antagonist (4 Cl-D-Phe^6, Leu^{17})-VIP did not produce any effect on spontaneous contractile activity. On the contrary, 14-GRF analog, which specifically interferes with VIP receptors, induced an evident increase in both tone and spontaneous contraction waves. The fact that the 14-GRF analog did not produce any increase in muscular tone after pharmacological blockade of the intrinsic nerve plexus, obtained by TTX administration, demonstrated that the action of this peptide is not myogenic, but is rather directed at the intrinsic nerve plexus. These findings suggest that a member of the glucagon-secretin family of peptides may be a neurotransmitter in the inhibitory plexus of guinea pig gallbladder, but VIP is not.

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Changes in red blood cell choline and choline-bound lipids with oral lithium

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Summary. The influence of oral lithium on the concentration of red blood cell choline (Ch), lecithin, glycerophosphorylcholine (GPCh) and phosphorylcholine (PCh) was studied. The concentration of RBC Ch was significantly elevated and the concentration of lecithin, GPCh and PCh significantly depressed in 16 patients on oral lithium compared to 9 age-matched controls. We conclude that lithium markedly depletes the red blood cell of choline containing compounds including lecithin. These changes may be responsible for both the therapeutic efficacy and the toxicity of lithium.
Key words. Lithium; mechanism; choline; lecithin; glycerophosphorylcholine; phosphorylcholine.

Lithium (Li) is the treatment of choice for patients with bipolar disorders and is utilized by millions of people yearly worldwide[1]. Its mechanism of action is unknown although Janowsky et al. have proposed that it changes the relative balance between the cholinergic/adrenergic systems[2]. In addition, it alters phosphatidylinositol turnover[3] and increases red blood cell (RBC) glycine[4]. Li has dramatic effects on the transport and concentration of choline (Ch) in RBC. Martin was the first to demonstrate that Li blocked the transport of Ch into and out of the RBC in human subjects[5] and Jobe et al.[6] showed that Li led to an approximately tenfold rise of RBC Ch. Jenden[6,7] and Hanin[8] also demonstrated that the expected rise of RBC Ch could be predicted with a kinetic model that they developed. No sustained increases in brain Ch have been demonstrated in animals on chronic Li therapy. However, animal models may not be appropriate to study the effects of Li in man as Miller et al.[9] have demonstrated that in ten different non-human species, Li did not elevate RBC Ch even though the dosage administered resulted in what would have been 'therapeutic levels' of Li in human subjects.

The long-term effect of Li on Ch metabolism in man is unknown. Specifically there have been no studies to demonstrate what prolonged block of Ch transport will do to the concentration of lecithin in the membrane or other Ch containing compounds such as glycerophosphorylcholine (GPCh) or phosphorylcholine (PCh). We have measured RBC Ch, lecithin, GPCh, and PCh in 9 normals and 16 patients on Li therapy for more than one week. The changes in these compounds are described and possible implications for therapy and toxicity are discussed.

Methods

A) *Selection of subjects*: Nine healthy young controls were compared to 16 patients with bipolar disorders. The patients with bipolar disease were physically healthy and all had therapeutic Li levels. All patients were carefully followed in the Psychiatric clinic at Harbor-UCLA Medical Center and plasma and RBC Li levels were monitored. The patients had been taking Li for a mean of 286.6 days with a range from 7 days to 5 years and a median of 90 days.

B) *Measurement of red blood cell choline*: Blood was drawn into a heparinized tube, immediately placed on ice and centrifuged at 3020 × g at 2 °C for 10 min to obtain packed RBC. The RBC were washed with 10 vols of saline and recentrifuged. The packed RBC (0.1 ml) were treated with 0.4 N $HClO_4$ (1 ml), and [$^2H_9$]-Ch (2 nmol) was added as an internal standard (I.S.). The sample was mixed and allowed to stand for 5 min. After thorough mixing, the suspensions were centrifuged at 12,100 × g for 10 min. Ch was measured in the supernatant by gas-chromatography-mass-spectrometry (GCMS) analysis as previously described [10].

C) *Measurement of choline bound mono and diesters and phospholipids*: RBC (100 µl) were mixed with 4 ml of 2:1 chloroform/methanol mixture. Distilled water (0.4 ml) was added and the sample was mixed thoroughly and centrifuged at 480 × g. The aqueous and organic phases were separated, the organic phase containing lecithin, while the aqueous phase contained free Ch, GPCh and PCh. To measure lecithin, 1 ml of organic phase was separated and evaporated to dryness. 6 N HCl (0.5 ml) was added and after mixing was heated for 1 h at 100 °C to hydrolyze the lecithin. To the solution was added 10 N NaOH (0.285 ml), [$^2H_9$]-Ch (40 nmol) and 0.4 N $HClO_4$ (1 ml). Ch was measured in the supernatant by GCMS analysis as described above. To measure GPCh, 0.1 ml of aqueous phase was added to 20 µmol [$^2H_9$]-Ch (0.1 ml). This was mixed with 3 N HCl (0.1 ml), heated at 100 °C for 30 min and cooled to room temperature. 4 N NaOH (0.1 ml) was added and mixed. Following this, 1 ml of 0.4 N $HClO_4$ was added, mixed and allowed to stand for 5 min. After thorough mixing, the suspension was centrifuged at 12,100 × g for 10 min. Ch was measured in the supernatant by GCMS analysis as previously described [10]. The Ch measured as described in section B was subtracted from the Ch measured by cleavage from GPCh. To measure PCh the aqueous phase (0.2 ml) was added to 1 M TAPS buffer at pH 9.2 (0.7 ml), 20 µmol [$^2H_9$]-CH (0.1 ml) and 0.1 ml of 20 mg/ml alkaline phosphatase (Sigma). The mixture was mixed, incubated at 38 °C for 2 h, and cooled to room temperature. 1 ml of 0.4 N $HClO_4$ was added, mixed and allowed to stand for 5 min. After thorough mixing, the suspension was centrifuged at 12,100 × g for 10 min. Ch was measured in the supernatant by GCMS analysis as previously described [10]. The Ch measured as described in section B was subtracted from the Ch measured by cleavage from PCh.

D) *Measurement of plasma and red blood cell lithium*: The concentration of plasma and RBC Li was determined by flame emission photometry. Potassium (K^+) was used as an internal standard. The emission intensities of Li and K^+ from the sample were compared as a ratio to the ratio obtained from standard reference solutions [11].

E) *Statistics*: The patients and controls were compared using Student's t-test. When distribution was skewed the Mann-Whitney U-test was used to compare groups. Results are expressed as mean ± standard deviation (degrees of freedom).

Results

The mean age of the patients was 37.5 ± 9.2 years (15) compared to 37.9 ± 3.4 years (8) for the controls. The plasma Li level ranged from 0.3 to 1.15 meq/l with a mean of 0.51 meq/l. In the patients the mean RBC Ch was 412.0 ± 238.4 nmol/ml (15) compared to 20.7 ± 18.9 nmol/ml (8) in the controls. The distribution was skewed and variance non-uniform so the Mann-Whitney U-test was performed ($U_{15,8} = 0$; $p < 0.00001$). The mean lecithin in the patients was 1270.3 ± 270.4 nmol/ml (15) compared to 1752.1 ± 307.0 nmol/ml (8) for the controls. This difference was highly significant t_{23} 4.08; $p < 0.005$. The GPCh was 6.1 nmol/ml ± 16.6 nmol/ml for the bipolar group compared to 14.7 ± 9.9 nmol/ml for the controls which was significant t_{23} 24.0; $p < 0.005$. The mean patient PCh was 7.3 ± 25.5 nmol/ml compared to 21.7 ± 13.9 nmol/ml for the controls. For PCh the distribution was skewed and variance non-uniform. The Mann-Whitney revealed a significant difference ($U_{15,8}$ 17.5; $p < 0.001$). These results are demonstrated in table 1.

Table 1. Comparison of RBC free and lipid-bound choline in the lithium-treated and normal groups (mean ± SD)

Variable	Patient group (n = 16)	Control group
Age (yr)	37.5 ± 9.2	37.9 ± 3.4
Ch (nmoles/ml)	412.0 ± 238.4 [1]	20.7 ± 18.9
Lecithin (nmoles/ml)	1270.3 ± 270.4 [2]	1752.1 ± 307.0
GPCh (nmoles/ml)	6.1 ± 16.6 [3]	14.7 ± 9.9
PCh (nmoles/ml)	7.3 ± 25.5 [4]	21.7 ± 13.9

[1] Difference between two groups is significant by Mann-Whitney U-test with $U_{15,8} = 0$, $p < 0.00001$. [2] Difference is significant by t-test with $t_{23} = 4.08$, $p < 0.005$. [3] Difference is significant by t-test with $t_{23} = 24.0$, $p < 0.005$. [4] Difference is significant by Mann-Whitney U-test with $U_{15,8} = 17.5$, $p < 0.001$.

Table 2. Comparison of RBC free and lipid-bound choline in patients with lithium therapy for greater and less than 30 days (mean ± SD)

Variable	Duration of Li therapy Over 30 days (n = 8)	Less than 30 days (n = 8)
Ch (nmoles/ml)	543.4 ± 206.8 [1]	280.5 ± 198.8
Lecithin (nmoles/ml)	1222.860 ± 302.977	1317.724 ± 244.360
GPCh (nmoles/ml)	8.088 ± 22.876	4.120 ± 7.689
PCh (nmoles/ml)	12.815 ± 36.246	1.813 ± 3.446

[1] Difference is significant by t-test with $t_{14} = 8.5$, $p < 0.001$.

The 8 patients with Li therapy for over 30 days were compared to the 8 who were treated for less than 30 days. In the < 30-day treatment group the mean Ch was 280.5 ± 198.8 nmol/ml (7) while the > 30-day group had a mean of 543.4 ± 206.8 nmol/ml (7). This difference was significant (t_{14} 8.5; $p < 0.001$). The duration of therapy did not correlate with the concentrations of lecithin, GPCh or PCh. These results are demonstrated in table 2.

Discussion

Previous studies of Li have demonstrated that it blocks RBC Ch transport causing a substantial rise in RBC Ch. The metabolic pathway leading to this Ch accumulation has never been defined although Chapman et al. [12] hypothesized that Li activated phospholipase D, cleaving Ch from lecithin. Work by Miller et al. indicates that RBC phospholipase D is activated [13] by oleic acid although this activation of phospholipase D is not calcium dependent. The accumulation of Ch and loss of lecithin in patients on Li is further evidence for Chapman's original hypothesis.

We demonstrated in this study that Li administration lead to a rise in RBC Ch levels of approximately 20-fold. This rise in Ch was associated with a marked loss of lecithin which approximately equalled the rise of Ch. GPCh and PCh also declined though this drop was of a lesser magnitude. The effect of Li on Ch containing molecules including lecithin, GPCh and PCh has not been previously documented. The loss of these compounds is consistent with the hypothesis that the rise of RBC Ch associated with Li use is due to the degradation of Ch-containing phospholipids. It is not known whether these changes seen in the RBC occur in other cell types, although Miller et al. [14] have shown that in normal elderly and patients with Alzheimer disease RBC Ch strongly correlated with Ch concentration in lymphocytes and granulocytes. It seems likely that similar changes occur within the brain.

These large changes in Ch containing phospholipids and phosphomono and diesters (GPCh and PCh) may have potential implications for both therapy and toxicity. Recent evidence demonstrates that potassium-selective channels in rat atrial cells are modified by arachidonic acid and lecithin [15]. These authors and others have suggested that fatty acids and lecithin may 'constitute a class of signal molecules that regulate ion channels'[16]. The large changes in lecithin seen in this study may mediate the pharmacologic action of Li.

Permanent neurological injuries have occurred with both acute and chronic Li therapy [17]. Although toxicity is not common and usually occurs in the setting of elevated plasma Li in patients on multiple medications [18], it can develop in patients on chronic therapy with therapeutic Li levels [19]. Common permanent sequelae include deficits in recent memory [20], ataxia, chorea and/or parkinsonism [17].

All of the patients in this study developed a marked rise in RBC Ch, although the changes in lecithin, GPCh and PCh were more variable. A prospective study is needed to see whether there is a relationship between treatment response or toxicity and changes in these compounds. However, there was no clear correlation between loss of lecithin, GPCh and PCh and treatment response in this small study.

Whether the changes that we have described in Ch containing phospholipids and Ch-bound mono and diesters contribute to the therapeutic efficacy and/or toxicity of Li is unknown. The demonstration that the changes in RBC Ch-bound compounds are present in other cell types will be important. Autopsy studies of brain phospholipids and possibly in vivo NMR spectroscopy analysis of patients on Li and in those with permanent neurological injuries from Li may help to answer these important questions.

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