Lithium does not alter the choline/creatine ratio in the temporal lobe of human volunteers as measured by proton magnetic resonance spectroscopy

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Objective: To study the effect of lithium administration on brain choline/creatine (Cho/Cr) ratios in healthy volunteers. **Design:** Double-blind, placebo-controlled, prospective study. **Setting:** The Nuclear Magnetic Resonance Research Unit at the University of Alberta. **Participants:** Sixteen healthy volunteers, recruited through advertisements. Subjects were excluded if they had a physical illness, or a personal or family history of psychiatric illness. The study period was from Feb. 6, 1996, to Mar. 21, 1996. **Interventions:** Subjects received a baseline proton magnetic resonance spectroscopy (^{1}H MRS) scan, and then were instructed to take either lithium (1 200 mg) or placebo at night for 7 days. On Day 8, the subjects returned for a second ^{1}H MRS scan. Study participants were seen by a physician at the beginning and at the end of the experiment, and had access to the physician throughout the study period. **Outcome measures:** Ratios of Cho/Cr measured in the temporal lobes by ^{1}H MRS. **Results:** There were no significant differences in the Cho/Cr ratios between the 2 groups on the test day (placebo 0.748 [standard deviation 0.29] versus lithium 0.811 [SD 0.25]; $F = 0.147$, $p = 0.72$), and there was no significant change from baseline in either group (0.003 above baseline for placebo; 0.056 above baseline for lithium; $F = 1.21$, $p = 0.32$). **Conclusions:** Lithium administration to healthy volunteers does not alter the Cho/Cr ratio in temporal lobe as measured by ^{1}H MRS. This result concurs with reports that differences in Cho/Cr ratios observed in patients with bipolar disorder are likely specific to the illness, and are not the result of lithium therapy. Hence, alterations in choline function are not involved in the clinical effectiveness of lithium.

Objectifs : Étudier l'effet de l'administration de lithium sur les ratios de choline/créatine (Cho/Cr) chez des volontaires en bonne santé. **Conception :** Étude prospective à double insu contrôlée par placebo. **Contexte :** L'unité de recherche en résonance magnétique nucléaire de l'Université de l'Alberta. **Participants :** Seize volontaires en bonne santé, recrutés au moyen d'annonces. Les sujets ont été exclus s'ils avaient une maladie physique ou des antécédents personnels ou familiaux de maladie psychiatrique. L'étude s'est étendue sur la période du 6 février 1996 au 21 mars 1996. **Interventions :** Les sujets ont été soumis à une spectroscopie de référence par résonance magnétique protonique (^{1}H SRM) et ont ensuite dû prendre du

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Medical subject headings: bipolar disorder; brain; choline; depressive disorder; lithium; nuclear magnetic resonance; temporal lobe

J Psychiatry Neurosci 1999;24(3):222-6.

Submitted Aug. 10, 1998
Revised Dec. 4, 1998
Accepted Dec. 8, 1998

lithium (1 200 mg) ou un placebo le soir pendant sept jours. Le huitième jour, les sujets sont revenus se soumettre à une deuxième ^{1}H SRM. Les participants ont été vus par un médecin au début et à la fin de l'expérience et avaient accès au médecin pendant toute la période d'étude. **Mesures de résultats :** Ratios de Cho/Cr mesurés dans les lobes temporaux par ^{1}H SRM. **Résultats :** Il n'y avait pas de différence significative entre les ratios Cho/Cr des deux groupes le jour de l'examen (placebo 0,748 [écart type, 0,29] ou lithium 0,811 [ET 0,25]; F = 0,147, p = 0,72), et il n'y avait pas de changement significatif par rapport au niveau de référence dans l'un ou l'autre des deux groupes (0,003 au-dessus de la ligne de référence dans le cas du placebo et 0,056 au-dessus de la ligne de référence dans celui du lithium; F = 1,21, p = 0,32). **Conclusions :** L'administration de lithium à des volontaires en bonne santé ne modifie pas le ratio Cho/Cr dans les lobes temporaux mesuré par ^{1}H SRM. Ce résultat correspond aux rapports selon lesquels des différences des ratios Cho/Cr observés chez des patients qui ont des troubles bipolaires sont probablement spécifiques à la maladie et ne sont pas causées par la thérapie au lithium. Les altérations de la fonction cholinique n'interviennent donc pas dans l'efficacité clinique du lithium.

Introduction

In bipolar disorder, there are severe swings in mood between depression and mania.[1] The standard treatment for bipolar disorder for many years has been the administration of lithium. Lithium is an effective short-term treatment for both the depression and mania seen in patients with bipolar disorder, as well as being an effective prophylactic treatment.[2,3] The mechanism of action of lithium is currently unknown, and has been proposed to affect protein kinase C and G-protein activity,[4,5] the sodium/potassium adenosine triphosphatase (ATPase)[6] and the phosphoinositide second messenger system.[7,8]

The suggestion that lithium may act through a cholinergic mechanism arose from findings that lithium inhibits membrane transport of choline,[9] increases the accumulation of erythrocyte choline in lithium-treated patients,[10–12] and inhibits membrane transport of choline in post mortem human brain tissue.[13] There have even been reports of using choline to treat mania,[14,15] and a cholinergic hypothesis of mania has been proposed.[15]

Choline levels in the brain can be measured using proton magnetic resonance spectroscopy (^{1}H MRS). ^{1}H MRS studies of choline levels in patients with bipolar disorder have been somewhat controversial, which may be due in part to the different patient populations and brain areas studied. Two studies that examined cortical areas found no differences between lithium-treated patients and controls.[16,17] However, studies of the basal ganglia have found increased choline/creatine (Cho/Cr) ratios in patients with bipolar disorder.[18–21] Interestingly, in the study by Kato et al,[20] patients receiving medication and not receiving medication were studied, and, although the patients with bipolar disorder as a whole had greater Cho/Cr ratios than the controls, the lithium-treated and non-lithium-treated groups did not differ from each other. This result suggests that the increase in Cho/Cr ratios may be specific to the disorder, and not a result of lithium treatment. The main problem in interpreting many studies is that it is difficult to separate the effects of the disorder from those of lithium or other drug treatment. This is especially relevant since the Cho/Cr ratio has been shown to be increased in patients with bipolar disorder receiving a variety of medications,[20] in patients with depression not receiving lithium,[22] and in the frontal lobes in male patients with schizophrenia.[23]

This study examined the effects of lithium on Cho/Cr ratios in the temporal lobes of healthy volunteers using ^{1}H MRS. Volunteers were treated with either lithium or placebo for 7 days in a double-blind study. Results of this study will to help to dissociate the effects of lithium on brain choline levels from those of bipolar illness.

Methods

Subjects and study design

The subjects, recruited by advertisement, were screened in detail. Full personal and family histories were obtained, and any diagnostic criteria used were taken from the *Diagnostic and Statistical Manual of Mental Disorders, fourth edition* (DSM-IV).[1] The subjects also provided a full medical history and underwent a physical examination. All potential subjects had blood tests to determine full blood count, urea and electrolyte levels, and to assess liver function. All potential subjects also had a urine test for drugs of abuse and an electrocardiogram. Subjects who had a personal or family history of psychiatric disease, who had physical disease, or who had abnormalities in the blood, urine or electro-

cardiogram tests, were excluded from the study. Sixteen volunteers aged 19 to 40 met the entry criteria and were included in the study. All subjects gave full informed consent. The study was approved by the local ethics committee.

This was a double-blind placebo-controlled study in which the subjects received either lithium (1 200 mg) at night (n = 10) or matching placebo (n = 6) for 7 days while at home. On the morning of Day 8, serum lithium levels were determined in all subjects. ^{1}H MRS scans were performed before lithium treatment, and again after 7 days of lithium treatment.

Magnetic resonance spectroscopy

All ^{1}H MRS measurements were made using a Magnex 3 Tesla magnet (Magnex Scientific, Abingdon, UK) with a 80-cm bore, equipped with actively shielded gradients. A Surrey Medical Imaging Systems (SMIS) spectrometer console (SMIS, Guildford, UK) was used for data acquisition. Signal transmission and reception were attained using a quadrature birdcage resonator.

Initially, magnetic resonance imaging (MRI) data were acquired using a gradient echo imaging sequence to produce multiple slice images along both coronal and transverse planes. This allowed a 2 × 2 × 3 cm volume to be registered in the temporal lobe, with the long axis aligned with the brain mid-line (Fig. 1). ^{1}H MR spectra were then acquired using the point-resolved spectroscopy (PRESS) localization method (echo time 30 ms, repetition time 3 s, 128 signal averages). A typical spectrum is illustrated in Fig. 2.

Volume registration for the ^{1}H MRS study was accomplished by aligning the selected brain volume (from MRI) with a fixed water reference sphere attached to the head support and aligned with the surface coil. The selected volume was positioned such that the centre of the long axis of the 2 × 2 × 3 cm volume was adjacent to the sphere and oriented parallel to the surface of the cortex at that location in the multislice transverse image. This alignment process was repeated using multislice coronal image data through the planes in which the sphere was observed. The volume edges were maintained at an average distance of 3 mm from the brain surface, to prevent overlap of the volume with cerebrospinal fluid.

Following filtering, fast Fourier transformation and phase correction of the raw data, baseline correction

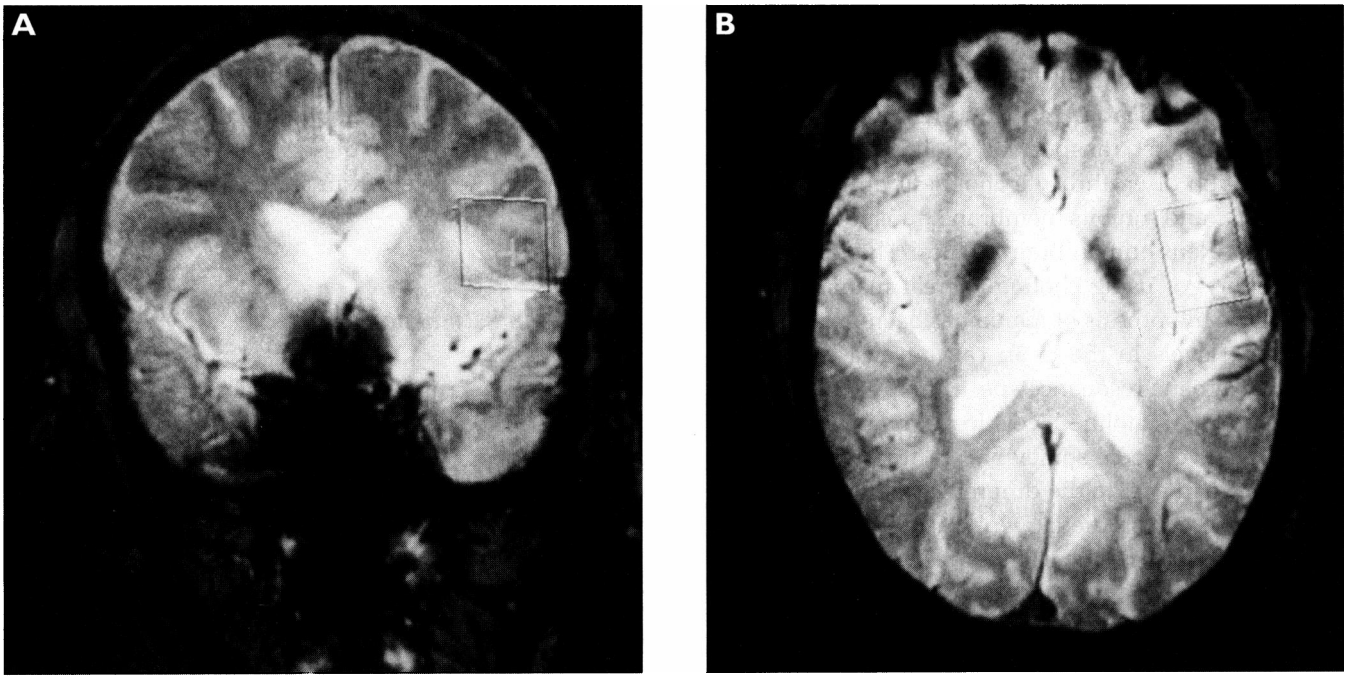

Fig. 1: Anatomical location of the brain region examined in this study. Coronal section (A) and horizontal section (B) from the brain of a typical volunteer. The box illustrates the location from which ^{1}H spectral information was acquired. The 2 × 2 × 3 cm volume was registered in the temporal lobe in reference to a fixed water sphere attached to the head support and aligned with respect to the surface coil.

and deconvolution of the spectra was accomplished using the Peak Research (PERCH) spectrum analysis software package (PERCH project, Department of Chemistry, University of Kuopio, Kuopio, Finland). All spectral analysis was carried out by investigators who were blind to the treatment that the subject had received.

Statistical analysis

A 2-way repeated measures multivariate analysis of variance (MANOVA) was used to test the statistical significance of changes in the peak area ratio. The 2 factors used were time (Day 0, Day 8) and drug treatment (lithium, placebo). The analysis was deemed significant at the 0.05 level for a main effect or for a drug treatment versus time interaction. *Post hoc* unpaired Student's *t*-tests were used to compare the daily results from placebo and lithium-treated groups. Paired Student's *t*-tests were used to examine the significance of any changes between scans. These analyses were deemed significant at the 0.05 level.

Results

Of the 16 subjects who met the eligibility criteria, 6 received placebo and 10 received lithium. There were no significant differences between these groups in terms of sex (there was 1 woman in the placebo group and there were 3 women in the lithium group), or age (mean age of placebo group 26.1 [standard deviation 2.6] years and lithium group 25.3 [SD 1.7] years). All subjects receiving lithium had a serum lithium level after 7 days of treatment of between 0.6 and 1.1 mmol/L (mean 0.74 [SD 0.7] mmol/L).

The mean Cho/Cr peak area ratio derived from the ^{1}H MRS scans for the placebo group was 0.745 (SD 0.30) before treatment and 0.748 (SD 0.29) after 7 days. In the lithium-treated group, the mean Cho/Cr peak area ratio was 0.755 (SD 0.24) before treatment and 0.811 (SD 0.25) after 7 days. The Cho/Cr peak area ratio was not statistically significant between the placebo and lithium-treated groups before treatment ($F[df\ 1] = 0.147, , p = 0.72$) or after 7 days ($F[df\ 1] = 1.21, , p = 0.32$). As well, there was no significant correlation between Cho/Cr ratios and lithium levels on Day 8 ($r = 0.30$, $p = 0.40$).

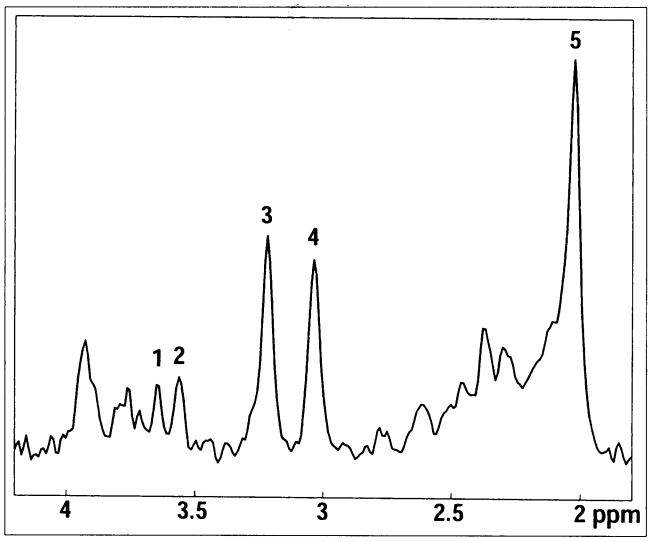

Fig. 2: ^{1}H MRS spectrum obtained from a 2 × 2 × 3 cm volume of temporal lobe brain tissue. The spectrum shown was obtained from a control subject before drug treatment. The peak assignments were as follows: 1 — *myo*-inositol 3.65 ppm; 2 — *myo*-inositol 3.56 ppm; 3 — choline; 4 — phosphocreatine/creatine; 5 — *N*-acetylaspartate.

Discussion

This study was designed to determine whether lithium administration for 7 days led to a change in Cho/Cr ratios in the brains of healthy human volunteers, and especially to dissociate the effects of lithium from the effects of bipolar disorder, since previous ^{1}H MRS studies have revealed increases in Cho/Cr ratios in patients with bipolar disorder.

Potential limitations to the interpretation of this data must be considered. First, the peak areas being measured contain signals from other compounds in addition to choline, such as taurine, *myo*-inositol, phosphorylcholine and glycerophosphorylcholine.[24] Thus, it is possible that changes in these other compounds masked any changes in choline. The singlet peak areas from the *N*-trimethyl moiety of the choline derivatives have only a relatively small echo-time dependence due to T_2 relaxation at the echo time of 30 ms used in this study. On the other hand, the taurine and inositol peaks are additionally modulated due to J-coupling, and could have a more significant effect on the peak areas measured for the choline peak. Clarification of this issue awaits the development of improved MRS techniques.

It is also possible that the duration of lithium treatment in this study (i.e., 7 days) was insufficient to observe any changes in brain choline ratio. While this may be the case, clinical effects of lithium can be

observed 6 to 10 days after the beginning of treatment,[25] and therefore, 7 days of lithium treatment should have been adequate to observe any changes in brain choline ratio, particularly in healthy volunteers.

Results of this study suggest that increased brain choline levels are not a major effect of lithium, indicating that this is probably not its major therapeutic effect. Although many studies have reported increased erythrocyte choline concentrations in lithium-treated individuals,[9–12] this effect may be secondary to other effects of lithium, or this effect may not be present in neuronal tissue.

The results of this study are in keeping with previous studies finding that lithium has no effect on Cho/Cr ratios in the cerebral cortex,[16,17] and that any observed changes in the Cho/Cr ratio in the basal ganglia may be due to the disorder itself, and not to drug treatment.[20] The current findings demonstrate that, in healthy control subjects, 7 days of lithium treatment have no significant effect on Cho/Cr ratios in the temporal lobe compared with placebo.

Acknowledgements

This study was supported in part by the Alberta Heritage Foundation for Medical Research and by a Canadian Psychiatric Research Foundation Post-Doctoral Fellowship to Dr. Rotzinger.

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