cytoplasmic constituents. To the nuclear pellet, solution B (20 mM HEPES, pH 7.9, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 0.4 M NaCl) with a cocktail of protease inhibitors (Roche) was added. Tubes were mixed and placed on a small rotatory shaker for 30 min. Finally, the mixture was centrifuged at 13 000 g for 3 min at 4 $^{\circ}\text{C}$. The supernatant containing the proteins from the nuclear extracts was transferred to a fresh tube. The protein concentrations of cytoplasmic and nuclear extracts were determined using Bio-Rad protein Reagent (Bio-Rad, Hercules, CA).

Electrophoretic Mobility Shift Assay (EMSA)

A gelshift assay was performed with 15 μg of nuclear extract, incubated with a nonradioactive (10 ng) biotin-labeled DNA oligo consensus (Panomics, Redwood city, CA) in gelshift buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM MgCl₂, 0.5 mM EDTA, 0.5 mM DTT, 4% glycerol, and 50 $\mu\text{g}/\text{ml}$ poly dI:dC) for 30 min on ice. The DNA-protein complex was separated on 5% TBE gel and electrophoretically transferred to a nylon membrane. Biotin-labeled oligonucleotide complexes were visualized using a streptavidin-HRP conjugate coupled with chemiluminescence on X-ray film (Kodak, Rochester, NY). The following oligonucleotide sequences were used for the gelshift assay: AP-1: CGCTTGATGAGTCAGCCGGAA; NF- κ B: AGTTGAGGGG ACTTTCCCGGC; AP-2: GATCGAACTGACCGCCCGCGG CCCGT, and PEA3: GATCT CGAGCAGGAAGTTCGA. The specificity of each transcription factor was determined by using 100 times excess unlabeled probe with a fixed amount of biotin-labeled DNA oligo consensus (10 ng) and nuclear extracts (15 μg). All the gelshift assay experiments were carried out twice with eight independent samples for each group. Optical densities of gelshift bands were quantified using Alpha Innotech software (Alpha Innotech, San Leandro, California). Values were expressed as percent of control.

Western Blot Analysis

In total, 60 μg of cellular or nuclear extract was separated on 10% SDS-polyacrylamide gels (Bio-Rad). Following SDS-PAGE, proteins were electrophoretically transferred to a nitrocellulose membrane. After overnight blocking in PBS containing 5% nonfat dried milk and 0.1% Tween-20, blots were incubated overnight with 1:1000 specific primary antibodies for AP-2 α , AP-2 β , or AP-2 γ (Santa Cruz, Santa Cruz, CA). PKC α and ε protein levels were also determined in cytoplasmic extracts from control and lithium-treated

groups using specific primary antibodies for PKC α and PKC ε (1:1000, Santa Cruz), followed by HRP-conjugated secondary antibodies (Bio-Rad). Blots were visualized using a chemiluminescence reaction (Amersham, Piscataway, NJ) on X-ray film (XAR-5, Kodak). All the experiments were carried out twice with eight independent samples for each group. Optical densities of the immunoblots were measured using Alpha Innotech software (Alpha Innotech). Optical densities were normalized to β -actin (Sigma, St Louis, MO) to correct for differences in loading. Data were expressed as percent of control.

Total RNA Isolation and Real-Time RT-PCR

Total RNA was isolated from control and lithium-treated brain samples using an RNeasy mini kit for brain and lipid tissue (Qiagen, Valencia CA). cDNA was prepared from total RNA using a high-capacity cDNA Archive kit (Applied Biosystems, Foster City, CA). Brain AP-2 α and AP-2 β mRNA levels in control and lithium-treated animals were measured by real-time quantitative RT-PCR, using ABI PRISM 7000 sequence detection system (Applied Biosystems). Specific primers and probes for AP-2 α and AP-2 β were purchased from the available Assays-on-Demand (Applied Biosystems), which consist of a 20 \times mix of unlabeled PCR primers and Taqman minor groove binder (MGB) probe (FAM dye-labeled). The fold change in gene expression was determined by using the $\Delta\Delta C_T$ method (Livak and Schmittgen, 2001). Data were expressed as the relative level of the target gene (AP-2 α -AP-2 β) in the lithium-treated animals normalized to the endogenous control (β -globulin) and relative to the control rats (calibrator), as previously described (Ghelardoni *et al*, 2004). Each sample was assayed in triplicate and all the experiments were carried out twice with six independent samples. Data were expressed as relative to expression of controls.

PKC Activity

Basal and AA-dependent PKC activities were measured in brain cytoplasmic extracts (10 μ g) using a nonradioactive PKC kinase assay kit (Stressgen Bioreagents, Victoria, BC, Canada). This is an enzyme linked immunoabsorbent assay that uses a specific synthetic peptide as a substrate for PKC and a polyclonal antibody that recognizes the phosphorylated form of the substrate. Briefly, total cytoplasmic extract protein (10 μ g) was incubated with immobilized substrate in the absence and presence of 40 μ M AA. The reaction was initiated by adding ATP. The kinase reaction was terminated after 60 min of incubation, and then a phosphospecific substrate antibody was added. A peroxidase-conjugated secondary antibody subsequently was bound to the phosphospecific antibody. The assay was developed by adding the tetramethylbenzidine substrate (TMB) and intensity of the color was measured at 450 nm. The intensity of color development is proportional to phosphotransferase activity. Individual sample reading was corrected by subtracting the corresponding blank reading (without cytoplasmic extracts) and dividing by the log μ g of protein. Seven to eight independent samples were used in each group and each sample was assayed in triplicate and

experiments carried out twice. All the values within the intra- and interassay coefficient of variation of <10% were taken for calculating PKC activities. Values were expressed in relative PKC activity.

Statistical Analysis

Data were expressed as mean \pm SEM. Statistical significance was calculated using a two-tailed unpaired *t*-test, and set as $p < 0.05$.

RESULTS

Physiological Parameters

Rats fed with lithium diet showed a significant decrease in mean body weight compared to control rats (240 ± 5 g *vs* 281 ± 6 g, $p < 0.0001$, $n = 16$). This effect has been ascribed to uncompensated lithium-induced polyuria (Teixeira and Karniol, 1982). Lithium feeding did not affect mean body temperature, heart rate, or arterial blood pH, pO₂, pCO₂, or blood pressure (data not shown).

The Effect of Lithium on the DNA-Binding Activity of the Transcription Factors that Regulate cPLA₂ Expression

To determine whether the reported downregulation of brain cPLA₂ mRNA by chronic lithium occurs at the level of transcription, we measured DNA-binding activities of different transcription factors that are known to regulate cPLA₂ gene transcription. Lithium significantly decreased by 42% the binding of nuclear proteins to the AP-2 consensus sequence ($p < 0.05$) (Figure 1a). In contrast, it increased AP-1 DNA-binding activity by 66% ($p < 0.05$) (Figure 1b), consistent with a previous report (Ozaki and Chuang, 1997). Binding activities of GRE, PEA3, or NF- κ B DNA were not significantly changed (Figure 1c). As shown in Figure 2, in the presence of an excess (100 times) of unlabeled specific oligonucleotides, the binding of labeled oligonucleotides was blocked, indicating the specificity of DNA-binding activities.

The Effect of Lithium on AP-2 α , AP-2 β , and AP-2 γ Expression

To further investigate whether the changes in brain AP-2 DNA-binding activities in lithium-treated animals were due to a change in protein levels of AP-2 subunits, the protein levels of AP-2 α and β were measured by Western blotting. Chronic lithium decreased the protein level of AP-2 α in both cytoplasmic and nuclear extracts (by 47 and 38%; respectively, $p < 0.05$) (Figure 3a). The protein level of AP-2 β also was reduced in the two extracts, by 44 and 46%, respectively ($p < 0.05$) (Figure 3b), compared to control levels. In contrast, the protein level of the AP-2 γ subunit was not significantly changed by lithium (Figure 3c).

To determine whether the changes in protein levels of AP-2 α and AP-2 β in lithium-treated rats were due to changes in their mRNA expression, mRNA levels of AP-2 α and AP-2 β were measured using real-time RT-PCR. We did not find any statistically significant change in the mRNA level of

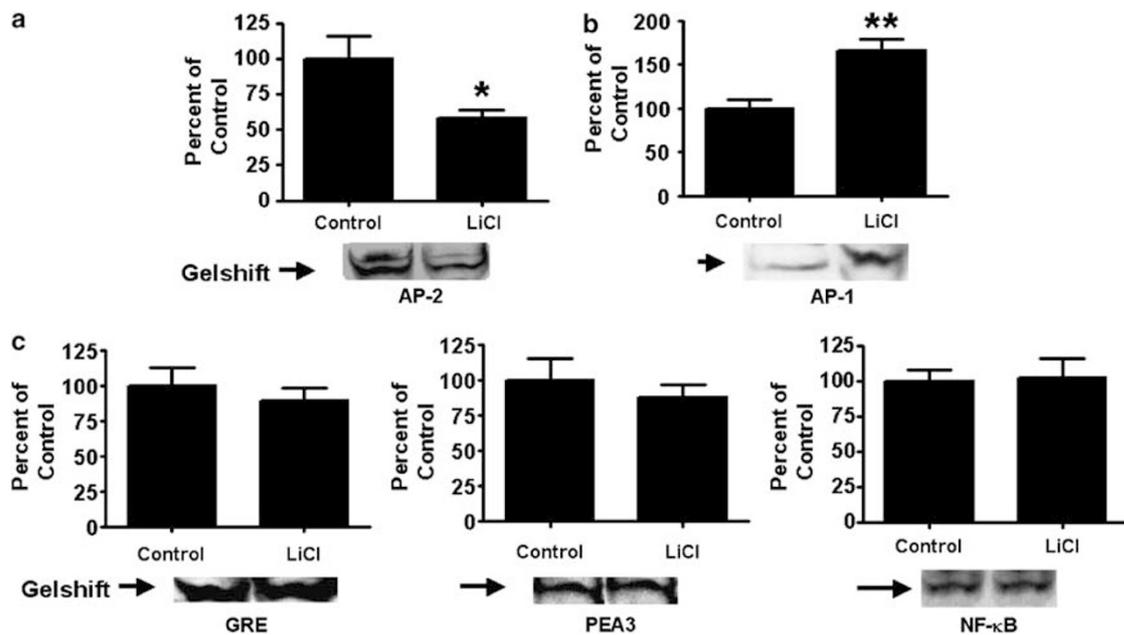


Figure 1 Representative gelshift bands of AP-2 (a), AP-1 (b), GRE, PEA3, and NF- κ B (c) in frontal cortex from control and lithium-treated rats. DNA-binding activity was measured in brain nuclear extracts, as described in the Materials and Methods. Data are mean \pm SEM expressed as percent of controls, and compared using an unpaired *t*-test ($n = 8$ independent samples). * $p < 0.05$; ** $p < 0.01$.

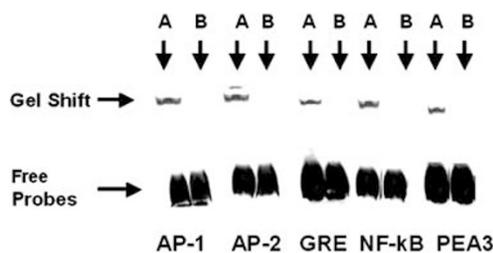


Figure 2 DNA-binding activities for AP-1, AP-2, GRE, NF- κ B, and PEA3 in rat frontal cortex. Specificity of DNA–protein-binding activities was tested in nuclear extracts in the absence (A) and presence (B) of excess (100 times) unlabeled probe, as described in the Materials and Methods. The unlabeled probe blocked the binding of labeled probe and decreased the gelshift.

either AP-2 α (0.89 ± 0.21) or AP-2 β (1.0 ± 0.22) in lithium-treated animals compared to controls (AP-2 α ; 1.0 ± 0.10 ; AP-2 β ; 1.0 ± 0.18).

The Effect of Lithium on PKC Expression and Activity

Since cPLA₂ and AP-2 can be activated by phosphorylation by PKC, which in turn can be activated by AA (Koide *et al*, 1992), we determined whether the reported downregulation by chronic lithium of brain PLA₂ activity (Chang and Jones, 1998) is accompanied by a change in PKC activity or in the protein level of PKC α or PKC ϵ . Chronic lithium decreased the protein levels of PKC α by 35% ($p < 0.01$) (Figure 4a) and of PKC ϵ by 27% ($p < 0.05$) (Figure 4b) in frontal cortex compared to control levels. To determine whether the above changes in protein level had affected basal and AA-dependent PKC activities, we also measured PKC activity in the presence and absence of AA. In pilot experiments,

different concentrations of AA in the range 0–100 μ M (0, 20, 40, 60, 100 μ M) were used to choose the optimal AA concentration changing PKC activity. AA increased PKC activity in a dose-dependent manner with a threshold of 40 μ M (data not shown). As shown in Figure 4c, total PKC activity in the presence of 40 μ M AA was decreased by 35% ($p < 0.001$) in lithium-treated rats (0.65 ± 0.05) compared to controls (1.0 ± 0.02). However, PKC activity, measured in the absence of exogenous AA, was not significantly changed.

DISCUSSION

In this study, we examined transcription factors known to regulate cPLA₂ gene expression in frontal cortex of rats treated for 6 weeks with lithium so as to produce a therapeutically relevant brain lithium concentration. We found a significant decrease in AP-2 DNA-binding activity in the lithium-treated compared with control rats. This change was accompanied by a significant decrease in the protein levels of AP-2 α and β subunits, but not in their mRNA expression. Chronic lithium also did not alter the protein level of AP-2 γ . In contrast, AP-1 DNA-binding activity was increased, whereas DNA-binding activities of other transcription factors on the promoter region of cPLA₂ gene—GRE, PEA3, and NF- κ B—were not changed significantly. Chronic lithium decreased the protein level of PKC α and PKC ϵ , and reduced AA-dependent PKC activity. Lithium-fed rats also showed a decrease in body weight, likely due to the lithium-induced polyuria, without a significant change in other measured physiological parameters.

Our results suggest that decreased AP-2 DNA-binding activity may be responsible for the reported downregulation

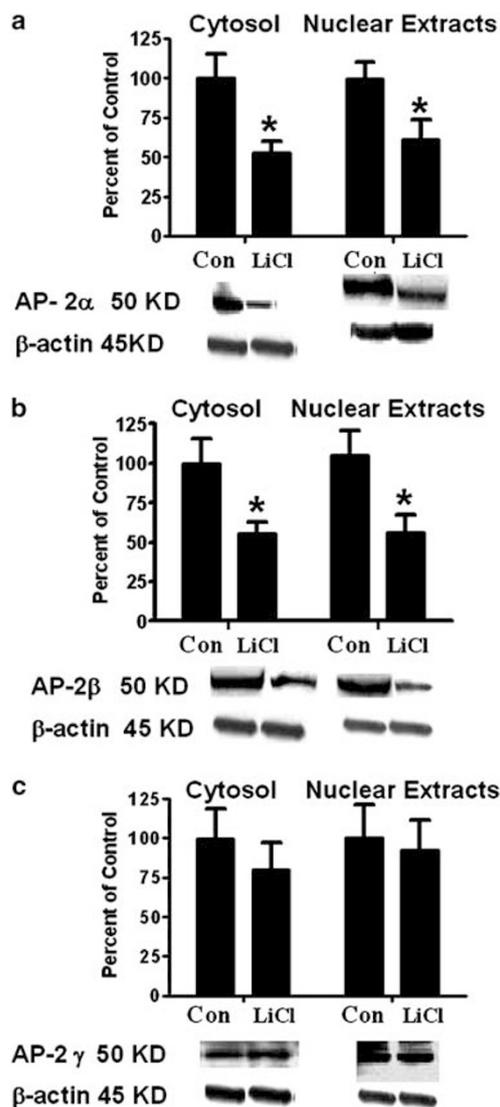


Figure 3 Cytosolic and nuclear protein levels of AP-2 α (a), AP-2 β (b), and AP-2 γ (c) in frontal cortex from control and lithium-treated rats. Immunolabeling of cytoplasmic and nuclear AP-2 proteins was performed as described in the Materials and Methods. Data are ratios of optical density of each AP-2 isoform to that of β -actin, expressed as percent of control and are compared using an unpaired *t*-test (mean \pm SEM, $n=8$ independent samples, * $p < 0.05$).

of brain gene and protein expression of cPLA₂ by chronic lithium (Rintala *et al*, 1999). The results agree with a study in which 11 days of lithium treatment decreased protein binding to AP-2 DNA consensus in nuclear extracts of rat brain (Damberg *et al*, 2000). However, because in that study the protein levels of AP-2 α and β were not changed, the decrease by lithium in AP-2 α or β might be dose- and/or time-dependent. Since the mRNA levels of these subunits remained unchanged, it is possible that lithium's effect on AP-2 occurs at a post-transcriptional level. The decreased protein levels of AP-2 α and β that we found after 6 weeks of lithium treatment could further contribute to the decreased AP-2 DNA-binding activity.

Activation of AP-2 requires phosphorylation of its subunits by PKC or PKA (Imagawa *et al*, 1987). Phos-

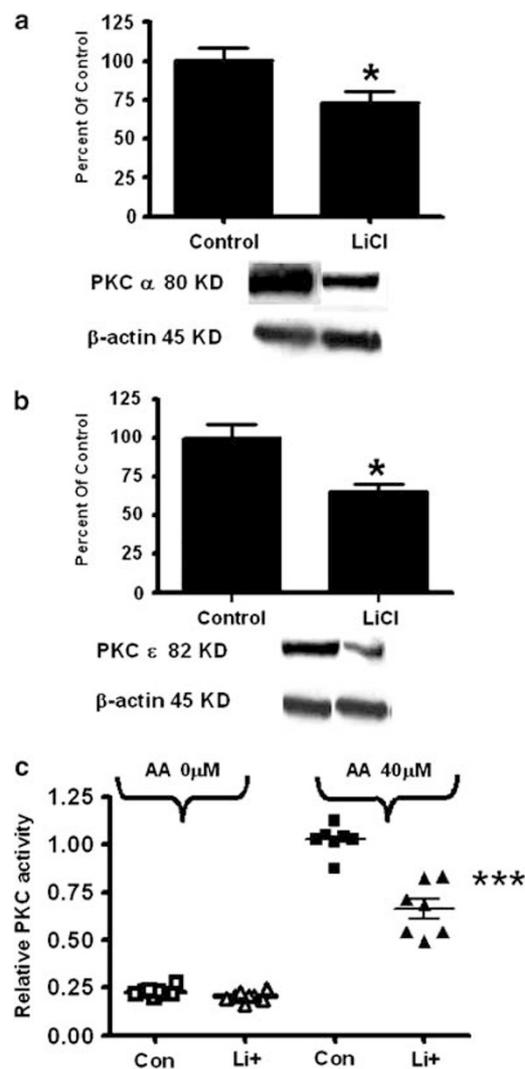


Figure 4 Protein expression of cytoplasmic PKC α (a) and PKC ϵ (b), and PKC activity (c) in frontal cortex from control and lithium-treated rats. Immunolabeling of PKC α and PKC ϵ was performed as described in the Materials and Methods. Data are the ratios of the optical densities of PKC α ($n=8$) and PKC ϵ ($n=8$) to that of β -actin in cytosolic fractions. Data are expressed as percent of control. Total PKC activity was measured in the absence (basal; 8 independent samples) and in the presence (activated) of 40 μ M AA. Data in panel (c) are mean \pm SEM ($n=7-8$ independent samples). Data are expressed as relative PKC activity and compared using an unpaired *t*-test. * $p < 0.05$; *** $p < 0.001$.

phorylated AP-2 subunits translocate to the nucleus, where they recognize a specific AP-2-binding sequence on chromatin to initiate transcription. Since PKC is involved in the activation of both cPLA₂ and AP-2, both of which are downregulated by lithium, we measured PKC activity in the absence of exogenous AA as well after adding 40 μ M AA. AA, which is released from cell membrane phospholipids by PLA₂ enzymes, is involved in many physiological processes including activation of PKC α and PKC ϵ in rat brain (Koide *et al*, 1992). Ours is the first study to show a significant decrease in AA-stimulated PKC activation by chronic lithium treatment, without a change in basal (unstimulated) PKC activity. This finding agrees with our report that the

phosphorylation state of rat brain cPLA₂ under basal conditions was not changed significantly by chronic lithium (Weerasinghe *et al*, 2003).

Earlier studies indicated that chronic lithium treatment altered PKC isoforms in rat frontal cortex, without changing total tissue PKC activity (Wang *et al*, 2001). This absence of change in total PKC activity was ascribed to a high abundance of PKC γ , a neuron-specific PKC, which is the major contributor to total PKC activity, thus masking changes in activities of the other PKC isoforms (Wang *et al*, 2001). The lack of effect on basal activity by lithium that we observed in this study may be due to the contribution of activity by other PKC isoforms, which masked the changes in the PKC α and PKC ϵ activities. In contrast to its effect on PKC, chronic lithium is reported not to change cAMP-dependent PKA activity in the cytosolic fraction of rat cortex (Jensen and Mork, 1997), suggesting that PKA may not be involved in lithium's effect on AP-2 activation.

Decreased brain expression of PKC α and PKC ϵ and of AA-dependent PKC activity following chronic lithium may contribute to the downregulation of AP-2 DNA-binding activity. The decreases also may lead to a reduced stimulus-mediated release of AA. This is supported by a report that prolonged exposure of mouse primary astrocytes to 100 nM phorbol myristate acetate caused a time-dependent downregulation of PKC α and PKC ϵ , as well as of AA release (Xu *et al*, 2002). Another report, however, did not find any change in protein levels of PKC isoforms in frontal cortex of 3-week lithium-treated rats (Manji *et al*, 1996). The discrepancy might be due to differences in the Western blotting procedures or in the duration of lithium treatment (3 weeks *vs* our 6 weeks).

Rapid activation of cPLA₂ through AA-stimulated PKC activation may play a role in cerebral ischemia and inflammation, where cPLA₂ activity is elevated and PKC is altered (Manji *et al*, 1996; Saluja *et al*, 1999; Stella *et al*, 1997). In contrast, modification of gene expression may require hours to days. This might involve the downregulation by chronic lithium of cPLA₂ gene expression via lithium's effects on AP-2, which also depend on PKC. In a fast response, PKC activates cPLA₂ activity. PKC can be activated by diacylglycerol, calcium, and AA. PKC in turn activates the AP-2 transcription factor. Chronic lithium reduces protein levels of PKC and AP-2 subunits in rat brain, which results in reduced AA-dependent PKC activity and AP-2 DNA binding. This may lead to a reduced expression of cPLA₂.

Our results suggest that chronic lithium decreased AA-dependent PKC activity in the frontal cortex leading to reduced activation of AP-2, which in turn may downregulate cPLA₂ gene expression (Rintala *et al*, 1999). This downregulation likely modifies neuronal signaling, considering cPLA₂'s role in several signal transduction pathways in brain (Sun *et al*, 2004). This effect also has clinical implications, as evidence indicates that PKC expression is upregulated in brains from patients with bipolar disorder (Hahn and Friedman, 1999; Wang and Friedman, 1996). PKC activation in bipolar disorder could trigger the activation of cPLA₂ and increase AA release and metabolism, supporting the hypothesis of a 'hyperactive AA cascade' in bipolar disorder (Rapoport and Bosetti, 2002). Additionally, AP-2 has been implicated in neurodevelop-

ment (Moser *et al*, 1997) and monoaminergic activity (Damberg *et al*, 2001).

The increase that we found in the AP-1 DNA-binding activity in the frontal cortex of chronic lithium-treated rats agrees with earlier reports (Asghari *et al*, 1998; Ozaki and Chuang, 1997; Yuan *et al*, 1999), and suggests that lithium independently modulates different transcription factors. The increase might be due to activation of c-Jun N-terminal kinases (Chen *et al*, 2003; Yuan *et al*, 1999), which phosphorylate one subunit of AP-1 (cJun-cFOS), or to inhibition of GSK-3 (Klein and Melton, 1996; Stambolic *et al*, 1996). GSK-3 β phosphorylates c-Jun at three sites adjacent to its AP-1 consensus sequence binding domains, thereby reducing AP-1-binding activity (Lin *et al*, 1993). The upregulation of AP-1 activity, in turn, might affect expression of other genes whose transcription is controlled by the AP-1 family of transcription factors, including genes for neuropeptides, neurotrophins, receptors, enzymes involved in neurotransmitter biosynthesis, and bcl-2 (Brunello, 2004).

The absence of a lithium effect on the DNA-binding activities of GRE, PEA3, and NF- κ B suggests that these transcription factors are not involved in the downregulation of cPLA₂ gene expression by chronic lithium. It remains unclear whether decreased AP-2 DNA-binding activity is solely responsible for the downregulation of cPLA₂ gene expression, or if there is crosstalk between increased AP-1 and decreased AP-2 in modulating cPLA₂ gene expression. It also is possible that lithium decreases cPLA₂ mRNA by affecting its stability, and studies in cultured cells may clarify this issue. Additionally, studying single cells might clarify the direct involvement of AP-2 in regulating cPLA₂ gene expression in lithium-treated neuronal cell lines.

There is evidence that a clinically relevant lithium level in plasma produces beneficial effects in animal models of depression (Angelucci *et al*, 2003). Lithium fed 42 days to Flinders-sensitive line rats produced significant changes in brain levels of NGF, BDNF, and GDNF compared to Flinders-resistant line rats (Angelucci *et al*, 2003). Changes in BDNF can rectify behavioral deficits in an animal model of depression in learned helplessness and in the forced swim test (Siuciak *et al*, 1997). Chronic lithium also corrects disrupted circadian clocks (Abe *et al*, 2000; Schibler, 1998) and can affect the endocrine system while decreasing CRF1 mRNA in the amygdala and frontal cortex (Gilmor *et al*, 2003). Overall, studies suggest that chronic lithium can alter many neurochemical and endocrinological pathways, which modulate neuronal plasticity, and normalize behavioral defects in animal models.

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