INTRODUCTION

Lithium, which is widely used in the treatment and prophylaxis of bipolar disorder, has very narrow therapeutic index and its toxicity is common in patients under lithium therapy. Accordingly, it has been found that the CNS is mainly affected area (Sheean, 1991; Suraya and Yoong, 2001; Oakley et al., 2001).

Enhanced elimination of lithium by various dialysis methods is the valid therapy of this toxicity despite the fact that sometimes sequelae or death can not be prevented (Apte and Langston, 1983; Waring, 2006). Severe memory impairment and a permanent seizure disorder have been reported as sequelae of acute overdose of lithium (el-Mallakh and Lee, 1987; Saxena and Mallikarjuna, 1988). Inositol and forskolin were used to ameliorate this toxicity regarding their possible interactions with lithium actions but they were not found effective (Kaplan et al., 1988).

Nevertheless, recent studies imply that nitrergic and glutamatergic systems, which are closely related to each other (East and Garthwaite, 1991), may be involved in lithium actions (Harvey et al., 1994; Harvey, 1996). In fact, it has been reported that lithium induces the activation of nitric oxide synthase (NOS) in hippocampus, cerebral cortex and cerebellum of the different groups of animals. GAD enzyme activity reduced in cerebral cortex but not altered in hippocampus or cerebellum by lithium as compared to the control (saline) group. We conclude that an interaction with nitrergic and glutamatergic systems may have a role in the acute toxicity of lithium in rats. The inhibition of glutamate metabolism may arise from this interaction and the involvement of GABA-ergic system should be further investigated in this toxicity.

Key words: Lithium toxicity, NO, Glutamate, GABA, Rats

Original Article

Nitrergic, glutamatergic and gabaergic systems in lithium toxicity

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ABSTRACT — We examined the role of nitrergic, glutamatergic and gamma-aminobutyric acid (GABA)-ergic systems in the mechanism(s) underlying lithium induced acute toxicity. With this aim, lithium (18 mEq/kg, i.p.) intoxicated rats were observed for 3 hr recording their clinical signs and death. Lithium exposure at the dose used produced central nervous system (CNS) depression. Pre-treatment of Nw-nitro-L-arginine methyl ester (L-NAME) a nonselective nitric oxide synthase inhibitor (10 mg/kg, i.p.), 7-nitroindazole (7-NI) a selective neuronal nitric oxide synthase inhibitor (25 mg/kg, i.p.), nitric oxide precursor L-arginine (1,000 mg/kg, i.p.) and MK-801 a noncompetitive antagonist of N-methyl-D-aspartic acid class of glutamate receptors (0.5 mg/kg, i.p.) all increased CNS depression and mortality in lithium group however, no change was seen in GABA receptor agonist GABA (1,000 mg/kg, i.p.) or D-arginine (1,000 mg/kg, i.p.) a biologically inactive enantiomer of L-arginine pre-treated rats. Glutamic acid decarboxylase (GAD) enzyme activity was measured in hippocampus, cerebral cortex and cerebellum of the different groups of animals. GAD enzyme activity reduced in cerebral cortex but not altered in hippocampus or cerebellum by lithium as compared to the control (saline) group. We conclude that an interaction with nitrergic and glutamatergic systems may have a role in the acute toxicity of lithium in rats. The inhibition of glutamate metabolism may arise from this interaction and the involvement of GABA-ergic system should be further investigated in this toxicity.

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in mouse cerebral cortex by presynaptic nerve endings (Dixon and Hokin, 1998). It produces subtype-specific alterations of glutamate receptor function (Karkanias and Papke, 1999) and displays an ability to potentiate glutamatergic pathways in the CNS (Weiss et al., 1990).

As the opposite of glutamatergic system, questions about the effects of lithium on GABA-ergic system have been raised, as well. A number of animal studies have found that GABA concentration increases in some brain areas following lithium administration depending on the dose and the length of treatment (Gottesfeld, 1976; Marcus et al., 1986; Otero Losada and Rubio, 1986). Moreover, it was reported that GABA<sub>B</sub> receptors are up-regulated by chronic lithium treatment (Motohashi et al., 1989).

Taken together, the accumulating data indicate that both nitrergic and glutamatergic systems may play a role in lithium actions. Besides, reported changes in GABA-ergic system suggest a relationship between lithium and GABA-ergic system.

One of the current challenges is to distinguish the critical effects of lithium from the many known biochemical actions, many which may lead to toxicity (Shaldubina et al., 2001). This study was conducted to clarify the involvement of nitrergic, glutamatergic and GABA-ergic systems in acute lithium toxicity. For this purpose, it was of interest to evaluate the effects of N<sup>ω</sup>-nitro-L-arginine methyl ester (L-NAME) a nonselective NOS enzyme inhibitor, 7-nitroindazole (7-NI) a neuronal NOS selective inhibitor and L-arginine NO precursor, MK-801 a non-competitive antagonist of N-methyl-D-aspartic acid (NMDA) class of glutamate receptors and GABA as an agonist of GABA receptors on the clinical signs and mortality of acute lithium toxicity in rats.

**MATERIALS AND METHODS**

**Animals**

Inbred male Wistar Albino rats (250-300 g) were used in this study. Until the experiments, animals housed in groups of five hold in a 12 hr light/dark cycle in temperature (21 ± 2°C) controlled room. They had free access to food and water.

The animals were handled according to the guidelines for the care of laboratory animals and in compliance with EEC Council Directive 86/609.

**Drugs and procedures**

Lithium chloride, L-NAME 7-NI, L-arginine, D-arginine, peanut oil and GABA were purchased from Sigma (Steinheim, Germany), MK-801 was purchased from Merck (Rahway, NJ, USA). All drugs were dissolved in saline while 7-NI was dissolved in peanut oil with sonication in ice-cold medium. Chemicals for the measurement of GAD activity with the exception of ninhydrin (BDH Chemicals, Leicestershire, England), triton X-100 (Merck, Darmstadt, Germany) and trichloroacetic acid (Merck, Darmstadt, Germany) were obtained from Sigma (Steinheim, Germany) Chemical Co.

Acute toxicity in rats was induced by administering lithium at a dose of 18 mEq/kg, intraperitoneally (i.p.). This dosage of lithium provided a toxic serum level within 6.9-8.6 mmol/l.

Rats received lithium, were pre-treated with L-NAME at the dose of 10 mg/kg (i.p.), 7-NI at the dose of 25 mg/kg (i.p.), L-arginine at the dose of 1,000 mg/kg (i.p.), D-arginine at the dose of 1,000 mg/kg (i.p.), MK-801 at the dose of 0.5 mg/kg (i.p.), peanut oil (vehicle) or saline (control) in equal volumes 15 min before lithium injection. In all groups, clinical signs and death were recorded within 3 hr. The rats survived after 3 hr were euthanized.

Some rats, received lithium, pre-treated with saline (i.p.), were used for determination of glutamic acid decarboxylase (GAD) enzyme activity in hippocampus, cerebral cortex and cerebellum. One group received saline, pre-treated with saline was added as control group to compare GAD activity. Three hours after the injections, rats in these groups were sacrificed by decapitation. Their brains were rapidly removed from the skull. The hippocampi, cortex and cerebella were separated. GAD activity was determined by the spectrofluorimetric method described previously (Lowe et al., 1958; Holdiness and Justice, 1980). In brief, frozen-dried tissue samples were then sonicated in 20 volumes of ice-cold 0.4 M sodium phosphate buffer (pH 6.4) containing 0.5 M KCl (potassium chloride), 0.01 M EDTA (ethylenediaminetetraacetic acid) and 0.5% Triton X-100. Then 100 μl of 1% (w/v) tissue homogenates were incubated with 100 μl substrate buffer containing 1 ml of 0.4 M sodium phosphate buffer (pH 6.4), 1 ml of 100 mM sodium L-glutamate in 0.4 M sodium phosphate buffer (pH 6.7) and 40 μl of 50 mM pyridoxal 5-phosphate dissolved in 0.4 M sodium phosphate buffer (pH 6.4), for two hours at 38°C and the reaction was terminated by adding 200 μl of 10% TCA (trichloroacetic acid). In a blank tube 200 μl of 10% TCA was added before tissue homogenate and substrate buffer. The tubes were centrifuged for 20 min at 950 x g and 200 μl of each supernatant was transferred to glass tubes containing 400 μl of 14 mM ninhydrin dissolved in 0.5 M sodium carbonate buffer (pH 9.95). After the incubation at 60°C for 30 min, 9 ml of copper tartrate reagent
containing 1.6 g sodium carbonate, 329 mg tartrate acid and 300 mg cupric sulphate in 1 L of distilled water, was added and after 20 min at room temperature the JAS- CO FP-750 spectrofluorometer (serial no:A001760557, Japan) was used on excitation wavelength of 375 nm and on emission wavelength of 450 nm for the determination of GABA formatted. The results were calculated according to the GABA standard and expressed as ng GABA/hr/mg protein. Protein content was determined by the method of Lowry (Lowry et al., 1951) with bovine serum albumin as standard.

**Statistical analysis**

Statistical analysis for clinical signs and death were carried out by means of Fischer’s exact test and for GAD enzyme activity by means of unpaired Student’s t-test. The level of significance was defined as P < 0.05.

**RESULTS**

**Clinical signs and death**

Table 1 shows clinical signs and death in rats received lithium three hours posttreatment. Decrease in motility, loss of righting reflex and diarrhea were observed as clinical signs.

There were no any statistically significant differences in the number of clinical signs and death in peanut oil (vehicle) or D-arginine (biologically inactive enantiomer of L-arginine) pre-treated groups, compared to the control (saline pre-treated) group (Table 1). In L-NAME, 7-NI, L-arginine (Table 1) and MK-801 (Table 2) pre-treated groups, the number of death and the number of rats with loss of righting reflex increased significantly comparing to the corresponding control group. Yet, GABA pre-treatment did not affect observed clinical signs and the number of death in rats intoxicated with lithium (Table 2). In all groups, there was no significant change in the number of rats with diarrhea.

The differences in the number of deaths in all groups were not statistically significant when we analysed for each hour within three hours (Tables 3 and 4).

**GAD enzyme activity**

Figure 1 shows cortical, hippocampal and cerebellar GAD enzyme activities in the lithium (saline pre-treated) and the saline (saline pre-treated) groups. Cortical GAD enzyme activity in the lithium group was found lower than the saline group while there was no significant difference in GAD enzyme activities of hippocampus or cerebellum between the lithium and the saline groups.

**DISCUSSION**

Present study was designed to determine the role of Table 1. The effects of NOS inhibitors and NO precursor on clinical signs and death in acute lithium intoxication.

<table>
<thead>
<tr>
<th>Pretreatment (mg/kg)</th>
<th>N</th>
<th>Decrease in motility</th>
<th>Loss of righting reflex</th>
<th>Diarrhea</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>8</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Peanut Oil</td>
<td>8</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>D-arginine (1000)</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>L-NAME (10)</td>
<td>6</td>
<td>6</td>
<td>6(^a)</td>
<td>0</td>
<td>6(^a)</td>
</tr>
<tr>
<td>7-NI (25)</td>
<td>6</td>
<td>6</td>
<td>6(^b)</td>
<td>1</td>
<td>6(^b)</td>
</tr>
<tr>
<td>L-arginine (1000)</td>
<td>6</td>
<td>6</td>
<td>6(^a)</td>
<td>0</td>
<td>6(^a)</td>
</tr>
</tbody>
</table>

Values represent the number of animals show the finding specified in groups. N = Total number of animals in each group.

\(^a\) P < 0.01 compared with saline, \(^b\) P < 0.05 compared with peanut oil (Fisher’s exact test)

Table 2. The effects of NMDA antagonist and GABA agonist on clinical signs and mortality in acute lithium intoxication.

<table>
<thead>
<tr>
<th>Pretreatment (mg/kg)</th>
<th>N</th>
<th>Decrease in motility</th>
<th>Loss of righting reflex</th>
<th>Diarrhea</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>8</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MK-801 (0.5)</td>
<td>6</td>
<td>6</td>
<td>6(^a)</td>
<td>1</td>
<td>6(^a)</td>
</tr>
<tr>
<td>GABA (1000)</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Values represent the number of animals show the finding specified in groups. N = Total number of animals in each group.

\(^a\) P < 0.01 compared with saline (Fisher’s exact test)
nitrergic, glutamatergic and GABA-ergic systems in acute lithium toxicity. Clinical signs in rats received lithium at toxic dose, were observed similar to the signs seen in mice (Alexander et al., 1982). According to the results obtained, some drugs used for the pre-treatments, intensified these signs in rats intoxicated with lithium. Administered doses of all pre-treatment drugs are commonly used doses in behavioural experimental models and are not lethal (Serrano et al., 1986; Stafstrom et al., 1997; Yamanturk et al., 1998).

NOS inhibitors intensified the acute toxic effect of lithium in rats. Consistently with this finding, Dehpour and co-workers (Dehpour et al., 2000) found some clues that there may be a synergism between NOS inhibitor L-NAME and lithium while an antagonism between NO precursor L-arginine and lithium in morphine withdrawal syndrome in mice. But, in contrast to acute toxic dose treatment of lithium in our study, they administered lithium at non-toxic dose and chronically. It has also been shown that L-NAME potentiates antidepressant-like effects of acute lithium administration at non-effective dose in the mouse forced swimming test. Furthermore, L-arginine at non-effective dose reverses the antidepressant-like effect of lithium (Ghasemi et al., 2008).

Table 3. The effects of NOS inhibitors and NO precursor on time course of mortality in acute lithium intoxication.

<table>
<thead>
<tr>
<th>Pretreatment (mg/kg)</th>
<th>N</th>
<th>Hours after intoxication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.</td>
</tr>
<tr>
<td>Saline</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Peanut Oil</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>D-arginine (1000)</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>L-NAME (10)</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>7-NI (25)</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>L-arginine (1000)</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

Values represent the number of animals died in groups. N = Total number of animals in each group.

Table 4. The effects of NMDA antagonist and GABA agonist on time course of mortality in acute lithium intoxication.

<table>
<thead>
<tr>
<th>Pretreatment (mg/kg)</th>
<th>N</th>
<th>Hours after intoxication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.</td>
</tr>
<tr>
<td>Saline</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Peanut Oil</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>D-arginine (1000)</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>L-NAME (10)</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>7-NI (25)</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>L-arginine (1000)</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

Values represent the number of animals died in groups. N = Total number of animals in each group.

Fig. 1. The effect of acute lithium intoxication on GAD activity in hippocampus, cortex and cerebellum. Data were presented as mean and standard error of the mean. Number of animals were 6 in each group. aP < 0.01 compared with control (Student’s t test).
frontal cortex and cerebellum measured ex vivo (Wegener et al., 2004). However, Bagetta and co-workers (Bagetta et al., 1993) reported that lithium at toxic dose induces NOS activity in hippocampus measured ex vivo. In another study of the same authors (Bagetta et al., 1995), there was no change in brain citrulline, the co-product of NO synthesis following lithium injection at the same toxic dose.

Surprisingly, NO precursor L-arginine also increased CNS depression and mortality in rats intoxicated with lithium. This effect could not be explained by nonspecific change since only L-arginine but not the biologically inactive enantiomer, D-arginine modified this parameter. On the other hand, the literature shows contradictory results regarding the dose-response relationship between L-arginine and NO production (Buchmann et al., 1996; Jayakumar et al., 1999). The dose of L-arginine what we used, has been found to produce NO in cerebral cortex of rats while half of this dose did not (Paul and Jayakumar, 2000). However, all these data must be interpreted with caution because the study of Buchmann and co-workers (Buchmann et al., 1996) indicates that pharmacological effects seen after arginine administration could be caused by arginine itself, by its conversion to NO in the midbrain and/or by changes seen with other amino compounds.

Our findings showed that antagonism of NMDA class of glutamate receptors increases CNS depression and mortality in acute lithium toxicity. This result differs from some published studies which report lithium displays an ability to potentiate glutamatergic pathways (Dixon and Hokin, 1998; Dixon et al., 1994; Karkanias et al., 1998; Weiss, 1990). Based on this, it would be expected that NMDA receptor antagonism would attenuate acute toxicity of lithium. Yet, in a recent article it has been reported that NMDA antagonists augment antidepressant-like effects of lithium at non-toxic dose (Ghasemi et al., 2010). This result is consistent with our data with the exception of the lithium dose suggesting the role of NMDA receptors in lithium’s acute effects. On the other hand, accumulating data indicate that lithium attenuates the effects of glutamate-mediated calcium signalling (Nonaka et al., 1998; Hashimoto et al., 2002; Sourial-Bassillious et al., 2009). Furthermore, the results of Antonelli and co-workers’ study (Antonelli et al., 2000) supports our interpretation as acute lithium administration at a high, non-therapeutic dose (4 meq/kg, s.c.) reduces frontal cortex dialysate glutamate levels long-lasting. These results may explain high mortality in MK-801 pre-treated group since it further decreases glutamatergic activity in lithium toxicity. The increase in mortality both in NOS enzyme inhibition and NMDA receptor antagonism may be due to the relation between glutamatergic and nitrergic systems described previously (East and Garthwaite, 1991). These interactions are discussed in more detail in the review of Ghasemi and Dehpour (Ghasemi and Dehpour 2011).

Interestingly, NO stimulation of the guanylyl cyclase by NO donors leading increase cGMP, decreases the exocytotic release of glutamate (Sistiaga et al., 1997; Sequeira et al., 1997). This effect of NO formation may be involved in the mechanism underlying L-arginine induced high mortality in acute lithium toxicity by decreasing the glutamate release more.

According to our results, GABA did not alter the intensity of acute lithium toxicity although some clues of the interaction between lithium and GABA-ergic system existed in the literature. Following the administration of a single dose of lithium (3 mEq/kg), GABA levels were found to increase in cerebral cortex and brain stem (Marcus et al., 1986). In the study of Antonelli and co-workers (Antonelli et al., 2000), lithium at toxic dose, which is found to reduce glutamate levels in frontal cortex, prolonged increase in dialysate GABA levels. Furthermore, they found that the reduction in glutamate levels was reversed by intracortical perfusion of a GABA\textsubscript{A} receptor antagonist. In contrast, it has been reported that administration of chronic lithium at non-toxic dose (2 meq/kg) does not alter GABA concentration in whole brain of rats (O’Donnell et al., 2003). In line with this study, Shibuya-Tayoshi and co-workers (Shibuya-Tayoshi et al., 2008) has shown that sub chronic lithium treatment does not change the levels of GABA in the human anterior cingulate cortex. Taken together, these findings are contradictory to judge about the interaction between lithium and GABA-ergic system. Our data may contribute the knowledge about this interaction indicating no relation at toxic dose of lithium. Nevertheless, obtained results do not give enough reason to replicate the existence of the role of GABA-ergic system in this toxicity.

In the acute lithium toxicity, GAD enzyme activity which is responsible for converting glutamic acid to GABA, was found to be reduced in brain cortex. In consistent with this finding, Otero Losada and Rubio showed that a single injection of lithium at the dose of 10 mEq/kg reduces the activity of GAD enzyme in cerebral cortex of rats (Otero Losada and Rubio, 1986). The effect of lithium on the activity of GAD enzyme may be a compensatory response to previously reported high dose lithium induced decrease in cortical glutamate (Antonelli et al., 2000). It would be better to investigate whether reduced cortical GAD activity is affected by the drug pre-treatments we applied. But this could not be realised in our experimental design since dying of NOS inhibitor,
NO precursor and NMDA antagonist pre-treated animals at different time points within 3 hr period.

The evaluation of consciousness state show that consistently with the high mortality in NOS inhibitor, NO precursor or NMDA receptor antagonist pre-treatment groups, the number of animals with complete loss of protective reflexes was found higher than saline pre-treated group.

The number of animals with diarrhea did not differ among experimental groups. This result may help to rule out possible pharmacokinetic interaction between lithium and the drugs pre-treated since higher lithium blood levels are expected to cause diarrhea more often.

To investigate possible mechanisms underlying the effects of lithium, it is administered to various species at different doses with various exposure times in most of the studies cited above. Methodological differences in the studies seem to cause discrepancy in the accumulating data. Thus, we have taken the dose used and exposure time in rats as reference to evaluate our results.

The present study extends the knowledge obtained from the previous in vitro experiments related with the interactions between lithium and both nitrergic and glutamatergic systems by its in vivo design focusing on acute lithium toxicity. A suggestion which may emerges from these findings is that previously observed interactions between lithium and GABA-ergic system seem not to be contributed in acute lithium toxicity. Nevertheless, possible role of GABA-ergic system should be further investigated. In conclusion, we can say that nitrergic and glutamatergic systems might be involved in lithium-induced acute toxicity in rats. Further studies should be done to clarify more the obtained results. These efforts may lead a new approach to the treatment of acute lithium toxicity.

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REFERENCES


Harvey, B.H., Carstens, M.E. and Taljaard, J.J. (1994): Evidence


Central mechanisms of lithium toxicity