Effect Of High Lithium Carbonate Doses On Rat Brain Content Of Some Neurotransmitters

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Abstract : We pursued the effect of high doses of lithium carbonate on rat brain neurotransmitters and their precursors, with spot on needed minerals for their synthesis at different periods. The use of high doses of anti depressants, was reported without reference advice, we tried toxic doses (150 mg/kg/d) on four groups of rats, 6/each. They were randomly recruited into, a control group, given only saline by same volume and duration (group 1), acute study, given a single dose, for 2 hours (group 2), sub-acute study group, given a dose each 3 days for 2 weeks (group 3) and a chronic study group, given dose each 3 days for a month (group 4). Brain content of γ- aminobutyrate (GABA), glycine, histamine precursor (histidine), dopamine precursors (tyrosine and phenyl alanine), were significantly decreased after 2 hours, 2 weeks and one month of administration, possibly assuming a role of lithium in depression management, with dual role in conversion of these amino acids into protein to maintain tissue integrity. These pathways, may explore better understanding for lithium actions with a non-harmful action of large doses at brain tissue level, apart from extra cranial tissues.

Key words: Lithium carbonate; High doses; Brain tissue; Antidepressant; Experimental study; Bipolar Disorders; Neurotransmitters; Amino acids.

1. Introduction

Lithium is a well established therapy for depression. Frequently, it is prescribed when other modalities are not effective in a number of affective disorders, as schizophrenia, and some psychiatric disorders in childhood [1, 2]. It decreases the suicidal risk in affective disorders [1-3]. Lithium slowed down the electro-encephalograph (EEG) with behavioral control [4], with satisfactory results when tricyclic medicines were ineffective, so lithium therapy restored emotional affections of manic-depressive (bipolar) patients. In addition, lithium treatment decreases the scores of depression [5, 6]. Lithium exhibits complex pharmacodynamic effects, so that different mechanisms contribute to its therapeutic or toxic outcomes. Its effects can be referred to maintaining cell membrane integrity, cell membrane transport, neurotransmitter synthesis and regulation of intracellular signaling, which can be characterized as communicated multilevel cascades[7]. Actually, lithium may elicit differential effects on tissues and/or brain regions that cannot be translated into individual effects in specific brain regions in affective patients [8]. Some reports correlate mitochondrial dysfunction to bipolar disorder, oxidative stress and reduced levels of glutathione. Lithium showed anti oxidant properties by up-regulating complex I and II of the mitochondrial electron transport chain[4, 9]. Lithium administration significantly decreased brain contents of some amino acids including phenylalanine and tyrosine in rats [10, 11]. Administration of lithium induced potential brain biochemical changes in treated rats, including serotonin synthesis from its precursors [12, 13]. In some animal studies, lithium has been shown to increase serotonin transmission by increased synthesis, increased uptake of tryptophan and elevated serotonin availability[14, 15]. Serotonergic effects of lithium have been suggested to be responsible for its antisuicidal and antiaggressive actions, as well as contributing to antidepressant augmentation. However, sub acute study in healthy volunteers did not support a prominent effect on serotonergic function, but found small changes in noradrenergic signaling, consistent with increased norepinephrine release[16, 17]. Lithium administration does not seem to reduce basal dopaminergic tone, but inhibits increased dopaminergic activity possibly via action on β-arrestin complexes[4]. This has been speculated as possibly contributing to the antimanic and even antipsychotic effects [18]. With respect to glutamatergic system, lithium shows several complementary actions. First, in acute administration it increases glutamate release, blocks glutamate reuptake by competing with magnesium (Mg2+) ion, while after several days these effects were reversed due to reduction of synaptic concentrations of glutamate by increasing and stabilizing its reuptake[19, 20]. The overall effect of lithium on neurotransmission is modulation of both inhibitory and excitatory signaling [21]. Calcium has multiple roles in neurons, it acts as a second messenger in cell bodies, triggers neurotransmitter release in presynaptic terminals and enters cells via several different mechanisms from both extracellular space and intracellular (endoplasmic reticulum) sites via voltage-gated and ligand-gated channels [22]. These gates can be regulated by lithium. Calcium abnormalities in bipolar disorders (BD) and the role of mood stabilizers in regulating calcium homeostasis have been well documented [20, 23]. This study was conducted to elucidate to what extent high doses of lithium carbonate affect the metabolic pathways at different durations of treatment and explore neurotransmitters levels and amino acids/neurotransmitters exchange at brain tissue level, to
judge whether these toxic doses are neurotoxic or only damage extra cranial tissues.

2. Materials and Methods

2.1. Animals
Twenty four male Swiss albino rats were used in this study, after a mortality of about 30%; kept on standard diet and drinking water ad libitum for one week before starting the experimental work. The animals were divided into 4 equal groups, group 1 served as normal control, received saline intra peritoneally, given normal saline by same volume and duration till the last corresponding dose of group 4, group 2 was used for the study of the acute effect of lithium carbonate; they received a single intra peritoneal dose of 150mg /kg body weight [24]. Group 3, they were injected by lithium carbonate (Sigma, USA) as 150 mg /kg/3 days for two consecutive weeks, i.e: a total of 5 doses (sub-acute study) and animals of group 4 were given 150mg/kg/3 days for a month, i.e: a total of 10 doses (chronic study). This dosing regimen was chosen after many trials made to reach the least mortality (30%, due to tested overdose). The experiment was executed in the Faculty of Pharmacy, Kafrelsheik University, Egypt.

2.2. Preparation of brain samples
The animals of each group were sacrificed, without anesthesia, to avoid expected effects on brain metabolism, their heads were put into ice immediately. The brains were separated, washed by saline, divided into different portions for each rat. The first aliquot of brain tissue was triturated with 80% ethanol at 4°C. The mixture was centrifuged at 3000 rpm, the supernatant was used for determination of free amino acids. The rest ethanolic brain extract portions were kept for determination of dopamine and histamine, while calcium and magnesium were determined in 0.1 N HCl brain tissue extract.

2.3. Chemicals
All chemicals were of analytical grade, obtained from Sigma-Aldrich, USA.

2.4. Methods
The ethanol extract was used for free amino acid estimation, using auto analyzer [25] and a fluorometric method was used for the determination of brain content of histamine [26] and dopamine [27] in the same ethanol extract. Calcium [28] and magnesium [29] were measured using enzymatic colorimetric Assay kits.

2.5. Statistical analysis
The statistical analysis of data was done using Graph Pad Prism 5 (Graph pad Software, San Diego California, USA). The results were expressed as means ± SEM, differences between the mean values for individual groups were assessed by one way ANOVA.

3. Results
Lithium carbonate induced a significant decrease in brain content of dopamine throughout the three time periods (p<0.001) (figure 1) while for histamine brain content lithium induced a significant increase after 2 hours (P<0.01) and one month (P<0.05) periods and significant decrease after 2 weeks of treatment (p<0.001) (figure 2).

Regarding γ-aminobutyrate, glycine histidine, phenylalaninine and tyrosine, lithium treatment induced a significant decrease in their brain content throughout the three time periods (p<0.001) (figure 3). Lithium induced a significant increase in brain content of magnesium after 2 hours and 2 weeks periods (p<0.001) while induced significant decrease after one month of treatment (p<0.001) (figure 1). Regarding brain content of calcium lithium induced a significant decrease after 2 hours and one month periods while induced significant increase after 2 weeks of lithium treatment (p<0.001) (figure 2).

4. Discussion
Brain tissue metabolism was reported to be disturbed, both quantitatively and qualitatively in some mood disorders, such as schizophrenia, BD and other depressive illnesses [30]. Moreover, low magnesium feeding induced alterations in brain protein contents, contributing to central deregulation, an apparent associate to affective disorders[31]. Lithium has been and continues to be the mainstay of BD pharmacotherapy, managing, prevention, prophylaxis, treatment and suicide protection. Although it has recently been studied in other psychoses as well as diverse neurodegenerative disorders, the drug cost effectiveness and availability make its use as a preferred choice [32]. Amino acids can affect brain functioning and mental health. Many of the neurotransmitters in the brain either are/ or made from amino acids. Lack of some amino acids is associated with depressed mood and aggressive behavior. The excessive buildup of amino acids may also lead to brain damage and mental retardation. For example, excessive buildup of phenylalanine in the individuals with disease called phenylketonuria can cause brain damage and mental retardation[33]. In the present work, it was important to study the effect of lithium on brain content of different free amino acids because some are neurotransmitters themselves or precursors for neurotransmitters. The decrease in γ-aminobutyric acid (GABA) and glycine during the different periods of administration indicated that lithium treatment preferentially depresses the formation of glycine and GABA, by inhibition of phosphat e-dependent glutaminase [34, 35]. The continuous decreasing level of glycine and GABA can be attributed to the antidepressant trait of lithium, being inhibitory neurotransmitters , thus, the mood disruptions may improve [36, 37]. High lithium dose exhibited prominent actions on amino acid content in the brain [38]. Meanwhile, histidine, phenyl alanine, tyrosine contents were significantly decreased allover treatment periods. In addition to a possible activation of incorporation of amino acids into polypeptides, lithium administration activates conversion of these three amino acids into active biogenic amines, as neurotransmitters, through hydroxylation [13, 39, 40]. The significant decrease in histidine content especially after one month of treatment could be parallel with increased content of histamine that indicates a possible activation of histamine synthesis in the brain, by activating histidine decarboxylae [41, 42]. Calcium ions are necessary for the release of catecholamines of the adrenal medulla and helps neurotransmitters to couple their receptors in the brain. The decrease in brain content of calcium after lithium treatment of one month was in accordance with [43, 44] that noticed elevated serum calcium during lithium
treatment showing calcium flux from the blood brain barrier to blood [45]. The decreased brain calcium content was associated with decreased dopamine content as the release of the catecholamines at the synapses is dependent on calcium ions [44]. Deficiency of magnesium brain content is responsible for hyper excitability and disturbed nervous performance, thus, patients with depression showed low plasma magnesium [46, 47]. Lithium treatment induced a significant increase in brain content of magnesium after 2 hours and 2 weeks, so it can provide a mechanism for alleviation of depressive symptoms. Chronic lithium treatment induced a significant decrease in brain content of magnesium that could be due to induction of magnesium renal excretion [46]. These potential benefits of lithium salts on neuroprotection and neuroregeneration, assures preclinical evidence underlying its mood stabilizing properties[48, 49].

5. Conclusion
Toxic doses of lithium although induced around 30% mortalities among treated animals, they were safe to brain tissue regardless other extra cranial tissue. The major pathway of lithium carbonate in controlling BD, seemingly through conversion of some amino acids into their corresponding stimulatory neurotransmitters after acute and chronic treatment, also control the level of inhibitory amino acids as GABA and glycine in direction of mood stabilization, managing neuro transmission and tissue stabilization.

Author Contributions
Nabil Mohie suggested the point and executed the experimental work. Both Montaha Al-Saffar and Michael A Fawzy contributed in statistical work and drafting the manuscript.

Declaration of Conflicting Interests
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Ethical Approval
All steps in this work are in agreement with the ethical standards of the University Research Committee and Helsinki declaration; no formal ethical review was required.

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**Nabil Mohie Abdel–Hamid.**
Professor of Cancer Biology at Department of Biochemistry, Ex- and First Dean and Founder of Faculty of Pharmacy, Kafrelsheikh University, Egypt. Had his Master in Biochemistry by 1989, and his PhD in biochemistry by 1999. Member of The Higher Consulting Organization of Egyptian Scientists Council. Deputy of Director of: The Egyptian Association of Cancer Research. Member in: The Arab Evaluators for Scientific Degree Promotions for Professorships. Member in: The Egyptian Evaluators for Scientific Degree Promotions for both associate and full Professorships. Research Interest: Cancer Biology, Principal Instructor and interested in Hepatocellular Carcinoma Research(Both Early Detection, Looking for New Sensitive and Specific Markers and Therapeutic Perusal), as New Trends in Management of Hepatocellular Carcinoma, Including Chemo and Radio Sensitization to Cancer Therapy. After long time of studying cell membrane, we now work on targeting liver cancer cell through membrane protein leads to minimize side effects, dose burdens, and treatment durations, with a maximal efficacy.

**Fig 1:** The effect of lithium carbonate (150 mg/kg) on dopamine and manesium contents in rat brain tissue extract after 2 hrs., 2 weeks and one month of oral administration (Values are expressed as mean± SE, number of rats= 6):
**Fig 2**: The effect of lithium carbonate (150 mg kg\(^{-1}\)) on histamine and calcium contents in rat brain tissue extract after 2 hrs, 2 weeks and one month of oral administration (Values are expressed as mean± SE, number of rats= 6):

![Histogram of histamine and calcium concentrations](image)

**Parameter**

**Fig 3**: The effect of lithium carbonate (150 mg kg\(^{-1}\)) on glycine \(\delta\)-aminobutyrate, histidine, phenyl alanine and tyrosine contents in rat brain tissue extract after 2 hrs, 2 weeks and one month of oral administration (Values are expressed as mean± SE, number of rats= 6):

![Bar chart of glycine, \(\delta\)-aminobutyrate, histidine, phenyl alanine, and tyrosine concentrations](image)