Effect of Lithium on Phosphoinositide Metabolism in Human Brain: A Proton Decoupled ^{31}P Magnetic Resonance Spectroscopy Study

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Background: *The objective of our study was to evaluate whether lithium increases brain phosphomonoester (PME) levels in human subjects.*

Methods: *Proton decoupled ^{31}P magnetic resonance spectra were obtained from eight healthy volunteers before and after the administration of lithium carbonate, 450 mg b.i.d., for 7 and 14 days.*

Results: *Pairwise comparisons of the mole percent PME revealed a significant increase from baseline at day 7 and day 14 of lithium administration.*

Conclusions: *An increase in PME concentration with 7 and 14 days of lithium administration in the human brain in vivo was observed. Because the inositol-1-monophosphate contributes to the PME peak, this result suggests that some of the initial actions of lithium may occur through a reduction of* myo-*inositol, which in turn may initiate a cascade of secondary changes at different levels of signal transduction process and gene expression in brain, effects that are ultimately responsible for the therapeutic benefits of lithium.* Biol Psychiatry 2001;50: 3–7 © *2001 Society of Biological Psychiatry*

Key Words: Lithium, inositol monophosphatase, proton decoupled ^{31}P MRS, inositol depletion

Introduction

Lithium, at therapeutically relevant concentrations, is an uncompetitive inhibitor of the enzyme inositol monophosphatase (IMPase; K_i = 0.8 mmol/L), and lithium administration is assumed to result in an accumulation of inositol-1-monophosphate (I1P), as well as a reduction in free inositol (Manji et al 1999; Renshaw et al 1986b). Thus, it has been hypothesized that a physiologic consequence of lithium's action is derived through a depletion of free *myo*-inositol in the brain (Berridge et al 1982, 1989).

A few human magnetic resonance spectroscopy (MRS) studies have investigated the effect of lithium on the enzyme IMPase. Using proton (^{1}H) MRS, Moore et al (1999) documented a lithium-induced decrease in *myo*-inositol concentrations in bipolar patients. Although subject to a Type II error by using ^{31}P MRS, Kato et al (1993), found no change in PME concentrations in six manic patients examined before and after initiation of lithium. Another study utilizing ^{31}P MRS found that in 21 psychotic patients given lithium for 2 weeks, there were no changes in PME concentrations (Keshavan et al 1995). Silverstone et al (1996, 1999) did not observe any significant effects of 7 days of lithium administration either on PME ratios or on *myo*-inositol levels in temporal lobe of healthy volunteers; however, they reported a significant increase on the PME ratios after the stimulation of the phosphoinositide cycle by amphetamine (Silverstone et al 1999).

In ^{31}P MR spectra of brain, it is estimated that inositol monophosphates constitute only about 10 to 15% of the total PME peak area (Renshaw et al 1987). Under normal circumstances, the concentration of I1P is 0.05 to 0.1 mmol/L, whereas with ^{31}P MRS the minimum concentration for detection is 0.5 to 1 mmol/L (Gyulai et al 1984; Tofts and Wray 1988). Thus, a 5- to 10-fold increase in I1P concentration would be necessary for detection as an increase in the PME resonance (Tofts and Wray 1988).

There are a limited number of ways by which the sensitivity of ^{31}P MRS experiments may be increased. In particular, proton decoupling (Kato et al 1998; Murphy-Boesch et al 1993) and increasing the volume of tissue examined are the easiest methods to implement on an existing scanner.

To investigate the effect of lithium on the enzyme IMPase, we collected proton decoupled ^{31}P MR spectra from human subjects before and after the administration of lithium. We hypothesized *a priori* that the decoupled ^{31}P MR spectra obtained from a large volume of brain would reveal increases in the PME concentrations after the chronic administration of lithium.

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Methods and Materials

Subjects and Study Design

Subjects were screened with the Structured Clinical Interview for Diagnosis (SCID), the Hamilton Rating Scale for Depression (Hamilton 1960), and the Young Mania Rating Scale (Young et al 1978) by a trained research psychiatrist. The subjects also had a medical history and physical examination, including an electrocardiogram (ECG); a blood chemistry panel for renal function tests, thyroid, and liver function tests; and a urine toxicology screen to determine surreptitious drug use and, for female subjects, pregnancy status.

Subjects were excluded for any SCID Axis-I diagnosis; family history of affective illness in first-degree relatives; neurologic illness; abnormalities in the blood, urine, or ECG; or for contraindication for MR scan.

All subjects gave full informed consent, and the Massachusetts General Hospital Institutional Review Board approved the study. Subjects received a baseline structural MRI and MRS scan and then were given lithium carbonate 900 mg/day (450 mg b.i.d.) for 14 days. Subjects received a second MRS scan on day 7 and the final MRS scan on day 14.

Our study included 8 subjects, 4 women and 4 men, who met eligibility criteria. They had a mean age of 38.9 ± 6.4 (18–45). Subjects had serum lithium level on day 7 (obtained 12 hours after last lithium dose) of between 0.40 and 0.72 (mean 0.57) mmol/L and on day 14 of between 0.40 and 0.70 (mean 0.58) mmol/L.

Magnetic Resonance Spectroscopy

Subjects were examined using a 1.5 T GE SIGNA MR system equipped for broadband spectroscopy. Proton MR images were acquired in the sagittal and axial planes to provide morphologic information and to select the slice to be studied in the MRS imaging exam. These images were acquired with the proton channel of a home-built, dual-tuned proton-phosphorous head coil (SPGR, 256 × 196, 1 NEX, 28 slices, slice thickness 5 mm with no gap, TE = 5 m/sec, TR = 40 m/sec, flip angle 40°). Phosphorous MRS data were acquired from 50-mm axial slice (~ 620 cm^3 brain volume) centered on the superior corpus callosum using a short TE (TE = 3 m/sec) slice selective spin echo pulse sequence (Lim et al 1994; Figure1).

Proton decoupling was applied using a Waltz-4 pulse sequence (Murphy-Boesch et al 1993). The spectra were fit using VARPRO/AMARES/MRUI time domain fitting package (van den Boogaart et al 1994; Figure 1). For each metabolite, the peak area was normalized by the total phosphorous signal, yielding mole percent metabolite values representing the percentage of the total ^{31}P signal intensity contributed by the metabolite (Klunk et al 1994).

Statistical Analysis

Paired samples Student's *t* test was used to examine changes within a group over days. Temporal changes in the PME concentrations also were assessed by using within-subjects repeated measures analysis of variance (ANOVA). Significance level was set at $p < .05$. All reported *p* values are two sided.

Results

When we made pairwise comparisons of the mole percent PME values over the decoupled ^{31}P MR spectra, there was a significant increase following chronic lithium administration both on day 7 ($p < .04$, $t_{df = 7} = 2.57$, SD (SD) of change = 1.67), and also on day 14 compared with baseline ($p < .04$, $t_{df = 7} = 2.58$, $SD_{change} = 1.34$; Figure 2, Table 1). These changes reflected a medium effect size (Cohen's *d*, for day 7 = 0.71 > 0.5; Cohen's *d*, for day 14 = 0.57 > 0.5; Figure 2, Table 1). We carried further analysis with ANOVA repeated measures. There was a significant change in PME values over the time following chronic lithium administration. [$F = 3.96(df = 2, 14)$ $p < .05$]. With post hoc comparisons, this difference was found to result from the increase in PME values from baseline to day 7.

Discussion

In this longitudinal study, we have shown for the first time, to our knowledge, that lithium treatment increases PME levels in human brain. This increase was observed at the 1st week and maintained through the 2nd week of lithium administration.

Prior studies showing an increase in the PME peak following lithium administration to cats and rats, using in vitro MRS, determined that this change was due to increases of I1P concentrations (Preece et al 1992; Renshaw et al 1986a, 1987).

Four prior MRS studies investigating effect of lithium in human brain detected no significant difference either on the substrate or on the product of the enzyme IMPase (Kato et al 1993; Keshavan et al 1995; Silverstone et al 1996, 1999); however, in the fourth study, Silverstone et al (1999), using a placebo-controlled double-blind protocol, detected an increased PME peak in volunteers given lithium for a week who were then subjected to amphetamine challenge. Our study, and Moore and colleagues' ^1H MRS study, demonstrate lithium's inhibitory effect on the enzyme IMPase in the human brain in vivo without any agonist stimulation (Moore et al 1999).

Inconsistencies in the effects of lithium on the phosphoinositide cycle on human systems in vivo may be due to variations in study populations, experimental procedures, the region of interest, and technical limitations of current MRS experiments. Our study addressed some of these methodologic shortcomings by studying a large brain volume and applying proton decoupling to increase the spectral resolution and the sensitivity of the ^{31}P MRS (Kato et al 1998). We believe this enabled us to detect a significant increase in the PME peak following lithium administration.

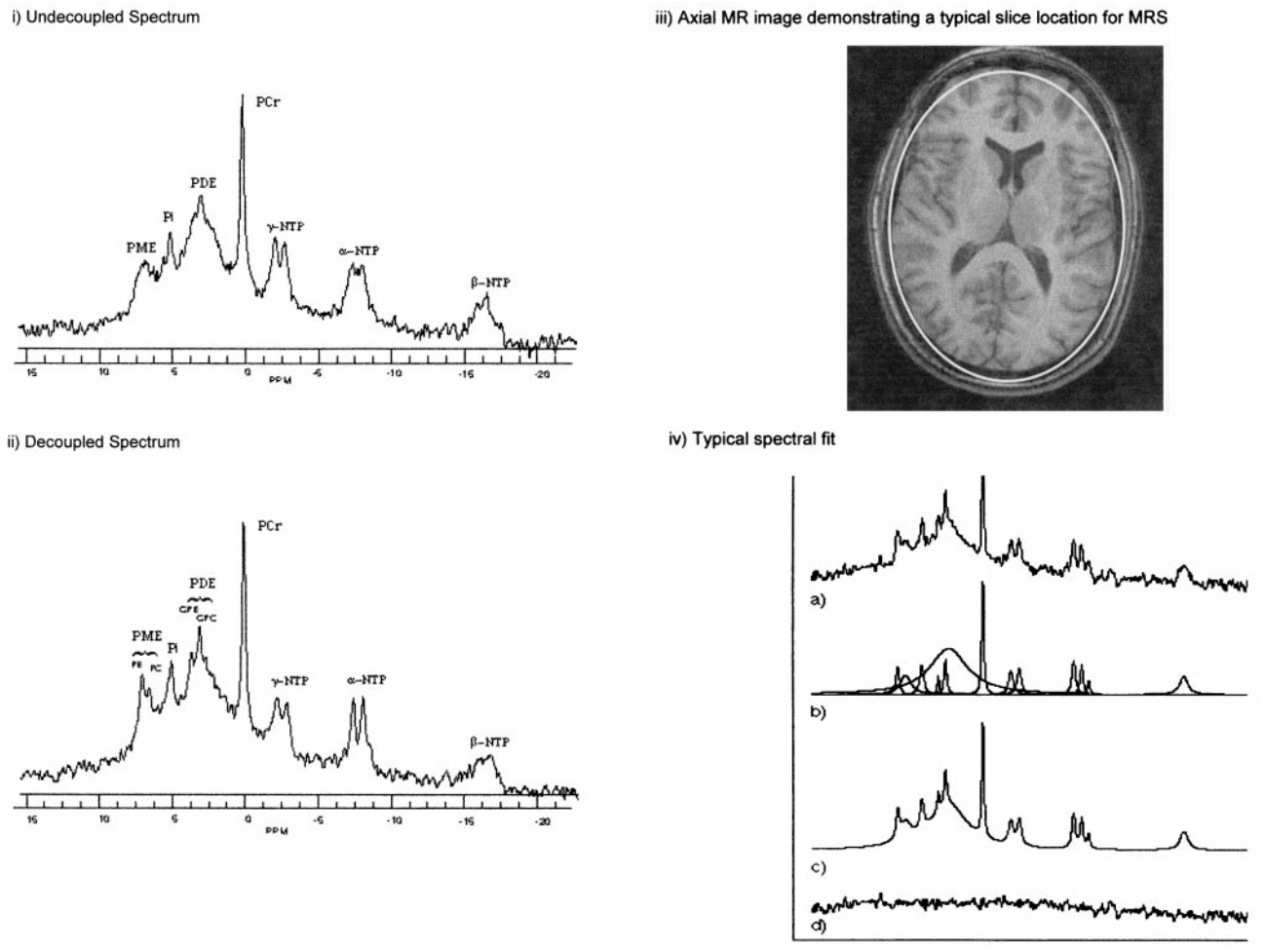

Figure 1. Typical ^{31}P-MR (magnetic resonance) spectra i) undecoupled, ii) decoupled, iii) axial MR image demonstrating a typical slice location for ^{31}P-MRS, and iv) a typical fit of the spectrum. Frequency units are expressed in parts per million (PPM). PME, phosphomonoesters; PE, phosphoethanolamine; PC, phosphocholine; Pi, inorganic phosphate; PDE, phosphodiesters; GPE, glycerophosphoethanolamine; GPC, glycerophosphocholine; PCr, phosphocreatine; γ, α, and β-NTP, gamma, alpha, and beta nucleotidetriphosphates.

Another in vivo study showed that chronic lithium administration caused a significant decrease in platelet membrane phosphatidyl inositol-4,5-biphosphate levels in bipolar subjects (Soares et al 2000). Although altogether these findings provide evidence for the inositol-depleting effects of lithium, it should be emphasized that inositol depletion per se may not be responsible for lithium's therapeutic effects. As recent findings suggest, however, some of the initial actions of lithium may occur through a depletion of inositol (either by inhibition of IMPase and/or *myo*-inositol transport; Lubrich and van Calker 1999), which in turn may initiate a cascade of secondary changes at different levels of signal transduction process and gene expression in brain, effects that are ultimately responsible for the mood stabilizing and neuroprotective effects of lithium (Acharya et al 1998; Chen and Manji 1997; Chen et al 1999; Klein and Melton 1996; Lenox et al 1996, 1998; Manji et al 1996, 1999, 2000; Moore et al 2000).

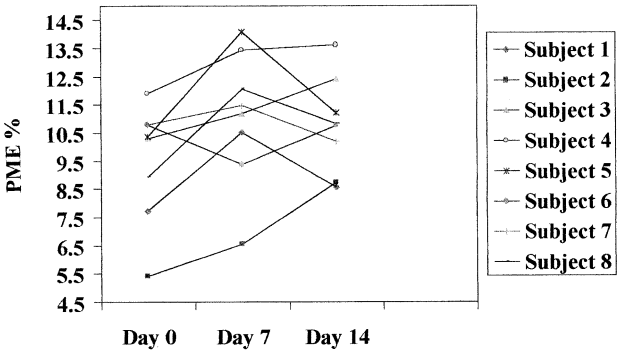

Figure 2. Mole percent phosphomonoesters (PME) values following lithium administration.

In conclusion, an increase in PME concentration with 7

Table 1. Changes in the Mole Percent PME Values Following Chronic Lithium Administration with Proton Decoupled ^{31}P MRS

	PME % Day 0	PME % Day 7	PME % Day 14
Subject 1	7.73	10.53	8.56
Subject 2	5.42	6.56	8.74
Subject 3	10.30	11.20	12.44
Subject 4	11.91	13.45	13.64
Subject 5	10.38	14.10	11.23
Subject 6	10.79	9.40	10.77
Subject 7	10.79	11.47	10.21
Subject 8	8.94	12.07	10.82
MEAN ± SD	9.58 ± 2.13	11.1 ± 2.37	10.8 ± 1.71

MRS, magnetic resonance spectroscopy; PME, phosphomonoester.

and 14 days of lithium administration in the human brain in vivo was observed. Future studies investigating the effects of lithium on IMPase in bipolar patients with different mood states, as well as its role as a predictor of lithium responsiveness and resistance, are warranted.

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