Lithium Regulation of Aldolase A Expression in the Rat Frontal Cortex: Identification by Differential Display

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Background: Substantial evidence indicates that lithium may exert its therapeutic effects through progressive adaptive changes at the level of gene expression; however, the study of lithium-regulated genes has been primarily undertaken with the “candidate gene” approach based on a specific testable hypothesis. The aim of our study was to identify lithium-regulated genes that would not be predicted a priori by the candidate gene approach.

Methods: Differential display polymerase chain reaction was used to isolate and identify messenger RNAs (mRNAs) that are differentially expressed in the frontal cortex of rats given lithium for 5 weeks to achieve plasma lithium concentrations of 0.6 to 0.9 mmol/L.

Results: A putative lithium-regulated complementary DNA fragment (LRG1) was identified. Northern blot analysis revealed that 5 weeks of lithium treatment, but not 1 week, significantly reduced LRG1 mRNA levels. LRG1 mRNA levels were similarly reduced by 5 weeks of carbamazepine, but not valproate administration. Sequence analysis and search of the GenBank database revealed that LRG1 is analogous to the sequence of the gene for rat aldolase A.

Conclusions: These results demonstrate that chronic administration of lithium, but not short-term administration, downregulates the levels of aldolase A mRNA, suggesting this effect may play a role in mediating the therapeutic action of this agent. Biol Psychiatry 2000;48: 58–64 © 2000 Society of Biological Psychiatry

Key Words: mRNA, differential display, aldolase A, lithium, carbamazepine, gene expression, bipolar disorder

Introduction

Although lithium is the primary drug used in the acute and prophylactic treatment of bipolar affective disorder, the neurobiological substrates underlying its therapeutic efficacy are still poorly understood. Recent evidence suggests that molecular and cellular changes in critical neuronal circuitry thought to be dysregulated in bipolar disorder may be involved in its mood-stabilizing effects (reviewed in Jope 1999; Li et al 2000; Manji et al 1995). These compensatory and homeostatic responses, which include modulation of intracellular signaling cascades, produce substantial and long-lasting changes in neuronal function, effects that reset the postulated signaling abnormalities in bipolar affective disorder to their normal functional range (Hudson et al 1993; Jope 1999; Li et al 2000; Manji et al 1995).

Since the clinical action of lithium is marked by a latency of onset, regulation of gene expression has been hypothesized to be one of the potential mechanisms by which lithium exerts its mood-stabilizing effects (Hyman and Nestler 1996; Jope 1999). Indeed, a growing body of evidence supports the notion that lithium, at clinically effective doses, is capable of regulating the expression of a number of genes in rat brain and cultured-cell models. For example, chronic lithium treatment reduces the mRNA levels of Gαs, Gαi1, and Gαi2 (Li et al 1991), but increases those of adenylyl cyclase types I and II in the rat cortex (Colin et al 1991). Long-term treatment with lithium also increases the mRNA levels of dynorphin A, prodynorphin, preprotachykinin (Sivam et al 1988, 1989), and dopamine D2 receptor (Dziedzicka-Wasylewska and Wedzony 1996) in the rat striatum. Moreover, maintained exposure to lithium increases the levels of 2',3'-cyclic nucleotide 3'-phosphodiesterase mRNA in rat C6 glioma cells (Wang and Young 1996) and the expression of nitric oxide synthase type 2 in rat astrocytes (Feinstein 1998). The transcriptional effects of lithium may be mediated by alterations in stimulus-transcriptional coupling through dampening of the intracellular second messenger systems.
Accordingly, it has been shown that chronic lithium treatment decreases basal c-fos mRNA levels and differentially modulates the stimulus-induced c-fos expression in the rat brain (Miller and Mathe 1997). Long-term administration of lithium also leads to increased transcription factor binding to activator protein-1 (AP-1) and cyclic AMP-responsive element (CRE) in the rat brain (Ozaki and Chuang 1997). Similarly, in vitro incubation of rat C6 glioma cells and human SH-SY5Y cells with therapeutically relevant concentrations of lithium altered AP-1 or CRE DNA binding activity (Asghari et al. 1998; Wang et al. 1999; Yuan et al. 1998). Thus, modulation by lithium of c-fos expression or transcription factor activity may have the potential to regulate downstream gene expression, ultimately promoting the cellular adaptation in critical neuronal circuits that lead to mood stabilization.

The changes in gene expression induced by lithium that have been tested to date have been primarily hypothesis driven. With the candidate gene approach, transcriptional changes in other genes may be overlooked unless they occur in the neurobiological cascade(s) of those genes for which expression changes have already been found. Differential display polymerase chain reaction (dd-PCR) is an emerging technique that permits systematic comparison of the entire transcript repertoire in various cells or tissues (Liang and Pardee 1992). As an initial step in uncovering other known or novel genes that display transcriptional changes following chronic lithium administration, we applied the technique of dd-PCR to screen and identify genes that are differentially regulated in the frontal cortex of rats treated chronically with lithium. We report here the downregulation of aldolase A expression after 5 weeks of lithium treatment but not after 1 week, an effect that was shared by chronic carbamazepine (CBZ) administration but not by valproate (VPA) administration.

Methods and Materials

Chemicals

Lithium carbonate (Li₂CO₃), CBZ, and sodium VPA were obtained from Sigma Chemical Co. (St. Louis). [α-³²P]Deoxy-cytidine triphosphate ([α-³²P]dCTP; ~3000 Ci/mmol) and [α-³¹P]Deoxyadenosine triphosphate ([α-³¹P]dATP; ~2000 Ci/mmol) were purchased from New England Nuclear (Boston). Other reagents were acquired as molecular biological grade from commercial sources.

Animal Treatments

Male Wistar rats (300–350 g; Charles River, St. Constant, Canada) were individually housed in a temperature-controlled room (21°C ± 1°C) and maintained on a 12-hour light/dark cycle with free access to food and water for at least a week before experiments. Animals were fed rat chow in pellet form containing Li₂CO₃ (2.2 g/kg diet; Bioserve, Frenchtown, NJ) for 1 or 5 weeks. Water and 2.6% saline were provided ad libitum to all animals. Rats receiving CBZ were fed with food pellets containing 0.25% CBZ for the first 4 days followed by 0.5% CBZ (Bioserve) for the next 31 days. In the VPA comparison group, animals were maintained on chow pellets containing 0.4% VPA (Bioserve) for 5 weeks. Separate control groups of rats were fed the regular rat chow for the indicated period. At the beginning of the experiment, there was no difference in weights of control (326 ± 6 g, mean ± SEM, n = 6) and mood-stabilizing drug treatment groups (lithium, 331 ± 8 g; CBZ, 323 ± 11 g; VPA, 328 ± 3 g; n = 6 in each group). Animals receiving mood-stabilizing drugs had weight gains [lithium, 104 ± 16 g; CBZ, 110 ± 10 g; VPA, 126 ± 5 g; F(3,20) = 0.865, p = .4] similar to those of control subjects (120 ± 7 g) during the treatment periods. Animals used in this study were cared for in strict accordance with guidelines of the Canadian Council on Animal Care, and the study was approved by the local Animal Care Committee.

At the end of the experiments, animals were decapitated. The brain was rapidly removed and the frontal cortex was dissected over ice, frozen on dry ice, and stored at −70°C until use. Blood samples were collected immediately after decapitation from the cervical trunk and put into heparinized test tubes. Plasma was separated by centrifugation (900 g, 20 min) for subsequent determination of drug levels. Plasma lithium levels were determined using the Vitros Li Slides (Johnson & Johnson, Mississauga, Canada), a colorimetric assay for lithium–crown–ether dye complex and ranged from 0.62 to 0.92 mmol/L in the lithium-treated rats. Mean (± SEM) plasma CBZ concentrations were 16 ± 4 μmol/L, as determined by the Vitros CRBM Slides (Johnson & Johnson), an immunoassay using anti-CBZ antibody. Plasma VPA levels were quantitated by the TDXFLX valproic acid assay system using fluorescence polarization immunoassay (Abbott Laboratories, Abbott Park, IL) and were 13 ± 4 μmol/L in the VPA-treated rats.

Extraction of RNA

Total RNA from the frontal cortex was isolated by the guanidinium isothiocyanate–cesium chloride method as previously described (Li et al. 1993). Residual chromosomal DNA was removed from total cellular RNA using the MessageClean kit (GenHunter, Nashville) according to the manufacturer's instructions. Ribonucleic acid quantitation was determined by absorbance at 260 nm, and the integrity evaluated by electrophoresis on 1% formaldehyde agarose gels and ethidium bromide staining.

mRNA Differential Display

Differential display was carried out essentially as previously described (Liang and Pardee 1992) using the RNAmap kit (GenHunter). DNase-treated total RNA (0.2 μg) was reverse transcribed to cDNA using SuperScript II reverse transcriptase (GIBCO-BRL, Canada) and one of the 3' composite anchor primers T₁₂MN (M = A, C, or G and N = A, C, G, or T). Two
microliters of the cDNA was subjected to PCR amplification in duplicate in 20 μL of PCR buffer (10 mmol/L Tris-HCl [pH 8.3], 50 mmol/L KCl, 1.5 mmol/L MgCl2, 0.001% [wt/vol] gelatin) with 2 μmol/L each of 2'-deoxynucleoside 5'-triphosphate (dNTP), 0.2 μmol/L of various arbitrary primers, 0.2 μmol/L of the respective anchor primer, 0.3 μL of [α-33P]dATP (2000 Ci/mmol, New England Nuclear), and 1 U of AmpliTaq (Perkin-Elmer, Branchburg, NJ). The PCR was conducted using the following program on an MJ Research (Waltham, MA) thermal cycler (model PTC-200): an initial denaturation at 95°C for 2 min followed by 40 cycles at 94°C for 30 sec, 40°C for 2 min, and 72°C for 30 sec, then a final extension at 72°C for 5 min, and rapid cooling at 4°C. The radiolabeled amplified cDNA fragments were then separated on a 6% (wt/vol) denaturing polyacrylamide sequencing gel. The gel was vacuum dried at 80°C with rapid cooling at 4°C. The radiolabeled amplified cDNA fragments were then separated on a 6% (wt/vol) denaturing polyacrylamide sequencing gel. The gel was vacuum dried at 80°C for 2 hours and autoradiographed (24 to 48 hours). Autoradiographic bands that were visually different in intensity between control and lithium-treated animals were excised from the dried gel and rehydrated, and the DNA was reamplified by PCR using the appropriate set of primers under the PCR conditions described above, except that the dNTP concentration was 25 μmol/L and no radioisotope was included.

DNA Cloning

The reamplified DNA was electrophoresed on a 1.5% agarose gel and the band of interest was excised and purified using a QIAEX Gel Extraction Kit (Qiagen, Chatsworth, CA) according to the manufacturer’s instructions. The recovered DNA was ligated into the pGEM-T vector system (Promega, Madison, WI). Ampicillin-resistant colonies were selected from ampicillin X-Gal LB agar plates and plasmid minipreps were prepared using standard procedures. Plasmids harboring inserts were identified by either restriction enzyme digestion using sites on the vector flanking the cloning sites or PCR screening using primers across the cloning sites.

Complementary DNA fragments isolated from plasmids were labeled with [α-32P]dCTP using a random-primed DNA-labeling kit (Boehringer Mannheim Canada, Laval) to a specific activity of 1 × 107 cpm/μg and used as probes for Northern blot analysis.

Reverse Northern Blot Analysis

Plasmids containing the cDNAs of interest or cyclophilin were denatured by boiling in 200 μL of 0.4 mol/L NaOH and 10 mmol/L EDTA for 10 min, and neutralized with an equal volume of 2 mol/L ammonium acetate, pH 7.0. Each plasmid was spotted onto two replicas of nylon membrane (GeneScreen Plus, New England Nuclear) at three concentrations ranging from 0.2 to 1.8 μg using the Bio-Dot microfiltration apparatus (Bio-Rad Laboratories, Richmond, CA). The duplicate blots were UV-crosslinked in a UV-Stratalinker 2400 (Stratagene, La Jolla, CA), and prehybridized for 4 hours at 42°C in 5X SSPE, 5% formamide, 5X Denhardt’s solution, 1% sodium dodecyl sulfate, 10% dextran sulfate, and 200 μg/mL denatured salmon sperm DNA, and subsequently hybridized with a 32P-labeled, random-primed cDNA probe (1 × 106 cpm/mL) prepared from a gel-purified insert. After stringent washing, hybridization signals were obtained by autoradiography using phosphoscreens. The blots were stripped and rehybridized with 32P-labeled rat cyclophilin cDNA to normalize for RNA loading and transfer.

DNA Sequence Analysis

The pGEM-T clone inserts were manually sequenced from both directions with T7 or M13-Reverse primer using the ThermoSequenase sequencing kit (Amersham Pharmacia Biotech, Baie d’Urfé, Canada). Homology search was performed using the GenBank DNA database and the BLAST algorithms.

Statistical Analysis

Statistical analysis of the data was performed using either one-way analysis of variance, followed by post hoc Tukey’s test to assess differences between cell means or two-tailed unpaired Student’s t test. Values of p < .05 were considered statistically significant. Data were expressed as mean ± SEM.

Results

mRNA Differential Display

With 80 combinations of the degenerate anchored oligo(dT) and arbitrary primers, 20 putative differentially expressed bands were identified in rats treated chronically with lithium. These 20 candidate cDNA fragments were subcloned and further screened by reverse Northern blot using cDNAs labeled by reverse transcription of the frontocortical RNA samples from control or lithium-
Effects of Chronic Treatment of Other Mood Stabilizers on LRG1 mRNA Levels

To assess whether the regulation of LRG1 mRNA level was unique to lithium or common to other mood-stabilizing agents, the effects of chronic lithium, VPA, and CBZ treatment on the levels of LRG1 mRNA were studied (Figure 3). The analysis of variance showed a significant main effect of drug on LRG1 mRNA levels \( F(3,16) = 5.70, p = .008 \). Post hoc analysis revealed that the levels of LRG1 mRNA were significantly reduced in the rat frontal cortex following either chronic CBZ \( t(8) = -4.92, p = .006 \) or lithium \( t(8) = -3.75, p = .04 \) treatment. There was a tendency for a decrease in LRG1 mRNA level (84% of control group) following chronic VPA administration; however, these changes were very small and not statistically significant \( t(8) = -2.15, p = .36 \).

Sequence Analysis and Homology

Results from the DNA sequencing revealed that LGR1 contained 540 bp and had the expected primers at its 5’ and 3’ ends. LRG1 contained a putative polyadenylation signal that was located 21 bp upstream from the poly A tail, further supporting the identity of this cDNA fragment as the 3’-untranslated end of the corresponding transcript. Using the BLAST algorithms to search the GenBank database, the sequence of LRG1 displayed a near perfect homology (>99%) to the nucleotide sequence (921–1441) for rat aldolase A mRNA (Figure 4).

Discussion

In our study the technique of dd-PCR was used to identify genes that show transcriptional changes in response to chronic lithium administration. In particular, the expression of a cDNA fragment (LRG1) was downregulated by lithium in the rat frontal cortex following a 5-week treatment period. Northern blot analysis confirmed the decreased expression of this transcript by chronic but not short-term lithium treatment.

Comparison of the sequence for LRG1 against those in the GenBank database revealed that this fragment corresponds to aldolase A, a key enzyme in glycolysis producing energy. This is further supported by the finding that the LRG1 probe hybridized to a single transcript of 1.4 kb, as previously reported for aldolase A (Joh et al 1985).

The biochemical role of aldolase A was originally described in relation to the glycolysis pathway catalyzing the aldol cleavage of fructose 1,6-diphosphate. Previous studies have shown that lithium in vitro inhibits the activity of regulatory metabolic enzymes such as fructose 1,6-bisphosphatase (Marcus and Hosey 1980) and glycogen synthase kinase-3β (GSK-3β; Klein and Melton 2000; 48:58–64). Thus, the reduction in aldolase A mRNA levels could conceivably be related to the effect of lithium on carbohydrate metabolism; however, the associations between the temporal effect of lithium on aldolase A expression and the apparent delay in the therapeutic response to lithium in bipolar affective disorder patients suggest that the changes in aldolase A expression may be therapeutically relevant. Indeed, aldolase A has been reported to modulate several other cellular functions that on closer perusal suggest effects that could be important to the therapeutic action (or side effects) of lithium. For example, aldolase A has been shown to interact physically.
with tubulin and actin filaments, an action that may contribute to the regulation of cytoskeletal structures and cell mobility (Kao et al 1999; Kusakabe et al 1997; Volker and Knull 1997). Second, the presence of aldolase A in the nucleus and its ability to interact with certain DNA sequences suggest a potential role of aldolase A in modulating transcriptional activity (Ronai et al 1992). Furthermore, the finding that aldolase A binds inositol 1,4,5-trisphosphate (InsP3) suggests a potential role for this enzyme in regulating the free intracellular concentrations of this second messenger, and subsequently intracellular calcium dynamics (Baron et al 1999; Koppitz et al 1986). This is of particular pathophysiologic importance given the well-documented observations of altered calcium homeostasis in bipolar affective disorder (Dubovsky et al 1992; Emamghoreishi et al 1997). Although it may seem difficult at first to ascribe any direct significance of these diverse cellular functions affected by aldolase A to the therapeutic action of lithium, the observations that chronic lithium treatment exerts modulatory effects on neuronal cytoskeletal architectures, transcription factors activity, InsP3 signaling, and intracellular calcium dynamics (Jope 1999) shed a different light on the role of aldolase A in these cellular changes regulated by lithium.

Moreover, it has recently been suggested that lithium regulation of cytoskeletal networks and AP-1 transcriptional factor activity may be mediated via inhibition of GSK-3β, another multifunctional metabolic enzyme (for a review see Jope 1999).

With the above considerations, it is not unreasonable to assume that some of these diverse cellular effects of lithium may also involve other metabolic enzymes such as aldolase A. The recent suggestion that GSK-3β may represent a potential target of lithium in the treatment of bipolar affective disorder (Agam and Levine 1998; Klein and Melton 1996) underscores the importance of lithium regulation of “multifunctional” metabolic enzyme(s) in its psychotherapeutic mechanisms. Of additional interest in this regard, increased serum aldolase A activity has been reported in a much earlier study of a small group of manic bipolar disorder patients (Meltzer 1968). Although the significance of the increased aldolase A activity was not clear at that time, it was suggested that an increase in cell permeability or decreased clearance of the enzyme from serum might be the peripheral manifestation of a pathologic process occurring in the central nervous system (Meltzer 1968). Although this has not yet been proven, our findings of reduced expression of aldolase A following chronic lithium treatment offer a more interesting possibility that dysregulation of aldolase A might occur in the brain of bipolar disorder patients.

Although an array of neurochemical, cellular, and mo-
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lecular actions of lithium has been described (Jope 1999; Li et al 2000; Manji et al 1995), it is often difficult to identify which of these targets underlie the mood-stabilizing effect of lithium and which reflect secondary or side effects of the drug. We have approached this question by determining whether alteration in aldolase A expression is shared by other mood-stabilizing agents. We found that chronic CBZ administration, but not VPA administration, also reduced the expression of aldolase A. The similar changes in aldolase A mRNA produced by chronic lithium and CBZ treatment may represent one of the molecular correlates of the therapeutic overlap (or side effects) of these mood stabilizers in the treatment of bipolar disorders. On the other hand, the lack of effect of chronic VPA administration on aldolase A expression may represent dissimilar molecular mechanisms of action for lithium and VPA, a notion supported by clinical evidence suggesting that VPA is more efficacious than lithium in the treatment of rapid-cycling bipolar affective disorder and mixed manic states (Calabrese et al 1994).

In conclusion, in this study we have provided the first evidence that chronic lithium administration regulates the expression of aldolase A, a multifunctional enzyme, in the brain. These findings raise the possibility that downregulation of aldolase A expression may play a role in mediating the therapeutic action of lithium.

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References


Colin SF, Chang HC, Mollner S, Pfeuffer T, Reed RR, Duman

![Figure 4. Nucleotide sequence homology of LRG1 and rat aldolase A (Gene Bank Accession No. M12919). Identical residues are shown as ‖, and the polyadenylation signal is underlined.](image-url)


