Attenuation of stress-induced behavioral deficits by lithium administration via serotonin metabolism

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Abstract:
Background: Although the mood stabilizing role of lithium is well established and the cognitive effects of lithium are also best demonstrated, but its primary effect on neurochemical profile and behaviors under stress remain ambiguous. Earlier studies have suggested that a single exposure to 2 h immobilization stress alters memory in various memory tasks, decreases exploratory activity in open field test and increases serotonin metabolism. This study is designed to investigate the stress relieving effect of lithium in rats.

Methods: Rats were orally administered with lithium carbonate (1 mg/kg/ml) while controls received an equal volume of water for 21 days. After 21 days, each group of rats was sub-divided into stressed and unstressed groups. Animals of stressed group received immobilization stress for 2 h and 24 h following stress behavioral analysis was performed, after which animals were decapitated and their brain samples were collected for neurochemical estimation by HPLC-EC.

Results: Results of the present study show that 2 h immobilization stress decreases locomotor activity while impairs memory performance. Prior administration of lithium attenuates memory impairment and locomotion suppressant effects of stress by reversing the stress induced brain serotonin metabolism in lithium treated rats.

Conclusion: Thus, the results of this study suggest that lithium may recover behavioral and neurochemical impairments induced by stress.

Key words: stress, lithium, 5-HT, behavior

Introduction

Stressful events in the life of humans are associated with many mental disorders such as depression, anxiety and cardiovascular diseases [36, 50]. This could damage certain brain areas which are normally involved in performing the crucial functions such as inhibition of memory formation and consolidation process by damaging the hippocampus [24]. Drugs that are capable of altering stress-induced responses have become useful in the treatment of these disorders [6]. Previous studies on experimental animals showed that an uncontrollable stress situation produced various neurochemical and behavioral deficits such as a decrease in food intake, growth rate and in exploratory
activity [1, 15]. Restraint stress has proven to be a very useful experimental procedure [20]; as immobilization induces stress in the animals, this experimental procedure is useful for the examination of both central and peripheral mechanisms of stress induced deficits, as well as for studying effect of drugs on these deficits [32]. It has been proposed as an animal model for psychological stress such as depression and anorexia nervosa, generally due to its ability to cause behavioral and physiological changes in rats [19, 21].

Lithium salts have been effectively used for the treatment of manic depressive illness and commonly prescribed as mood stabilizing drugs [25]. Lithium has been used for its anti-manic, antidepressant, and anti-suicidal effects [23, 37, 48]. Previous studies have suggested a neuroprotective and neurotrophic role of lithium in animals [4, 5, 48, 49] as well as in human [29, 30]. Moreover, it was also reported to enhance memory in various tasks [18, 46], or to attenuate memory impairments induced by other factors [21]. It has been reported that lithium may exhibit the cognition enhancing effects under stress condition [45] and may have protective role against the deleterious effect of stress on behaviors and cellular function [42]. Serotonin neurotransmission is an important element of stress response [3]. An exposure to single 2 h immobilization stress results in increased serotonin levels in whole brain [30, 38]. The effect of lithium on serotonin metabolism is also well known [40]. Increased brain tryptophan, 5-HT and 5-HIAA levels [41], as well as increased brain serotonin synthesis rate [33] following lithium treatment has been reported previously, therefore, an important goal of our study was to elucidate the behavioral and neurochemical profile of repeated administration of lithium carbonate in immobilized rats.

Materials and Methods

Animals and treatment

The experiments were carried out on locally breed Albino Wistar rats weighing 180–200 g. Animals were caged individually in plastic cages and were exposed to a natural day-night cycle with free access to cubes of standard rodent diet and tap water for 3 days before starting the experiment. For the natural light and dark cycle windows were present in the animal room. Body weight and food intake of all rats were monitored in both pre- and post-experimental period. All experiments were performed according to a protocol approved by local animal care ethical committee.

Drug preparation

Four hundred mg tablet of lithium carbonate was crushed and dissolved in 100 ml of deionized water. The drug was given orally at the dose of 1 mg/kg/ml for 21 days.

Experimental protocol

In the beginning of experiment, animals were divided in two groups: water and drug treated. Rats were given the standard diet and water throughout the experiment. Fresh drug was prepared before starting the experiment. Lithium carbonate (1.0 mg/kg/ml) was orally administered to drug treated animals and equal volume of water was orally administered to water treated rats for 21 days. After 21 days of administration, both groups were divided into unstressed and stressed groups. Animals in the stressed groups were immobilized for 2 h. Behavioral analysis were carried out 24 h following immobilization procedure. Animals were decapitated after behavioral analysis and brain samples were stored at −70°C for neurochemical estimation.

Immobilization stress procedure

At the end of 21st day of the treatment, animals of stress groups (both water and drug treated) were subjected to single exposure of immobilization stress for 2 h. Immobilization was done in separate room to prevent unstressed animals from being under stressful condition due to disturbance. The animals were immobilized by approved procedure as described earlier [10, 14, 18]. Wire grids of 10" × 9" fitted with a Perspex plate of 9" × 6.5" as described earlier [12, 14] were used. Immobilization was affected by pressing the legs of the rats through the gaps in the metal grid and tapping them together with zinc-oxide plaster. Hind limbs were also tapped and the head of animal rested on the Perspex plate. After 2 h immobilization stress, animals were released by applying acetone to the tape and returned to their home cage.
Behavioral analysis

Open field testing

The locomotor activity of control and lithium treated rats were monitored in an open field apparatus. Open field is the square area of 76 × 76 cm with opaque walls of 42 cm height. The floor was divided by lines into 25 equal squares. The test was performed in a quiet room under white light to avoid any noise effect as described earlier [12, 20]. Animals were placed in the center square of the open field (one at a time). Activity in open field was determined by counting number of squares crossed for 5 min as described earlier [13]. Exploratory activity of control rats and drug treated rats were monitored in a balance design to avoid order effect.

Novel object recognition task

The object recognition task is based on the natural tendency of animals to investigate a novel object rather than a familiar object when both are simultaneously present in an area. This test was monitored and described by Ennaceur and Delacour [8]. The test was performed in a box having an area of 45 × 45 cm with 42 cm high walls constructed of wood. The objects to be discriminated were two transparent glasses (used as novel object). Objects were heavy enough so that rats could not move them. It should be made sure that objects have no natural significance of rats. The two objects should always be placed at the same location within the area during the training and test.

Neurochemical analysis

At the end of the experiment, animals were decapitated using guillotine. Brain was removed immediately and stored at −70°C for the determination of 5-HT and 5-HIAA by HPLC-EC as described earlier [11]. A 5-II Shim-Pack Octadecylsilane separation column of 4.0 mm internal diameter and 150 mm length was used. Separation was achieved by a mobile phase containing methanol (14%), octyl sodium sulfate (0.023%) and ethylenediaminetetraacetic acid (0.0035%) in 0.1 M phosphate buffer at pH 2.9 on Shimadzu Electro Chemical 6A Detector at an operating potential of 0.8 volts.

Statistical analysis

Behavioral and neurochemical data were analyzed by two way ANOVA. Post-hoc analysis was done by Newman-Keul’s test and results are presented as the means ± SE; p values > 0.05 were considered non-significant.

Results

Data for square crossed in an open field analyzed by two-way ANOVA (df = 1, 20) showed a significant effect of drug (F-lithium = 12.176, p < 0.01), significant effect of stress (F-stress = 24.349, p < 0.01) and non-significant interaction between two factors (F-interaction = 0.315). However, post-hoc analysis by Newman-Keul’s test showed that 2 h immobilization stress significantly decreased (p < 0.01) locomotor activity in water treated as well as in lithium treated rats. Repeated administration of lithium significantly increases the open field activity in unstressed (p < 0.05) and stressed rats (p < 0.01) (Fig. 1).

Data for recognition memory analyzed by two-way ANOVA (df = 1, 20) showed a significant effect of drug (F-lithium = 35.784, p < 0.01), significant effect of stress (F-stress = 34.5338, p < 0.01) and significant interaction between two factors (F-interaction = 11.491, p < 0.01). However, post-hoc analysis by Newman-Keul’s test showed that 2 h immobilization stress significantly decreased sniffing time of new ob-

![Fig. 1. Effects of 2 h immobilization stress on open field activity in water treated and lithium treated rats. Values are the means ± SE (n = 6) of unstressed and stressed rats. Significant differences by Newman-Keul’s test. ** p < 0.01 from respective unstressed rats, + p < 0.05, + + p < 0.01 from respective water treated rats](image-url)
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Fig. 2. Effects of 2 h immobilization stress on recognition memory in water treated and lithium treated rats. Values are the means ± SE (n = 6) of unstressed and stressed rats. Significant differences by Newman-Keul’s test. ** p < 0.01 from respective unstressed rats, ++ p < 0.01 from respective water treated rats.

Fig. 3. Effects of 2 h immobilization stress on brain 5-HT in water treated and lithium treated rats. Values are the means ± SE (n = 6) of unstressed and stressed rats. Significant differences by Newman-Keul’s test. ** p < 0.01 from respective unstressed rats, ++ p < 0.01 from respective water treated rats.

Fig. 4. Effects of 2 h immobilization restraint stress on brain 5-HIAA in water treated and lithium treated rats. Values are the means ± SE (n = 6) of unstressed and stressed rats. Significant differences by Newman-Keul’s test. * p < 0.05 from respective unstressed rats, + p < 0.05 from respective water treated rats.

Studies on the experimental animals show that an uncontrollable stress situation produced neurochemical changes and behavioral deficits like decrease in locomotor activity, food intake and growth rate [15]. Stress induced behavioral deficits in experimental animals are widely used as animal model of depression [27]. Studies performed on animals showed that prior administration of lithium attenuated the stress-induced depressive behavior [27] and cellular function [42] demonstrating that lithium can exhibit stress...
relieving properties. Uncontrollable stress has been shown to affect recognition memory by influencing hippocampal synaptic plasticity in rats [2]. Our findings indicate that single 2 h immobilization stress impairs non-spatial recognition memory significantly in object-recognition task, which is strongly attenuated by lithium [17, 22, 44]. Attenuation of stress induced alteration in cognition, learning and memory, and emotional responses following lithium treatment has been reported previously [46]. Immobilization-induced deficits in locomotor activity were not observed in lithium treated rats. A decrease in locomotor activity following single episode of 2 h immobilization stress has been reported previously [9]. The results of this study show that pretreatment of lithium attenuate the stress induced decrease in locomotor activity.

Serotonin levels have been reported to increase following immobilization stress in whole brain [39] and various brain regions [14, 28] of rats. An ample evidence indicate that dysfunction of serotonergic neurotransmission in CNS is involved in the development of depression, anxiety and memory disorders [16, 31]. Increased level of brain 5-HT enhances memory [10] whereas decreased level of brain 5-HT impairs cognitive performance [35]. Hence, it can be suggested that administration of lithium increases cognitive performance due to increase in 5-HT levels in unstressed animals. It was observed in this study that administration of lithium increases 5-HT levels in unstressed but not in stressed animals. This enhancing effect of lithium on 5-HT largely support the previous data [7]. Lithium increases the 5-HT turnover rate [41] and the levels of 5-HT, 5-HIAA as well as its precursor tryptophan in the brain [33]. However, under immobilization stress primary actions of repeated treatment with lithium salts on 5-HT may be presynaptical, which stimulates serotonin synthesis [33] and 5-HT release in raphe neurons [43]. This may stimulate the regulation of 5-HT release via presynaptic 5-HT auto receptors in rat hippocampus causing the decreased release of 5-HT. These actions of lithium may serve to correct 5-HT function abnormalities under stress.

Immobilized animals also exhibited an increase in 5-HT metabolism. It may be noted that lithium induced increase of 5-HT did not occur in stressed animals. Exposure to a stress inducing situation increases the effectiveness of negative feedback control over 5-HT metabolism via 5-HT$_{1A}$ receptors [39]. When these receptors are desensitized, their negative feedback action would become less effective. The serotonergic system in CNS is known to inhibit dopamine neurotransmission at the level of the origin of dopamine system in the midbrain as well as in the terminal regions. This action of serotonin is due to the stimulation of 5-HT$_{2C}$ receptors located at the dopaminergic neurons [26, 34]. An increase in 5-HT functions decreases motor activity [47]. On the other hand, stimulation of these receptors could decrease the availability of 5-HT, releasing dopamine neurons from the inhibitory effects of 5-HT and producing hyperactivity. It is therefore suggested that administration of lithium increases brain 5-HT metabolism at the somatodendritic region to decrease the availability of 5-HT at DA neurons and elicits hyperactivity in unstressed rats. However, the brain regional studies are required to further elucidate the role of 5-HT in lithium induced hyperactivity in both the stressed and unstressed animals.

The findings of our study show that lithium administration attenuates the 5-HT metabolism in immobilized rats, it is therefore suggested that stress relieving effects of lithium are expressed under conditions of functional or biological challenge to the nervous system. Hence, it may be effectively used in stress-related neurological disorders. Further studies regarding the effect of lithium on serotonin metabolism in specific brain regions would be helpful in understanding the underlying mechanism following lithium administration.

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