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Lithium-induced alterations in nucleoside triphosphate levels in human brain: a proton-decoupled ^{31}P magnetic resonance spectroscopy study

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Abstract

We examined how lithium's demonstrated effects on various cellular processes in human brain would be reflected in the ^{31}P magnetic resonance spectra of living human beings with respect to brain high-energy phosphate metabolites. Eight healthy volunteers received a baseline ^{31}P magnetic resonance spectroscopy (MRS) scan, after which they received lithium carbonate, 900 mg/day, for 14 days. Follow-up MRS scans were obtained on day 7 and on day 14. We detected a lithium-induced decrease in alpha-, beta-, gamma- and total nucleoside triphosphate NTP levels with chronic administration of lithium. On day 7, significant decreases were noted in gamma-NTP (14%) and total NTP (11%) levels. There was a trend for a decrease in beta-NTP (11%) levels. On day 14, significant decreases were noted in alpha-NTP (7%) and total NTP (8%) levels. There was a trend for a decrease in beta-NTP (16%) levels. Lithium caused a 25% reduction in inorganic phosphate (P_i) levels on day 14. The theoretical relevance of the lithium-induced alterations on brain high-energy phosphates to the lithium-induced modifications of neuroplasticity is discussed.

Keywords: Lithium; Proton-decoupled ^{31}P MRS; Nucleoside triphosphates

1. Introduction

It has been estimated that in most industrialized countries, one person in a thousand is undergoing lithium treatment for bipolar mood disorder (Schou, 1991). In spite of the common use of lithium and the

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uniqueness of its efficacy, the mechanisms by which this cation exerts its long-term beneficial effects in bipolar illness are not yet clear (Moore et al., 2000b).

Most recent data from molecular and cellular studies suggest impairments of neuroplasticity and cellular resilience in mood disorders and enhancement of these highly organized functions of human brain by lithium (Manji et al., 2000b). Magnetic resonance spectroscopy (MRS), by allowing the assessment of brain chemistry in vivo, gives us the opportunity to build on research data coming from molecular and cellular studies to the situation in living individuals. With this in mind, we wanted to examine how lithium's effects on human brain would be reflected in the ^{31}P magnetic resonance (MR) in vivo human brain spectra with respect to the brain high-energy phosphate metabolites.

Initial ^{31}P magnetic resonance spectroscopy (MRS) data on brain high-energy phosphate metabolites come from the serial studies of Kato et al. (1992, 1994, 1995), who reported a decrease of phosphocreatine (PCr) in bipolar depression. Later, Murashita et al. (2000) detected decreased PCr levels after photic stimulation in lithium-resistant patients with bipolar disorder. These findings led to the hypothesis that an impairment of mitochondrial function may be implicated in bipolar mood disorders (Kato and Takahashi, 1996; Kato et al., 1997; Murashita et al., 2000). More recent ^{31}P MRS studies documented lower than normal levels of beta- and total nucleoside triphosphate (NTP) levels in both the basal ganglia (−16% and −6%, respectively; Moore et al., 1997) and the frontal lobes (−17% and −8%, respectively; Volz et al., 1998) of depressed adults. Most recently, Renshaw et al. (2001) found beta-NTP levels to be lower by 33% in depressed relative to comparison subjects. This profile of metabolic changes is unusual since NTP concentration is usually maintained at the expense of phosphocreatine because of the higher phosphate group transfer potential of phosphocreatine (Renshaw et al., 2001). However, the lower mole percent beta-NTP in depressed subjects, in the presence of the constant PCr mole percent values, may be linked to disturbed metabolic processes of high-energy phosphates in depression (Moore et al., 1997). To investigate the matter further, in the present study, we examined lithium-induced alterations in brain high-energy phosphate metabolites in healthy volunteers.

Of the currently available methods for the assessment of brain high-energy phosphate metabolites, ^{31}P MRS, which provides a direct measure of NTP levels, is the most studied (Renshaw et al., 2001). An important limitation of ^{31}P MRS for assessing brain chemistry is the relatively low sensitivity of the method, approximately 5% that of hydrogen MRS (Renshaw et al., 2001). There are a limited number of ways by which the sensitivity of ^{31}P MRS experiments may be increased. In particular, proton decoupling (Murphy-Boesch et al., 1993; Kato et al., 1998) and increasing the volume of tissue examined are the easiest methods to implement on existing scanners. Thus, we collected proton-decoupled ^{31}P MR spectra from healthy 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 phosphomonoester (PME) concentrations, as well as alterations in brain high-energy phosphate—in particular, NTP concentrations—after the chronic administration of lithium.

In an earlier report, we looked at the effects of lithium on PME levels in the context of the "inositol depletion hypothesis" (Yildiz et al., 2001). Here, we report our observations on the effects of lithium on nucleoside triphosphate (total, alpha-, beta-, and gamma-), inorganic phosphate (P_i), phosphocreatine and phosphodiester (PDE) levels in the context of intracellular systems other than the inositol system.

2. Methods

2.1. Subjects and study design

Subjects were screened with the Structured Clinical Interview for Diagnosis (SCID), the Hamilton Rating Scale for Depression and the Young Mania Rating Scale by a trained research psychiatrist. Healthy subjects were excluded for any SCID axis-I diagnosis, a family history of affective illness in first-degree relatives, a neurological illness or abnormalities in the blood, urine or electrocardiogram or for a contraindication for MR scan.

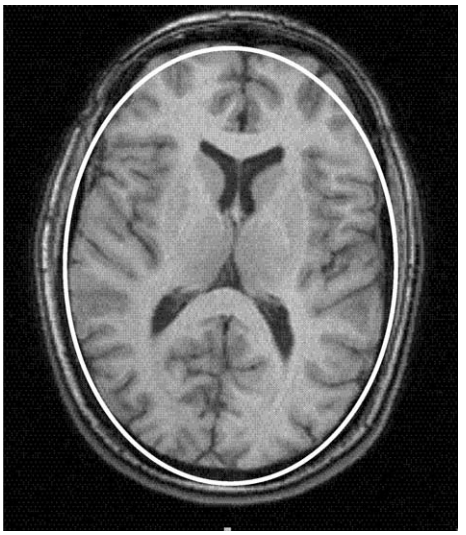

Fig. 1. Axial MR image demonstrating a typical slice location for MRS.

All subjects gave full informed consent, and the Massachusetts General Hospital Institutional Review Board approved the study. Subjects received a baseline structural MRI and ^{31}P MRS scan and then were given lithium carbonate 900 mg/day (450 mg b.i.d.) for 14 days. Subjects received a second ^{31}P MRS scan and a ^{7}Li MRS scan on day 7, followed on day 14 by the final ^{31}P MRS and ^{7}Li MRS scans.

Eight subjects, four women and four men, who met eligibility criteria, took part in the study. They had a mean age of 38.9±6.4 (18–45) years. Subjects had serum lithium levels on day 7 (obtained 12 h after the last lithium dose) of between 0.40 to 0.72 (mean 0.57) mM/l and on day 14 of between 0.40 to 0.70 (mean 0.58) mM/l. For more details, please refer to our previous report (Yildiz et al., 2001).

2.2. 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 morphological information and to select the slice to be studied in the MRS imaging examination. These images were acquired with the proton channel of a home-built, dual-tuned (^{31}P and ^{7}Li: Asia Instruments, Highland Heights, OH) head coil (SPGR, 256×196, 1 NEX, 28 slices, slice thickness 5 mm with no gap, TE=5 ms, TR=40 ms, 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 ms) slice selective spin echo pulse sequence (Lim et al., 1994; see Fig. 1).

Proton decoupling was applied using a Waltz-4 pulse sequence (Murphy-Boesch et al., 1993). The

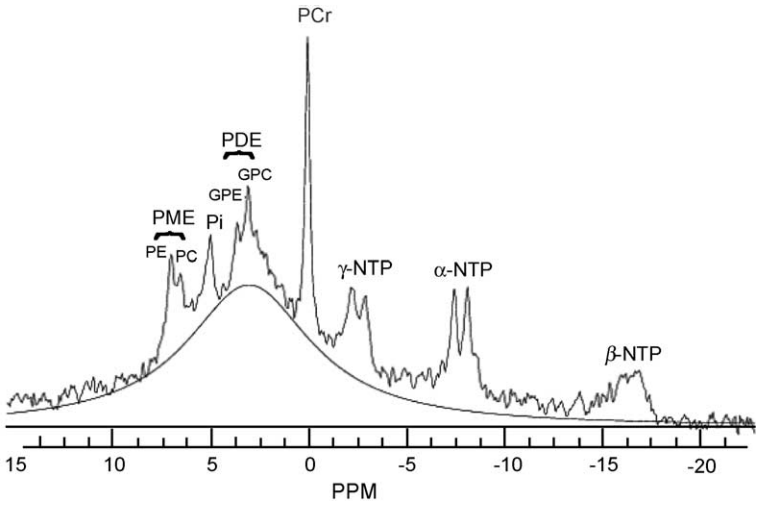

Fig. 2. Typical decoupled ^{31}P MR spectrum. Frequency units are expressed in parts per million (ppm). PME—phosphomonoesters; PE—phosphoethanolamine; PC—phosphocholine; P_i—inorganic phosphate; PDE—phosphodiesters; GPE—glycerophosphoethanolamine; GPC—glycerophosphocholine; PCr—phosphocreatine; γ-, α- and β-NTP—gamma-, alpha- and beta-nucleoside triphosphates.

spectra were fit using the VARPRO/AMARES/MRUI time domain fitting package (van den Boogaart et al., 1994; Fig. 2). 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).

^{7}Li spectra were acquired from a 60-mm axial slice centered on the superior edge of the ventricles. Serum lithium levels were acquired before the MRS examination and 12 h after the last dose of lithium. The spectra were fit using SA/GE (General Electric Medical Systems, Milwaukee, WI), and the images were segmented to determine the percent contributions to the lithium signal from the brain, cerebrospinal fluid and muscle using CINE software (Johnson et al., 1993). Finally, brain lithium concentrations were calculated according to the method of Gonzalez et al. (1993).

2.3. Statistical analysis

The Wilcoxon signed ranks test was used to examine within-group changes over days. Temporal changes in the NTP concentrations were also assessed with a within-subjects repeated measures analysis of variance (ANOVA). Simple linear regression analysis was used to assess the associations of the brain lithium levels and the NTP concentrations. The significance level was set at $P<0.05$. All reported P values are two-sided. We hypothesized a priori that the decoupled ^{31}P MR spectra obtained from a large volume of brain would reveal alterations in the NTP levels after the chronic administration of lithium. Since we were looking primarily for changes in the NTP resonances based on the findings in depressed subjects, no correction for multiple comparisons was made.

3. Results

We made pairwise comparisons of the mole percent total, alpha-, beta- and gamma-NTP; PCr; PCr/total-NTP; PCr/beta-NTP; PCr/P_i; and P_i values. There was a significant decrease in total NTP levels compared with baseline on both days 7 and 14 (Z=2.240, P=0.025; Z=2.100, P=0.036, respectively). Repeated measures ANOVA confirmed the significance of this change in total NTP levels (F=4.466, P=0.032). For individual measurements of alpha-, beta- and gamma-NTPs, we found a significant decrease of alpha-NTP on day 14 (Z=2.240, P=0.025) and gamma-NTP on day 7 (Z=2.240, P=0.025) compared with baseline. For the measurements of the mole percent values of beta-NTP, on the other hand, we observed a trend on both day 7 and day 14 indicating a decrease in this metabolite peak compared with baseline (Z=1.682, P=0.092; Z=1.680, P=0.093, respectively). While there was no significant change in the PCr mole percent values, we found a significant increase in PCr/P_i and PCr/total NTP ratios (Z=2.380, P=0.017; Z=2.383, P=0.017, respectively) and a nonsignificant trend for the PCr/beta-NTP ratios (Z=1.680, P=0.093) on day 14 compared with baseline. The increase in the PCr ratios was primarily due to the changes in NTP and P_i values. Finally, we found a significant decrease in the P_i metabolite peak on day 14, again in comparison to baseline (Z=2.100, P=0.036). Table 1 shows the mole percent values of the metabolite ratios and corresponding standard deviations.

Table 1
Mole percent concentrations of the high-energy phosphate metabolites in the human brain before and after the administration of lithium

	Day 0 mean±SD	Day 7 mean±SD	Day 14 mean±SD	P value* days 0–7	P value* days 0–14
Total NTP	37.17±2.85	32.97±4.00	34.16±3.84	0.025	0.036
α-NTP	14.29±1.05	13.04±2.19	13.33±1.10	0.161	0.025
β-NTP	8.38±1.49	7.47±0.66	7.06±1.96	0.092	0.093
γ-NTP	14.51±1.84	12.46±1.61	13.78±2.18	0.025	0.123
PCr	10,17±2,05	9.70±3.00	10.67±2.20	1.000	0.123
PCr/total NTP	0.28±0.07	0.30±0.09	0.32±0.08	0.141	0.017
PCr/β-NTP	1.28±0.47	1.32±0.50	1.72±0.97	0.779	0.093
PCr/P_i	2.04±0.62	3.13±2.15	2.97±1.27	0.263	0.017
P_i	5.37±1.98	4.07±2.25	4.01±1.48	0.327	0.036

* Wilcoxon signed ranks test.

Table 2
Brain lithium levels as measured by ^{7}Li MRS

	Brain lithium levels, day 7	Brain lithium levels, day 14
Subject 1	0.1389	0.1638
Subject 2	0.1832	0.2735
Subject 3	0.4548	0.4682
Subject 4	0.5030	0.3626
Subject 5	0.1224	0.1415
Subject 6	0.5338	2.5637
Subject 7	0.2030	0.3246
Subject 8	0.1723	2.6483

The ^{7}Li MRS experiments revealed brain lithium levels of the eight subjects on both days 7 and 14 (Table 2). While two subjects showed a remarkable increase in their brain lithium levels on day 14 compared with day 7, the other subjects showed only modest changes in their brain lithium concentrations. We applied a simple linear regression model for the total NTP levels and brain lithium concentrations on days 7 and 14. In this analysis, we could not detect a significant association between the total NTP levels and brain lithium concentrations on either day 7 or day 14 (B=0.274, P=0.511; B=0.270, P=0.517).

No significant change has been detected on the mole percent PDE values on either day 7 or day 14 (Z=−0.560, P=0.575; Z=−1.680, P=0.093, respectively; Table 3).

4. Discussion

Nucleotides (purines: adenosine triphosphate [ATP]; guanosine triphosphate [GTP]; and pyrimidines: cytidine triphosphate [CTP]; uridine triphosphate [UTP]; thymidine triphosphate [TTP]) are constituted from a nitrogen-containing base, a pentose ring and a phosphate group (Nelson and Cox, 2000). The most fundamental use of nucleotides in a cell is their role as subunits of nucleic acids. However, they have a variety of other functions in every cell—as energy carriers, components of enzyme cofactors and chemical messengers (Nelson and Cox, 2000).

While our data indicated only a trend for a decrease in beta-NTP levels, which is likely to be related to the small sample size, we observed a lithium-induced decrease in the total, alpha-, beta- (a trend) and gamma-NTP levels in the presence of constant PCr levels (Table 1). The magnitude of the signal decrease, in relative terms, was indeed similar for beta-, alpha-, gamma- and total NTP, a finding that is compatible with a decrease in NTP levels. The failure of the beta-NTP decrease to reach significance may reflect the lower signal-to-noise ratio for this peak relative to the others. Subtherapeutic serum/brain lithium levels in several of the subjects may also have contributed to the failure of the beta-NTP decrease to reach significance.

Intriguingly, our findings on lithium's effect on the brains of healthy human subjects are compatible with the ^{31}P MRS data in unipolar depression but not in bipolar depression. This may relate to variations in the physiological mechanisms underlying bipolar versus unipolar depression, experimental procedures, the region of interest and technical limitations of the MRS experiments. It is also possible that lithium may exert differing effects on the brains of mood disorders patients and healthy volunteers.

Lithium's demonstrated effects on various cellular processes in the brain (Manji et al., 2000a,b, 2001; Phiel and Klein, 2001; Coyle and Manji, 2002) may be examined in the light of the present results to discuss possible mechanisms underlying the lithium-induced decrease in NTP levels. NTPs can function in a cell as the subunits of nucleic acids, as energy supplies and as components of enzyme cofactors/chemical messengers; thus, the present findings may be interpreted differently depending on the specific role of the NTPs.

Table 3
Changes in the mole percent PDE values following chronic lithium administration with proton-decoupled ^{31}P MRS

	PDE% day 0	PDE% day 7	PDE% day 14
Subject 1	33.90	45.32	38.83
Subject 2	36.98	62.61	42.55
Subject 3	41.44	39.47	42.54
Subject 4	37.53	37.37	37.51
Subject 5	37.56	36.88	48.65
Subject 6	34.92	35.41	32.76
Subject 7	38.92	38.01	39.16
Subject 8	40.34	42.26	40.90
Mean±SD	37.70±2.54	42.17±8.86	40.36±4.60

4.1. NTPs as the subunits of nucleic acids

It has been demonstrated that chronic lithium administration increases mRNA levels of the polyomavirus enhancer binding protein 2 (PEBP2; Chen et al., 1999a,b), B-cell lymphoma protein 2-bcl-2 (Chen et al., 1999a,b), tyrosine hydroxylase (Manji et al., 1999), adenylate cyclase (Newman and Belmaker, 1987; Risby et al., 1991) and inositol monophosphatase (Renshaw et al., 1986; Shamir et al., 1998). Given the role of NTPs in DNA decoding and transcription/translation processes (mRNA, tRNA, rRNA), it can be speculated that lithium, by increasing the consumption of the NTPs (as the subunits of nucleic acids), may give rise to a transient decrease in the NTP levels in human brain.

4.2. NTPs as energy carriers

Lithium has been demonstrated to increase the levels of a major antiapoptotic protein bcl-2 in several brain regions (Manji et al., 2000b). Bcl-2 has been shown to promote the regeneration of axons in the mammalian brain (Chen et al., 1997), to regulate neurite sprouting and outgrowth (Chen et al., 1997) and to increase axonal growth rate (Hilton et al., 1997). Besides, chronic lithium administration was found to significantly increase *N*-acetyl-aspartate (a molecule synthesized within mitochondria indicating neuronal viability and function) concentrations in human brain in vivo (Moore et al., 2000a,b). Taken together, these data indicate that lithium stimulates neurogenesis and, thus, the synthesis of proteins/lipoproteins, enzymes and some structural elements in the human brain. Obviously, the synthesis of proteins/lipoproteins, lipids and enzymes is an energy-consuming process that may contribute to the observed decrease in the NTP levels of lithium-treated individuals.

In addition, lithium-induced acceleration of the Na, K ATPase (Wood et al., 1989) and Ca ATPase activity (Mork, 1993) may account at least in part for the consumption of energy and the decrease of NTPs we have observed.

Lithium has also been shown to inhibit GSK-3β, which inhibits glycogen synthesis in human brain (Manji et al., 2001). This effect of lithium in directing the brain glucose for the synthesis of glycogen instead of glycolysis may also add to the observed decrease in NTP levels.

4.3. NTPs as the components of enzyme cofactors/chemical messengers

Among the NTPs, CTP is used in the resynthesis of phosphatidylinositol 4,5-biphosphate (PIP2), which is the initial molecule in the phosphoinositide cycle (Majerus et al., 1988). Lithium, through its chronic effects on the phosphoinositide cycle, may cause an increased demand for the resynthesis of PIP2 (Renshaw et al., 1986; Shamir et al., 1998; Manji et al., 1999; Yildiz et al., 2001) so that may lead to an increased consumption of CTP. Another nucleotide, GTP, on the other hand, is utilized for the transmission of lithium-induced signal through the G protein-receptor complex (Mork, 1993). Finally, lithium-induced enhancement of basal and stimulated adenylate cyclase activity may also contribute to the decrease in the NTPs via utilization of ATP for the synthesis of cyclic adenosine monophosphate (Gould and Manji, 2002).

It is also possible that the brain, under the influence of lithium, may display an increased demand for the nucleotides (as the subunits of nucleic acids, energy carriers, chemical messengers and components of enzyme cofactors). An altered mitochondrial function and creatine phosphokinase (CPK) activity (favoring formation of PCr at the expense of ATP), on the other hand, instead of compensating for this heightened demand, may fail to regenerate NTPs (at least for the energy counterpart, mainly in the form of ATP) and may contribute to the observed reductions in the NTP-ATP levels in presence of constant PCr (Erecinska and Silver, 1989; Volz et al., 1998).

Another finding in this study is the lithium-induced decrease in inorganic phosphate levels. It is known that protein phosphorylation plays an important role in neuronal function. Several classes of proteins undergo phosphorylation by means of various enzymes called kinases. Among these are the enzymes (e.g., tyrosine hydroxlase, tryptophan hydroxylase, adenylate cyclase and kinases), neurotransmitter receptors (e.g., β-adrenergic receptors and nicotinic cholinergic receptors), ion channels (Na^+, K^+, Ca^{++} channels), proteins involved in regulation of transcription and translation (e.g., RNA polymerase) reactions and

cytoskeletal proteins (e.g., actin and tubulin; Erecinska and Silver, 1989). Lithium, through its effects on various kinases, seems to be involved in phosphorylation, thereby in the regulation of ion channels, cytoskeletal elements, transcription factors, enzymes, neurotransmitter receptors, signaling molecules, and proteins regulating apoptosis and scaffolding (Mork, 1993; Manji et al., 2000a; Gould and Manji, 2002). It could be speculated that the end result of the lithium-facilitated regulatory phosphorylation/dephosphorylation reactions may be in the favor of phosphorylation and may be associated with the lithium-induced decrease in inorganic phosphate levels we have observed.

4.4. Limitations of the study

There are a number of significant limitations affecting the generalizibility of the present findings to the situations in bipolar subjects. First of all, this is a study in a small number of healthy human subjects. It is quite possible that lithium may have different effects on the brains of mood disorders' patients and healthy human subjects. Recently, it has been suggested that absolute concentrations of the metabolite peaks should be used in MRS experiments instead of metabolite ratios in which PCr or the total phosphorus signal is taken as the denominator. However, using mole percent metabolite values is still a common practice, and this method would not be expected to cause an inflation of the observed changes since it tends to underestimate metabolite changes as the denominator decreases in the same direction with the numerator. No segmentation from MRI has been made to correct the ^{31}P MRS data. However, in an earlier report from our laboratory (using the same MR scanner), use of an image segmentation program revealed that muscle tissue comprised less than 5% of the tissue volume (Christensen et al., 1996). As reflected in the serum and brain lithium levels, not all the subjects in this study achieved therapeutic lithium levels. This factor, together with the time delay needed to observe lithium's effects in clinical situations, may have confounding effects on the interpretation of the observed changes in high-energy phosphate metabolite levels. In addition, the observed changes in high-energy phosphates associated with lithium are compatible with the findings in unipolar depression but not bipolar depression or mania. Although this disparity may in part relate to the limited number of studies in bipolar depression/mania, it limits the interpretation of findings in the light of lithium's demonstrated effects on various cellular systems in brain.

Nonetheless, a decrease in total NTP concentration after 7 and 14 days of lithium administration in the human brain in vivo was observed. Given the antimanic properties of lithium (lithium is mainly effective for the treatment and prevention of manic episodes, while its efficacy in treatment and prophylaxis of depression is controversial), the similarity of lithium-induced alterations in brain energy metabolism to depression is intriguing. Although its wider implications can only be speculated on, this report provides a thought-provoking discussion on the relationship of lithium-induced modifications in various cellular events and NTP concentrations in human brain. Future investigations of the high-energy phosphate metabolites in mood disorder patients and healthy volunteers who are being treated with lithium or the other antimanic or antidepressant agents are needed for a better understanding of the issue.

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