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Behavioral and neurochemical responses to 8-OH-DPAT in restrained and unrestrained animals treated with lithium carbonate in drinking water

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Abstract: Lithium has been suggested for mood disorders and neurodegenerative diseases. Its ability to increase the gray matter and provision of protection against neuronal death makes it tempting to be marketed as brain food. Moreover it also ameliorates the effects of stress on brain dendrites; however lithium has a narrow therapeutic range. Brain serotonin (5-HT) neurotransmission may mediate the actions of lithium. Preclinical studies have shown that single restraint stress produces behavioral and neurochemical deficits. The present study was designed to investigate a potential role of Lithium in attenuation of stress induced behavioral and neurochemical deficits in rats. Moreover the study also monitored the responsiveness of pre and post synaptic serotonin 1A receptor following restraint and administration of lithium carbonate. Pre stress behavioral activities were monitored after 15 and 30 days of consumption of 0.1% lithium carbonate in drinking water while post stress were monitored on day 31. Pre and post synaptic 5-HT-1A responsiveness was monitored by injecting 0.25mg/ml/kg of 8-OH-DPAT. Although lithium produced hypo activity but attenuated stress induced behavioral deficits. Whole brain neurochemical analysis revealed that its administration increased tryptophan, 5-HT and 5-Hydroindoleacetic acid (5-HIAA). 8-OH-DPAT elicited hyperactivity and fore paw treading were enhanced in lithium treated rats. Lithium induced pre synaptic changes together with the super sensitivity of post synaptic receptors may be able to produce antidepressant effect.

Keywords: Lithium carbonate, stress, 8-OH-DPAT, 5-HT metabolism, RAM, EPM, exploratory activity.
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INTRODUCTION

Literature survey has shown that Lithium is being used as brain food. Lithium is an antipsychotic, antimanic medication used for those with affective disorders. Its mood stabilizing action[1,2] has emerged as a robust neuroprotective agent in preventing apoptosis of neurons[3], brain damage in Alzhiemer[4]. It not only protects against neuronal death caused by excessive amounts of glutamate; the brain's most prevalent neurotransmitter[5] and also promotes the stimulation of New Neuronal growth, a process called Neurogenesis[6]. Moreover Moore and his co-workers [7] have also demonstrated lithium induced increase in human brain gray matter. Chronic treatment with a low dose of lithium protects the brain against Ischemic injury by reducing Apoptopic death[8].

Preclinical studies have shown that lithium alters sodium transport in nerve and muscle cells and affects a shift towards intraneuronal metabolism of monoamine and indoleamines. Chronic lithium treatment also prevents the stress induced decrease in dendritic length[9]. Preclinical studies have also arrived to a conclusion that subchronic lithium treatment could enhance the anxiolytic like effects of serotonergic drugs by facilitating central 5-HT neurotransmission at clinically therapeutic plasma lithium levels [10] Moreover it has been also suggested by authors that the anxiolytic action of MAO inhibitors may be enhanced by lithium[11]. Bell and his coworkers [12] have demonstrated that pretreatment with lithium can attenuate dextroamphetamine induced changes in cognitive tasks such as word generation paradigm and spatial attention task in healthy volunteers. It is also known to improve short term memory[13]. Lithium treatment may provide protection to rat cerebrum in protein-deficient cases[14]. Lithium can be effectively used for an augmentation therapy in case of antidepressant non-responders[15,16] and also prevents relapse in cases of unipolar major depressive disorders[17]. Lithium could also be used as an adjunct to manage clozapine-induced neutropenia in Schizophrenic cases[18]. Several lines of evidence suggest that the mode of action for the lithium augmentation of antidepressants is mediated by lithium-induced increase of serotonin neurotransmission. First, in vivo microdialysis studies have shown that subchronic lithium treatment increases extracellular serotonin levels in medial prefrontal cortex and hippocampus[19-22].

Second, lithium has been shown to alter the dynamics of neurotransmission within the serotonergic pathways in the central nervous system with reference to presynaptic and post synaptic effects [23]. Third, in electrophysiological studies, lithium enhances serotonin neurotransmission through its presynaptic action on serotonin nerve terminals [24] .Lithium has narrow therapeutic dose range (0.5-1.2 ηM), below which it is largely ineffective and above it is severely toxic[25, 26].

Research in our laboratory show constantly that single restraint stress produces behavioral and neurochemical deficits[27-30] 8-OH-DPAT (a selective 5-HT-1A agonist) stimulates post synaptic receptors to produce hyperactivity syndrome and pre synaptic receptors to decrease 5-HT synthesis[31,32]. The present study was designed to investigate a potential role of Lithium in attenuation of stress induced behavioral

and neurochemical deficits in rats. Moreover the study would also monitor the responsiveness of pre and post synaptic serotonin 1A receptor following restraint stress and also long term administration of Lithium Carbonate.

Experimental protocol

Forty eight albino Wistar rats weighing 180-230gm purchased from Aga Khan University Hospital, Karachi, Pakistan were housed individually and maintained under a 12h light /dark cycle in a temperature controlled environment. Animals were given free access to standard rodent diet and tap water. After acclimatization animals were divided into controls and Lithium treated groups (24 animals in each group). The test animals received 0.1% lithium carbonate (Sigma) in drinking water for 32 days [33] while the controls were given tap water for drinking.

Exploratory activity[34] in an open field was monitored on day 15th at 09 00 as described in material and method section. Home cage[34] activity was also monitored on day 15 at 13 00 as described in material and method section. Activity in an EPM was monitored on day 16 at 09 00 and light/ dark box[35] activity were monitored on day 17 at 09 00 to determine the index of fear/fearlessness. Radial maze testing (RAM)[36] was done on the 29th day after the training of the animals. On day 31 the animals of each group were further divided into unrestrained and restrained. Unrestrained animals were left in their home cages while their restrained counter parts were immobilized on wire grids for 2 h (0900-1100).The restraining procedure was essentially the same as described earlier [28]. Home cage, exploratory, EPM and Light/dark activities were monitored as post stress behavioral activities 24 h after the last stress as described previously[34-36].

The animals of each group were further divided into two groups (6 animals in each group) were treated with saline or 0.25mg/ml/kg of 8-OH-DPAT[37]. Intensity of 5-HT syndrome elicited by 8-OH-DPAT (Sigma) was scored from 5-30 min post injection. On day 32 the animals were decapitated by cervical dislocation. Brains were dissected out and stored at -70° C for neurochemical estimation by HPLC-EC. All the experiments were carried on as approved by the local Ethical Committee.

Statistical analysis

Pre stress data was analyzed using student's t test while post stress by 2-Way ANOVA. The effects of lithium carbonate administration, restraint stress and 8-OH-DPAT injection were carried using 3-Way ANOVA. Post hoc analysis was done by Neuman Keul's test.

RESULTS

Figure 1 shows the effects of chronic lithium carbonate on cumulative food intake (a), body weight (b) and fluid intake (c). Data computed using t test revealed that lithium carbonate had no effects on food intake and body weight, while fluid intake was increased ($P<0.05$).

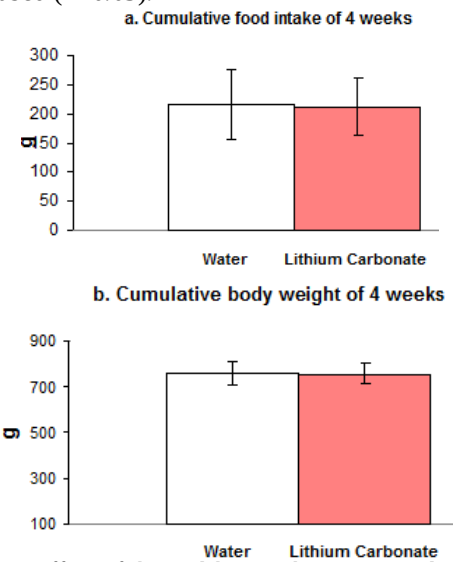

Figure 1: Effects of chronic lithium carbonate on cumulative food intake (a), body weight (b) and fluid intake.

Figure 2 shows the effects of chronic lithium carbonate on home cage (a) and open field activity (b) monitored on the 15th day. Data analyzed by student t test showed that lithium carbonate decreased activity in home cage and open field ($P<0.01$).

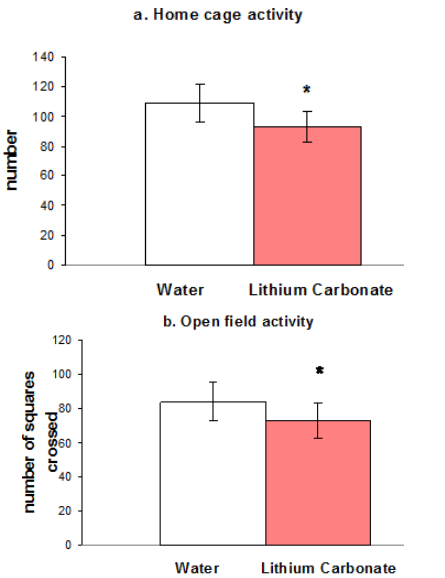

Figure 2: effects of chronic lithium carbonate on home cage (a) and open field activity (b) monitored on the 15th day.

Figure 3 shows the effects of chronic lithium carbonate on time spent (a) and the number of entries (b) in the lit compartment of the light/dark box on the 16th day. Data analyzed by student's t test revealed no effect of lithium carbonate on the number of entries and the time spent in the lit compartment (P>0.05).

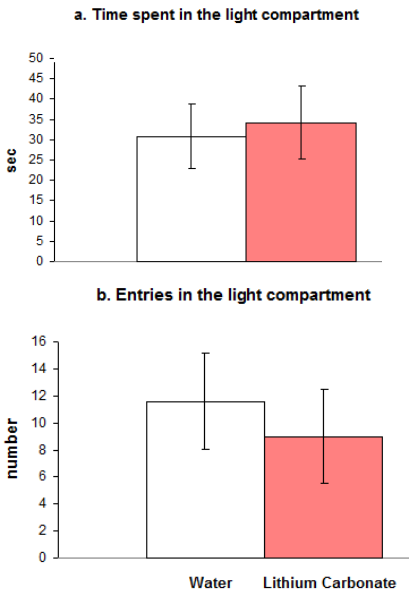

Figure 3: Effects of chronic lithium carbonate on time spent (a) and the number of entries (b) in the lit compartment of the light/dark box on the 16th day.

Figure 4 shows the effects of chronic lithium carbonate on the time spent (a) and the number of entries (b) in the open arm of the elevated plus maze on the 17th day. Data analyzed by t test showed no significant effects of lithium carbonate on the time spent (a) and the number of entries in the open arm (b) of the elevated plus maze.

Figure 5 shows the effects of lithium carbonate in radial arm maze (RAM) test monitored on the 29th day. Data computed using t test revealed a significant decrease in the cut off time (P<0.05).

Figure 6 shows the effects of single restraint stress (2 h/day) on the activity of animals in home cage in water and lithium carbonate treated rats. Data analyzed by 2-Way ANOVA revealed significant effects of stress (F=374, d.f. 1, 44, P<0.01), significant effects of lithium carbonate (F=6.9, d.f. 1, 44, P<0.05) and a significant interaction (F=6.9, d.f. 1, 44, P<0.05) between the two factors. Post hoc analysis showed that acute restraint decreased activity in home cage in both water and lithium treated rats. Home cage activity was greater in lithium treated restrained as compared to water restrained group but were comparable in lithium treated unrestrained and water treated unrestrained group.

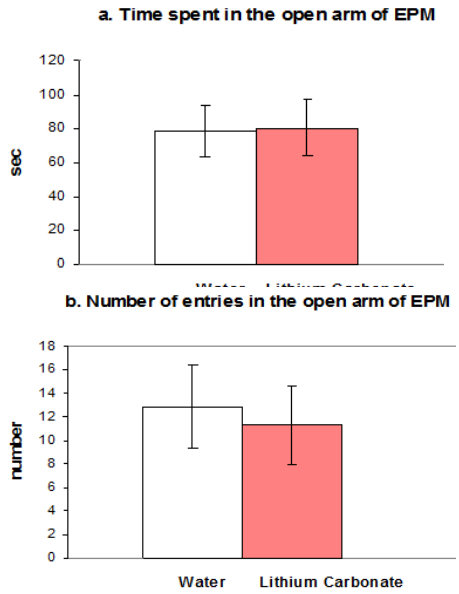

Figure 4: Effects of chronic lithium carbonate on the time spent (a) and the number of entries (b) in the open arm of the elevated plus maze on the 17th day.

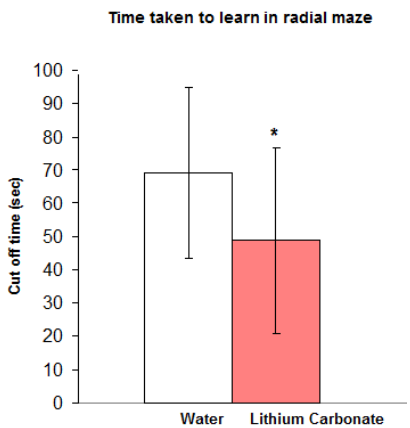

Figure 5: Effects of lithium carbonate in radial arm maze (RAM) test monitored on the 29th day.

Figure 7 shows the effects of restraint stress (2 h/day) on open-field activity in water and lithium treated group. Data analyzed by 2-Way ANOVA revealed significant effects of stress (F=518, d.f. 1, 44, P<0.01). Effects of lithium treatment were not significant (F=2.26, d.f. 1, 44, P>0.05). Interactions between the two factors were significant (F=35, d.f. 1, 44, P<0.01). Post-hoc analysis showed that acute restraint decreased ambulatory activity in both water and lithium treated rats. Number of squares crossed were smaller in lithium unrestrained than water unrestrained group. Number of squares crossed were greater in lithium restrained as compared to water restrained group.

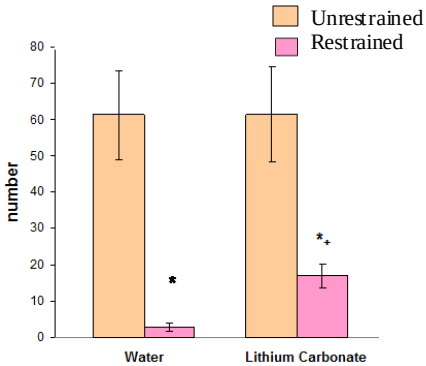

Figure 6: Effects of single restraint stress (2 h/day) on the activity of animals in home cage in water and lithium carbonate treated rats.

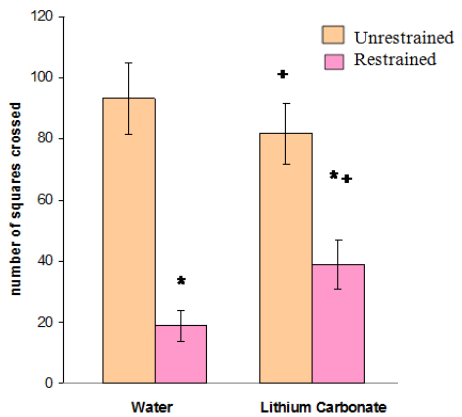

Figure 7: Effects of restraint stress (2 h/day) on open-field activity in water and lithium treated group.

Figure 8(a) shows the effects of restraint stress (2 h/day) on the number of entries in the lit compartment of the light/dark box in water and lithium treated rats. Data analyzed by 2-Way ANOVA revealed significant effects of stress ($F=97$, d.f. 1, 44, $P<0.01$). Effects of Lithium treatment were not significant ($F=2.23$, d.f. 1, 44, $P>0.05$). Interaction between the two factors was significant ($F=9.98$, d.f. 1, 44, $P<0.01$). Post-hoc analysis showed that restraint stress decreased the number of entries in the lit compartment of both water and lithium treated rats. The numbers of entries was greater in lithium restrained rats as compared to water restrained group but were comparable in lithium treated unrestrained and water treated unrestrained group.

Figure 8(b) shows the effects of restraint stress (2 h/day) on the time spent in the lit compartment of the light/dark box of water and lithium treated rats. Data analyzed by 2-Way ANOVA revealed significant effects of stress ($F=355$, d.f., 1, 44 $P<0.01$) and significant effect of lithium treatment ($F=4.42$, d.f., 1, 44, $P<0.05$). The interaction between the two factors was not significant ($F=3.97$, d.f., 1, 44, $P>0.05$). Post hoc analysis showed that restraint stress decreased the time spent in the lit compartment of water and lithium treated group. Time spent was increased in lithium treated restrained group as compared to water treated restrained group but was comparable in lithium treated unrestrained and water treated unrestrained group.

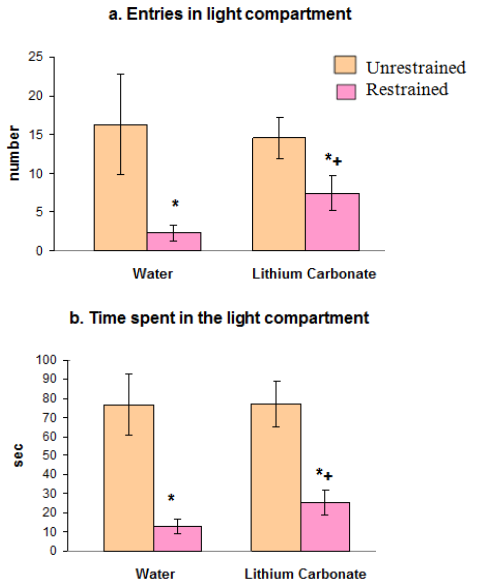

Figure 8: Effects of restraint stress (2 h/day) on the number of (a) entries and (b) time spent in the lit compartment of the light/dark box in water and lithium treated rats.

Figure 9(a) shows the effects of restraint stress (2 h/day) on the number of entries in the open arm of the elevated plus maze of water and lithium treated group. Data analyzed by 2-Way ANOVA revealed significant effects of stress ($F=132$, d.f., 1, 44, $P<0.01$) and significant effects of lithium ($F=11.37$, d.f. 1, 44, $P<0.01$) while the interaction was not significant ($F=0.74$, d.f.,1, 44, $P>0.05$). Post hoc analysis showed that restraint stress decreased the number of entries in open arm of the elevated plus maze of both water and lithium treated group. Number of entries was greater in lithium treated restrained as compared to water treated restrained group but were comparable in lithium treated unrestrained and water treated unrestrained group.

Figure 9 (b) shows the effects of restraint stress (2 h/day) on the time spent in the open arm of the elevated plus maze of water and lithium treated group. Data analyzed by 2-Way ANOVA revealed significant effect of stress ($F=147$, d.f., 1, 44, $P<0.01$). Lithium administration ($F=3.9$, d.f., 1, 44, $P>0.05$) effect was not significant. The interaction between the two factors was also not significant

(F=1.13, d.f., 1, 44, P>0.05). Post hoc analysis showed that restraint stress decreased the time spent in open arm of the elevated plus maze of both water and lithium treated group. Time spent was greater in lithium treated restrained as compared to water treated restrained group but was comparable in lithium treated unrestrained and water treated unrestrained group.

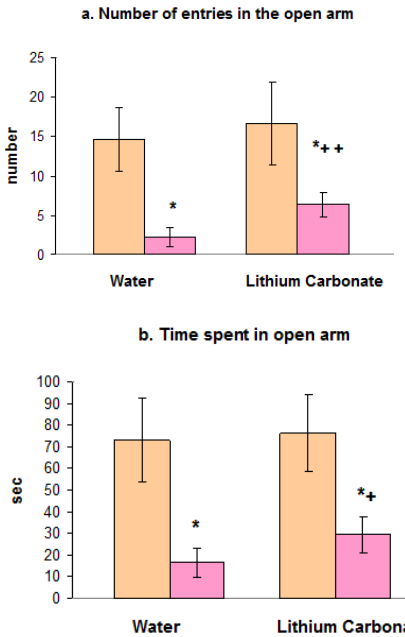

Figure 9: Effects of restraint stress (2 h/day) on (a) the number of entries and (b) time spent in the open arm of the elevated plus maze of water and lithium treated group.

Figure 10 (a, b and c) shows 8-OH-DPAT induced hyperactivity (a) forepaw treading (c) flat body posture in water+unrestrained, water+ restrained, lithium+unrestrained, lithium + restrained. Data on fore paw treading, hyperactivity syndrome and flat body posture were analyzed in 8-OH-DPAT injected rats by 2-Way ANOVA. Behavior of saline injected rats was not monitored. Data on 8-OH-DPAT induced hyperlocomotion (a) revealed significant effects of stress (F=13.4, d.f., 1, 20, P<0.01) and lithium carbonate (F=26.67, d.f., 1, 20, P<0.01). Interaction between the two was not significant (F=0.03, d.f. 1, 20 P>0.05). Post hoc analysis showed that restraint stress decreased cage crossings in both water and lithium treated group. Cage crossings were greater in lithium treated unrestrained than water treated unrestrained group. Lithium carbonate restrained rats exhibited greater cage crossings than water restrained group.

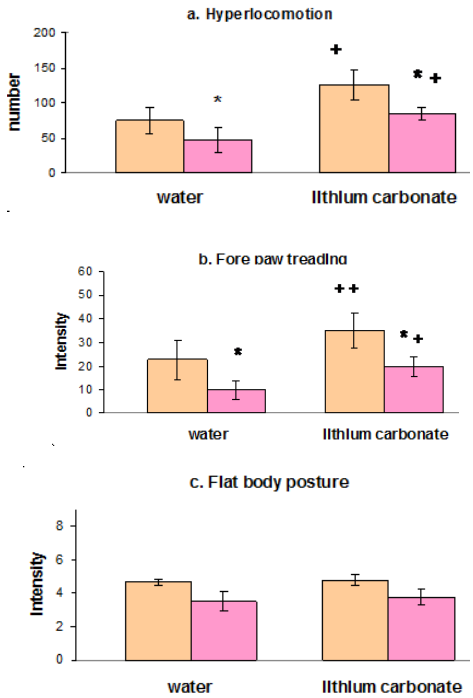

Figure 10: 8-OH-DPAT induced hyperactivity (a) forepaw treading (c) flat body posture in water+unrestrained, water+restrained, lithium+unrestrained, lithium+restrained.

Statistical analysis of 8-OH-DPAT induced fore paw treading (b) revealed significant effects of lithium carbonate (F=16.8, d.f. 1, 20, P<0.01), significant effects of stress (F=25, d.f. 1, 20, P<0.01) while the interaction between the values was not significant (F=0.20 d.f., 1, 20 P>0.01). Post hoc analysis showed that restraint stress decreased fore paw treading in both water and lithium carbonate treated rats. Lithium carbonate unrestrained rats showed greater forepaw treading as compared to unrestrained water treated rats. Restrained lithium carbonate treated rats exhibited greater forepaw treading as compared to water restrained rats.

Statistical analysis of 8-OH-DPAT induced flat body posture (c) revealed that effects of lithium carbonate (F=2.25, d.f. 1, 20 P>0.05), stress (F=1.06, d.f. 1, 20, P>0.05) and the interaction between the two values was not significant (F=2.31, d.f. 1, 20, P>0.05).

Figure 11 shows the effects of 8-OH-DPAT (0.25 mg/ml/kg) on brain TRP in chronic Lithium Carbonate unrestrained and restrained animals. Data analyzed by 3-Way ANOVA revealed that effects of stress (F=105.04, d.f. 1, 40, P<0.01) and Lithium Carbonate (F=333.05, d.f. 1, 40, P<0.01) were significant. Effects of 8-OH-DPAT were not significant (F=0.13, d.f. 1, 40, P>0.05). 8-OH-DPAT x Lithium Carbonate (F=15.811, d.f., 1, 40, P<0.01),

stress x 8-OH-DPAT (F=72.72, d.f., 1,40, P<0.01), Lithium Carbonate x stress (F=223.0, d.f., 1,40, P<0.01), and stress x 8-OH-DPAT x Lithium (F=46.47, d.f., 1,40, P<0.01) were significant. Post-hoc analysis showed that 8-OH-DPAT injection decreased brain TRP concentrations in both unrestrained and restrained water treated rats but not in Lithium Carbonate treated unrestrained and restrained group.

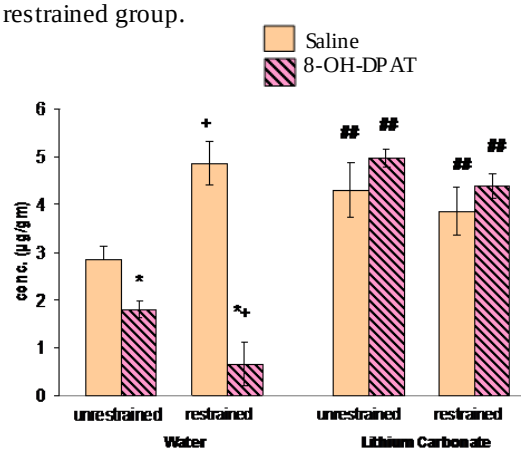

Figure 11: Effects of 8-OH-DPAT (0.25 mg/ml/kg) on brain TRP in chronic Lithium Carbonate unrestrained and restrained animals.

Water treated restrained group injected with saline exhibited higher brain TRP concentration than water treated unrestrained group injected with saline. Water treated restrained group injected with 8-OH-DPAT exhibited lower brain TRP concentrations than water treated unrestrained group injected with 8-OH-DPAT. The values of brain TRP were comparable in Lithium Carbonate treated unrestrained group injected with saline or 8-OH-DPAT. Exposure of animals to 2 h per day restraint increased brain TRP concentrations in water treated but not in Lithium Carbonate treated animals. Lithium Carbonate treated unrestrained and restrained animals exhibited greater TRP concentrations than their water treated counterparts. Values in Lithium Carbonate treated restrained animals and water treated restrained animals were not significantly different.

Figure 12 shows the effects of 8-OH-DPAT (0.25mg/ml/kg) on brain 5-HT in chronic Lithium Carbonate unrestrained and restrained animals. Data analyzed by 3-Way ANOVA revealed that effects of stress (F=263.82, d.f., 1, 40, P<0.01), 8-OH-DPAT (F=12.60, d.f., 1, 40, P<0.01) and Lithium Carbonate (F=160.45, d.f., 1, 40, P<0.01) administration were significant. Interactions between stress x Lithium Carbonate (F=170.36, d.f., 1, 40, P<0.01) and stress x 8-OH-DPAT (F=33.61, d.f., 1, 40 P<0.01) were significant. Interactions between 8-OH-DPAT x Lithium Carbonate (F=2.64, d.f., 1, 40, P>0.05) was not significant. Interactions between stress x 8-OH-DPAT x Lithium Carbonate (F=28.60, d.f., 1, 40, P<0.01) were significant. Post-hoc analysis showed that 8-OH-DPAT injection decreased brain 5-HT concentrations in both unrestrained and restrained water treated rats but not in Lithium Carbonate treated unrestrained and restrained group. Water treated restrained group injected with saline exhibited higher brain 5-HT concentration than water treated unrestrained group injected with saline. Water treated restrained group injected with 8-OH-DPAT exhibited lower brain 5-HT concentrations than water treated unrestrained group injected with 8-OH-DPAT. The values of brain 5-HT were comparable in Lithium Carbonate treated unrestrained group injected with saline or 8-OH-DPAT. Exposure of animals to 2 h per day restraint increased brain 5-HT concentrations in water treated but not in Lithium Carbonate treated animals. Lithium Carbonate treated unrestrained and restrained animals exhibited greater brain 5-HT concentrations than their water treated counterparts. Values in Lithium Carbonate treated restrained animals and water treated restrained animals were not significantly different.

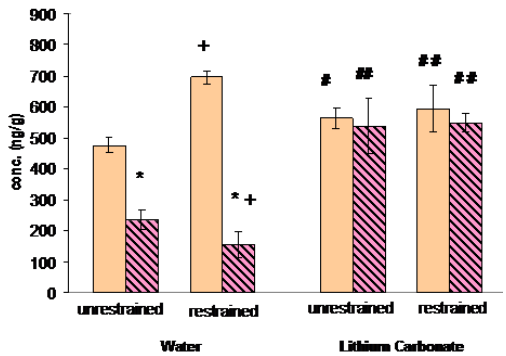

Figure 12: Effects of 8-OH-DPAT (0.25mg/ml/kg) on brain 5-HT in chronic Lithium Carbonate unrestrained and restrained animals.

Figure 13 shows the effects of 8-OH-DPAT (0.25mg/ml/kg) on brain 5-HIAA concentrations in chronic Lithium Carbonate unrestrained and restrained animals. Data analyzed by 3-Way ANOVA revealed that effects of stress (F=75.66, d.f., 1, 40, P<0.01), 8-OH-DPAT (F=7.5, d.f., 1, 40, P<0.01) and Lithium Carbonate (F=39.76, d.f., 1, 40, P<0.01) were significant. Interactions between 8-OH-DPAT x Lithium Carbonate (F=8.72, d.f., 1,40, P<0.01), stress x Lithium Carbonate (F=14.9, d.f., 1,40, P<0.01), stress x 8-OH-DPAT (F=5.97, d.f., 1,40, P<0.05) and stress x 8-OH-DPATxLithium Carbonate (F=24.97, d.f., 1,40, P<0.01) were significant. Post-hoc analysis showed that 8-OH-DPAT injection decreased brain 5-HIAA concentrations in both unrestrained and restrained water treated rats but not

in Lithium Carbonate treated unrestrained and restrained group.

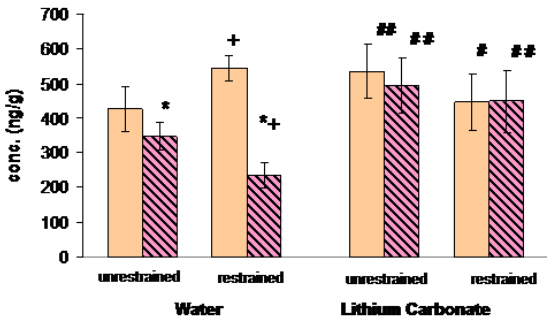

Figure 13: Effects of 8-OH-DPAT (0.25mg/ml/kg) on brain 5-HIAA concentrations in chronic lithium carbonate unrestrained and restrained animals.

Water treated restrained group injected with saline exhibited higher brain 5-HIAA concentration than water treated unrestrained group injected with saline. Water treated restrained group injected with 8-OH-DPAT exhibited lower 5-HIAA TRP concentrations than water treated unrestrained group injected with 8-OH-DPAT. The values of brain 5-HIAA were comparable in Lithium Carbonate treated unrestrained group injected with saline or 8-OH-DPAT. Exposure of animals to 2h per day restraint increased brain 5-HIAA concentrations in water treated but not in Lithium Carbonate treated animals. Lithium Carbonate treated unrestrained and restrained animals exhibited greater 5-HIAA concentrations than their water treated counterparts. Values in Lithium Carbonate treated restrained animals and water treated restrained animals were not significantly different.

DISCUSSION

Behavioral effects of lithium

Much of the work on the behavioral actions of lithium was accomplished in the history of the drug[38]. Certain behaviors are modified or remain unchanged upon lithium administration. In the present study treatment with lithium carbonate did not influence the amount of food consumed (Figure 1a). The body weights were also comparable in control and lithium treated group (Figure 1b); our reports being consistent with the previous reports[39], show that animals were healthy. A current study has shown that lithium treatment to protein deficient cases provided protection against protein deficiency[14]. In the present study fluid intake was increased in lithium treated group, the findings are consistent with the previous studies[39]. Lithium also exhibits antiagressive actions in both animal model of rodents[40,41] and humans[42,43]. A study has shown that[44] administration of lithium chloride had no effect on locomotor activity. The results in the present study demonstrate decreased activity in home cage and ambulatory activity in an open field in lithium treated rats. Our findings do not agree with Gould and his co workers[44], but consistent with Cappeliez[39] could be explained in terms of increased 5-HT. Increasing 5-HT functions decreases ambulatory activity[45]. Thus lithium induced increases in 5-HT (Fig. 5.2) could account for lithium induced decreases in ambulatory activity in open field and home cage.

Lithium's effect on cognitive functioning is ambiguous. Some have reported an improvement in spatial reference memory in a Morris water maze[46]; however other reports have yielded inconclusive result[47]. Lithium also improves performance in passive avoidance test[48], thereby improving short term memory. In the present study memory function of rats as assessed on the radial maze after chronic lithium treatment was increased. Lithium treatment has been shown to reverse helplessness behavior in the learned model of depression [49]. Tests of anxiety evaluated on an elevated plus maze and light/dark box showed no effects of lithium on the number of entries in the light compartment of the light/dark box and open arm of the elevated plus maze (Figures 3 a, b). Similar results were obtained in the time spent in both the tests of anxiety (Figures 4 a, b).

Behavioral and neurochemical effects of single restraint in chronic lithium treated rats

Restraint (2 h/day) induced suppression of activities in home cage (Figure 6) and ambulation in an open field (Figures 5 and 7) largely agree with the previous studies[37,28]. The anxiogenic effects of acute restraint in the two anxiety paradigms exhibiting a fearless response consistent with the previous authors [38,51] can be explained in terms of increased 5-HT metabolism (Figures 8 and 9).

Chronic lithium has been shown to decrease hypoactivity (generally measured in terms of locomotion) induced by immobilization stress[52]. Restraint induced hypokinesia attenuated in chronic lithium treated rats in the present study (Figures 7 and 8) is consistent with previous studies[52,53]. Lithium treatment has been shown to reverse helplessness behavior in the learned helplessness model of depression[49]. Many studies converge to a point that increasing 5-HT functions is antidepressant[54-56]. Attenuation of stress-induced behavioral deficits in rats treated with lithium carbonate may occur because of an increase in 5-HT metabolism in lithium treated rats. However these results are not in agreement with the reports from other authors who have reported that lithium

treatment did not prevent stress deficits in plus maze and open field[9].

The results obtained in the present study coincide with the reports of certain authors; however do not agree with others due to the underlying factors as follows.

Genetic differences between mouse strains that are responsive and unresponsive to lithium induced behavior in different experiments[44]. Dose, route and duration of administration of lithium during the experimental design could also cause variations in results [47,40,46,57]. Effect of lithium administration can also be altered due to species differences[58,59,44,60].

Neurochemical effects of lithium

A number of workers have continuously contributed in understanding the mechanisms of actions of lithium and explore it to treat a multitude of somatopsychic disorders ranging from mania to depression [61,62,63] .Previous studies have shown that sub chronic lithium treatment showed enhanced brain 5-HT levels in the medial prefrontal cortex and hippocampus[20-22]. Short and long term treatment enhanced 5-HT efflux in rat limbic regions[64-66], increased the turnover of 5-HT in frontal cortex or hippocampus[67]. Chronic (21 days) intraperitoneal administration of lithium enhanced 5-HT release in the hippocampus but not in the frontal cortex[68].The present study shows that chronic (32 days) oral lithium carbonate administration increased 5-HT and 5-HIAA in whole brain. It has been reported that chronic lithium resulted in increased TRP uptake into cat brain[69]. The present result shows that lithium administration increased TRP concentrations (Figure 11). TRP being the precursor of 5-HT can modulate the synthesis of 5-HT as enzyme TRP hydoxylase which is confined to the serotonergic neurons and catalyses the rate limiting step of 5-HT synthesis is not saturated at normal brain TRP concentrations. Thus lithium induced increases in uptake of TRP by[69] by serotonergic neurons in the brain[70] could have resulted in increased 5-HT synthesis and 5-HIAA metabolism (Figure 11 and 12 as observed in the present study. Our results do no exhibit compatibility with the results of Bhalla and his coworkers[48] who have observed a significant decrease in serotonin content after Lithium administration. The discrepancies in the result could be due to the duration of experiment as they observed the decreases after 2 and 4 months while we observed these increases after one month of oral administration. Thus we could suggest that oral administration of lithium could have facilitated the release of serotonin from 5-HT nerve terminals and enhanced 5-HT neuronal activity in the whole brain thus producing antidepressant effect, as a hyposerotonergic system is known to exist in depression.

Restraint induced behavioral and neurochemical effects of 8-OH-DPAT

An episode of 2 h restraint stress attenuated the intensity of 8-OH-DPAT induced 5-HT syndrome (Figure 10) consistent with the previous study[37], thus providing evidence that post-synaptic 5-HT-1A receptor dependent functions are attenuated following exposure to an uncontrollable stressor and are involved in stress -induced behavioral deficits[29]. The availability of 5-HT at terminal/post synaptic sites is under the control of an effective feed back mechanism. 5-HT-1A receptors located on the cell body and dendrites of the serotonergic neurons and 5-HT1B receptors located on the nerve terminal end are mediators of the control mechanism. 8-OH-DPAT, a full 5-HT-1A agonist activates somatodendritic as well as post synaptic 5-HT-1A receptors to decrease 5-HT synthesis and release in the nerve terminals [71,72,31]. A decrease in whole brain 5-HT and regional 5-HT metabolism 1 h after the administration of 8-OH-DPAT (0.25-1.0 mg/kg) has been shown in other studies[50,73]. The present study consistent with the study in our laboratory[37] shows that 4 h after the injection of 8-OH-DPAT (0.25 mg/kg) decreased 5-HT metabolism in whole brains of restrained animals. Thus the present study also strengthens the notion that stimulation of somatodendritic 5-HT-1A receptor decreases 5-HT and 5-HIAA concentrations (Figure 12 and 13) in whole brain regions of restrained than unrestrained animals. It is therefore suggested that as in the previous study[37] that an increase in the effectiveness of somatodendritic 5-HT-1A receptors leading to a decrease in the functional activity of 5-HT involved in precipitation of behavioral deficits observed in restrained animals. Conversely a decrease in the effectiveness of somatodendritc 5-HT-1A receptors that occurred following exposure to restraint stress may be involved in adaptation to stress[74].

Behavioral and neurochemical effects of 8-OH-DPAT following exposure to restraint stress in lithium treated rats

Previous studies have shown that 8-OH-DPAT, a selective 5-HT-1A agonist, stimulates the post synaptic receptors to produce hyperactivity syndrome[31,32] and pre synaptic receptors to decrease 5-HT synthesis and release by a feed back mechanism[29,75,32]. Studies with chronic lithium treatment enhances the post synaptic 5-HT-1A receptor mediated behavioral syndrome induced by 8-OH-DPAT in rats[76.77]. The present study consistent with the previous studies that drug (0.25 mg/kg) elicited hyperactivity and fore paw treading enhanced in lithium treated rats (Figures 10 a, b). Thus the

present study suggests that the sensitivity of 5-HT post synaptic receptors is increased in lithium treated rats.

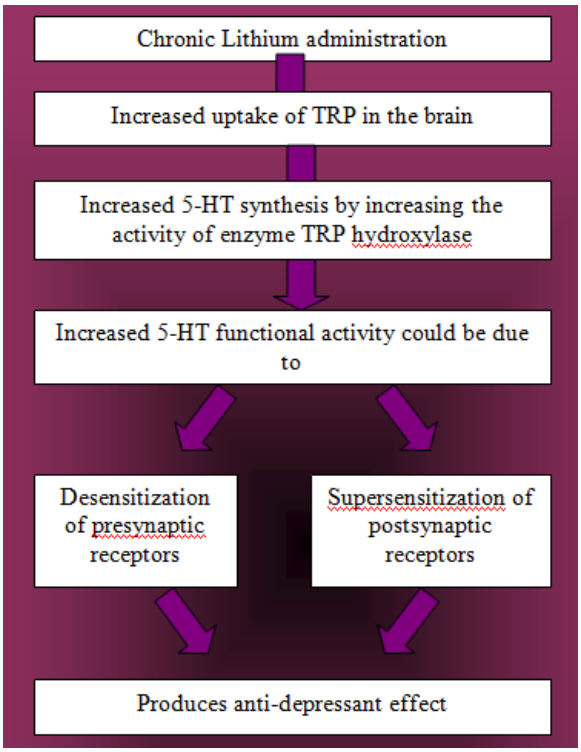

Figure 14: Proposed mechanisms for lithium in producing anti depressant effect.

Studies on the mechanism of the antidepressants have shown that each major class of antidepressants on repeated administration increased 5-HT neurotransmission either by increasing the sensitivity of post synaptic receptor[24] or by decreasing the sensitivity of presynaptic receptors. Lithium which is known to be an effective treatment for the prevention of recurrent episodes in patients with manic depressive illness[78], could have caused increased 5-HT neurotransmission as depicted by increased 5-HT metabolism by inducing desensitization of pre synaptic 5-HT-1A somatodendritic auto receptors in the raphe nuclei and terminal 5-HT1B auto receptors [79,80,55]. Thus the present results could suggest that when the pre synaptic receptors are desensitized, their negative feed back action would become less effective. This would lead to a larger increase in 5-HT concentration at the functional sites. So lithium induced pre synaptic changes together with the super sensitivity of the receptors could be able to produce antidepressant effect (Figures 10 and 11). This could be relevant to the studies from our laboratory showing an increase in the responsiveness of post synaptic 5-HT-1A responsiveness and a decrease in the responsiveness of presynaptic 5-HT-1A receptors in adaptation to stress[81].

The present and the previous studies demonstrate the intensity of 5-HT syndrome was increased in lithium treated rats[76,77]. Radio ligand binding studies have shown the down regulation of 5-HT-1A receptors in the hippocampus but not in the frontal cortex[76,23,68]. It has always been a point of discussion that an increase in 5-HT functions may not necessarily depend on extra synthesis or turnover[81,28]. Greater availability of the neurotransmitter at the functional sites and/ or regulation of pre or post synaptic receptors could produce extra function[28]. Thus it is possible that although the number of receptors are decreased but are super sensitized to produce lithium's antidepressant effect as antidepressants increase 5-HT neurotransmission by increasing the sensitivity of post synaptic receptors[24](Figure 14).

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