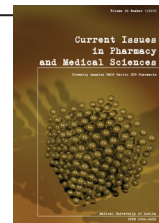


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# The effect of short-term lithium treatment on head twitches induced by 5-hydroxytryptophan in mice

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### ABSTRACT

The effectiveness of lithium in the treatment of affective disorders is well documented. However, the mechanism of this effect is still unknown. The purpose of this study was to investigate the effect of lithium on serotonergic neurons. The evaluation of the serotonergic system activity was performed on the basis of an experimental model of head twitch response triggered by direct or indirect stimulation of serotonin 5-HT<sub>2</sub> receptors in the brain. The obtained results indicated that the lithium chloride co-applied with a direct precursor of serotonin – 5-hydroxytryptophan used in a threshold dose and with carbidopa, generated head twitch response in mice. What is more, an enhancement of head twitch response in mice was observed after repeated 5-hydroxytryptophan application in head twitch-evoking doses. Moreover, inhibition of the serotonin storage in nerve endings in mice was evoked by reserpine administration. Furthermore, lithium increased the effect of 5-hydroxytryptophan given in a threshold dose and a head twitch-evoking dose, respectively. In addition, when P-chlorphenylalanine (pCPA), an inhibitor of the serotonin synthesis within the serotonergic neurons, was given simultaneously with the lithium chloride, carbidopa and 5-hydroxytryptophan in the threshold dose, as well as with the lithium chloride and 5-hydroxytryptophan given at head twitch-evoking dosage, pCPA administration decreased the number of head twitches responses in both experimental models, as well as in the reserpinized mice subjected to the lithium chloride and 5-hydroxytryptophan application. Finally, 5,7-dihydroxytryptamine-evoked serotonergic nerve endings destruction led to absolute inhibition of head-twitch response when observed after the lithium and 5-hydroxytryptophan application. Moreover, the increase by lithium 5-hydroxytryptophan-evoked head twitch response was inhibited by administration of the ritanserine – a 5-HT<sub>2</sub> serotonin receptor blocking agent.

In summary, our data show that lithium induced an enhancement of serotonergic neurotransmission due to its action on presynaptic serotonergic terminals.

### INTRODUCTION

Lithium is a unique drug in its clinical profile usage within the field of psychiatry, as it holds both antidepressant and antimanic properties and also prophylactic actions in bipolar and unipolar affective disorder [12]. The mechanism involved in this clinical profile of lithium have been a subject of much behavioral and biochemical investigation. It has been proposed that the catecholaminergic, serotonergic and cholinergic neuron systems in the brain may be involved [10].

Researchers have demonstrated that lithium potentiated the 5-hydroxytryptophan induction of head twitches in rats that was abolished by pretreatment with the serotonin synthesis inhibitor – p-chlorphenylalanine [15]. This effect is thought to be brought about by lithium bringing about an increase in the tryptophane uptake inside the brain and inducing an increase in serotonin synthesis within the brain's neurons [2,8].

Many authors have noted that the behavioural syndrome in rats and mice deemed 'head twitching' is induced by the administration of serotonin precursors (5-hydroxytryptophan) or serotonin agonists (e.g. DOI), and have utilized this effect as a model for response as mediated by 5-HT<sub>2</sub>

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receptors [6,14]. Head twitching may be also induced by lithium alone in rats or by lithium in combination with reserpine in mice [18]. Both effects are strongly inhibited by the administration of antiserotonergic drugs (e.g. ritaserine) or by administration of a serotonin synthesis inhibitor (p-chlorphenylalanine) [13].

The present study was carried out to obtain further information about the lithium effect on the functional pool of serotonin in the serotonergic nerve terminals.

## MATERIALS AND METHODS

**Animals.** Albino Swiss male mice weighting 20-28g were used in this experiment. The animals were housed under natural light/dark cycles, having free access to food and water, and were randomly assigned to experimental groups consisting of 8 mice. All behavioural observations were performed between 11 a.m. and 5 p.m. The mice were tested only once.

All experiments were carried out according to the National Institute of Health Guidelines for the care and use of laboratory animals, and the European Council Directive on 24 November 1986 for the Care and Use of Laboratory Animals (86/609/EEC), and were approved by the Local Ethics Committee.

**Drugs.** The following drugs were used: lithium chloride (Merc, Germany), 5-hydroxytryptophan (Sigma, USA), p-chlorphenylalanine (Sigma, USA), carbidopa (Sigma, USA), 5,7-dihydroxytryptamine (Sigma, USA), reserpine (Ciba, Switzerland), ritanserin (RBI, USA). In this experiment, for administration, 5-hydroxytryptophan, carbidopa and p-chlorphenylalanine were suspended in Tween 80 (Sigma, USA), while lithium chloride, reserpine and ritanserin were dissolved in distilled water. Moreover, 5,7-dihydroxytryptamine was dissolved in physiological saline containing 0,1% ascorbic acid (Pliva, Poland). The drugs were injected intraperitoneally (i.p.) in a volume of 10 ml/kg, subcutaneously (s.c.) – 5 ml/kg of body weight, and intracerebroventricularly (i.v.c.) – in a volume of 5 µl. The mice assigned to control received an injection of the appropriate vehicle. The drug solutions were prepared just prior to starting the experiments.

**The head-twitch response.** The head twitch response is defined as a spontaneous rotation of the head, neck and shoulders of the mice. Such response was counted for a 60 minute period – 6 times for 1 minute at 10 minute intervals following injection of this drug. The doses of 5-HTP in this study were: at 50 mg/kg with carbidopa (at 10 mg/kg) or 5-HTP alone – 150 mg/kg. Lithium chloride (LiCl) was given in doses of 100 or 200 mg/kg i.p., 30 minutes before administration of the 5-HTP, or 5-HTP and carbidopa.

**Depletion of serotonin in the brain.** For the study of endogenous serotonin depletion, the mice were pretreated with reserpine (blockage of amine storage) or p-chlorphenylalanine (pCPA) once daily for 3 days.

**Lesioning of brain serotonergic neuron.** The selective destruction of serotonin containing nerve terminals in the brain was induced by administration of

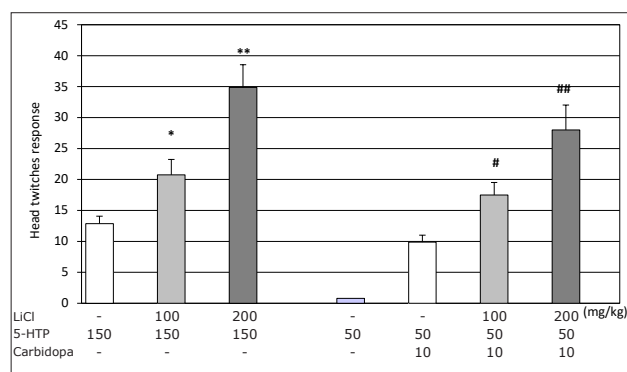
5,7-dihydroxytryptamine (5,7-DHT – 25, 50, 75 µg) injected intraventricularly (i.v.c.) with a Hamilton syringe, at a volume of 5 µl – as described by Herman. For control purposes, control animals were injected with the 5 µl saline ascorbate vehicle (0,1%) alone. The mice used in this study were allowed 8 days to recover from the lesioning procedure. After that time, LiCl was given at doses of 100 and 200 mg/kg, and 30 minutes later, 5-HTP was administered in a dose of 150 mg/kg alone or 50 mg/kg with carbidopa at 10 mg/kg.

**Statistical analysis.** All results were statistically analyzed using Student's t-test. Differences with  $p < 0.05$  were considered statistically significant.

## RESULTS

### The head twitch response induced by lithium in combination with 5-hydroxytryptophan and with 5-hydroxytryptophan and carbidopa

Lithium chloride given at doses of 100 and 200 mg/kg in combination with either 5-HTP (150 mg/kg) alone or with 5-HTP (50 mg/kg) and carbidopa (10 mg/kg), significantly enhanced head-twitch response in mice ( $p < 0.02$ ,  $p < 0.001$  vs 5-HTP, and  $p < 0.01$ ,  $p < 0.001$  vs 5-HTP, carbidopa) (Fig.1).



\* $p < 0.02$ , \*\* $p < 0.001$  vs 5-HTP, # $p < 0.01$ , ### $p < 0.001$  vs 5-HTP+carbidopa

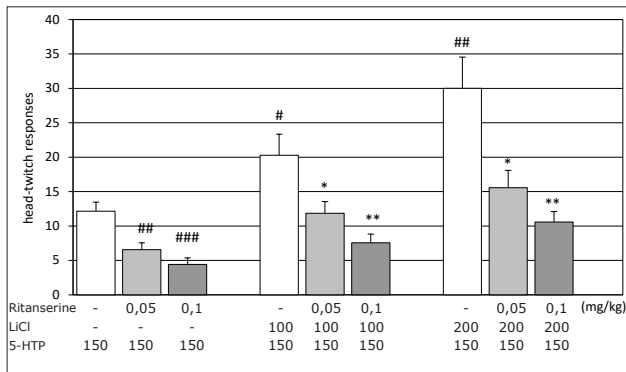
Mice were injected with lithium chloride (LiCl) at doses of 100 and 200 mg/kg, and 30 minutes later 5-hydroxytryptophan (5-HTP) in the dose causing head twitches – 150 mg/kg.

LiCl was administered at doses of 100 and 200 mg/kg, and after 30 minutes, mice were given carbidopa (10 mg/kg), and after another 30 minutes – 5-HTP at the threshold dose of 50 mg/kg. All injections were made i.p.

**Figure 1.** Effects of lithium chloride on the head twitches produced by 5-hydroxytryptophan and carbidopa with 5-hydroxytryptophan in mice

### Effects of serotonin receptor blocking agent

The head twitch response induced by lithium (100 and 200 mg/kg) in combination with 5-HTP (150 mg/kg) was significantly reduced by the administration of the 5-HT<sub>2</sub> selective antagonist – ritanserin – given at both doses of 0.05 and 0.1 mg/kg ( $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$  vs 5-HTP and  $p < 0.05$ ,  $p < 0.01$  vs LiCl+5-HTP) (Fig.2).

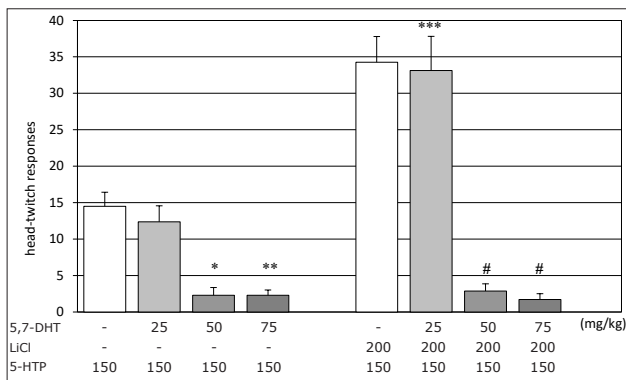


# p < 0.05, ### p < 0.01, #### p < 0.001 vs 5-HTP, \* p < 0.05, \*\* p < 0.01 vs LiCl+5-HTP  
Mice were injected with ritanserine at the doses of 0.05 and 0.1 mg/kg i.p. After 30 minutes LiCl was administered at the doses of 100 and 200 mg/kg, and after another 30 minutes – 5-HTP at the dose causing head twitches (150 mg/kg) i.p.

**Figure 2.** Effects of ritanserine on the enhanced lithium chloride head twitches produced by 5-hydroxytryptophan in mice

### Effects of pretreatment with 5,7-dihydroxytryptamine

The intraventricularly injection of 5,7-DHT (50 and 75 µg/mouse) 8 days before the test, significantly decreased the head-twitch response induced by 5-HTP (150 mg/kg) alone or by the combination of lithium (200 mg/kg) and 5-HTP (150 mg/kg) (p < 0.05, p < 0.01, p < 0.001 vs 5-HTP and p < 0.001 vs LiCl+5-HTP). However, 5,7-DHT given i.v.c. at the dose of 25 µg/mouse 8 days before the test did not alter the 5-HTP and lithium – 5-HTP induced head-twitch response (Fig.3).



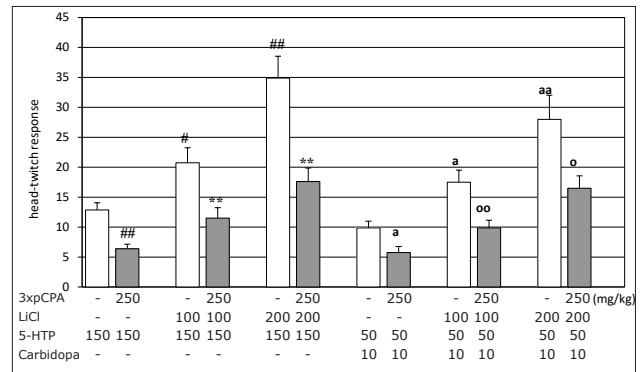
\*p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 vs 5-HTP, #p < 0.001 vs LiCl+5-HTP  
5,7-dihydroxytryptamine (25, 50, 75 µg) was given eight times, every 24 hours, i.v.c. On the eighth day mice were injected with LiCl (100 and 200 mg/kg) and after 30 minutes – 5-HTP (150 mg/kg) i.p.

**Figure 3.** Effects of 5,7-dihydroxytryptamine on head twitches produced by 5-hydroxytryptophan and lithium chloride in mice

### Influences of drug affecting serotonin synthesis and storage of serotonin in nerve terminals

PCPA pretreatment of mice at the dose of 250 mg/kg given 3 times every 24h, significantly inhibited the head-twitch response induced by 5-hydroxytryptophan (150 mg/kg) administration alone or by administration of lithium (100 and 200 mg/kg) with 5-HTP (150 mg/kg) or by administration of the combination of lithium (100 and 200 mg/kg), 5-HTP (50 mg/kg) and carbidopa (10 mg/kg) (p < 0.02,

p < 0.001 vs 5-HTP or 5-HTP+carbidopa and p < 0.05, p < 0.01 vs LiCl+5-HTP or LiCl+5-HTP+carbidopa) (Fig.4).

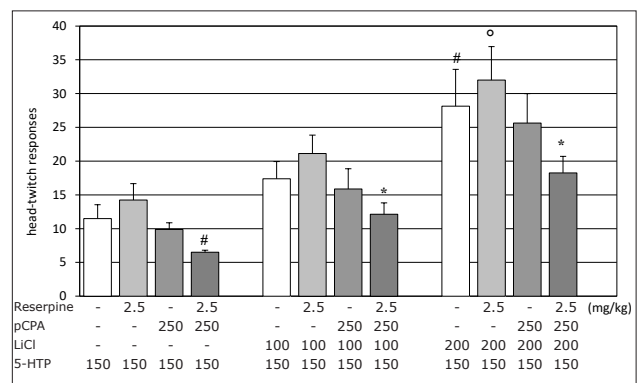


# p < 0.02, ## p < 0.001 vs 5-HTP, \* p < 0.05, \*\* p < 0.01 vs LiCl+5-HTP, # p < 0.02, ## p < 0.001 vs 5-HTP+carbidopa, # p < 0.05, ## p < 0.01 vs LiCl+5-HTP+carbidopa

Mice were injected with p-chlorophenylalanine (pCPA) at the dose of 250 mg/kg, three times every 24 hours i.p. 72 hours after the last dose of p-CPA, LiCl was given at doses of 100 and 200 mg/kg. 30 minutes after the administration of LiCl were injected: 5-HTP (150 mg/kg) or carbidopa (10 mg/kg) and after the next 30 minutes – 5-HTP (50 mg/kg), causing head twitches.

**Figure 4.** Effects of p-chlorophenylalanine on the enhanced by lithium chloride head twitches produced by 5-hydroxytryptophan or 5-hydroxytryptophan and carbidopa in mice

Reserpine (2.5 mg/kg 20h prior to the test) and pCPA (250 mg/kg 2h prior to the test) given alone did not change the head-twitch response induced by 5-HTP (150 mg/kg) or by the combination of lithium (100 mg/kg) and 5-HTP (150 mg/kg). However, lithium given in a dose of 200 mg/kg with 5-HTP (150 mg/kg) significantly enhanced head-twitch response in reserpinised mice. When the mice were pretreated with reserpine (2.5 mg/kg) and pCPA (250 mg/kg), the head-twitch response produced by 5-HTP (150 mg/kg) or by a combination of lithium (100 and 200 mg/kg) with 5-HTP (150 mg/kg) was significantly inhibited (p < 0.01 vs 5-HTP and p < 0.05 vs LiCl+5-HTP and p < 0.01 vs reserpine+5-HTP) (Fig.5).



# p < 0.01 vs 5-HTP, \* p < 0.05 vs LiCl+5-HTP, # p < 0.01 vs reserpine+5-HTP  
Mice were injected with reserpine at the dose of 2.5 mg/kg, only once, subcutaneously (s.c.).

After 20 hours pCPA was administered at the dose of 250 mg/kg i.p. Two hours later LiCl was administered at the doses of 100 and 200 mg/kg, and after 30 minutes – 5-HTP at the dose of 150 mg/kg i.p.

**Figure 5.** Effects of p-chlorophenylalanine in combination with reserpine on the enhanced lithium chloride head twitches produced by 5-hydroxytryptophan in mice

## DISCUSSION

Numerous reports in the literature indicate that lithium has an affect upon the neurons of the cholinergic, adrenergic and serotonin systems in the CNS. Indeed, biochemical studies have shown that lithium enhances the release of serotonin, norepinephrine and acetylcholine from nerve endings. This is confirmed by the researches of many authors, who showed the stimulating effect of lithium on serotonergic transduction [1,3]. However, studies carried out so far do not provide a single image of the changes induced by lithium administration in these neurotransmitter systems.

In behavioral studies, in order to assess the impact of drugs on serotonergic transduction, the experimental model of head twitch response is employed. Head twitching is provoked by the direct precursor of serotonin (5-hydroxytryptophan, 5-HTP), and by selective ( $[(+/-)-1-(2.5\text{-dimethoxy-4-iodophenyl})-2\text{-aminopropane}]$ , DOI) and non-selective ( $\alpha$ -methyl-5-hydroxytryptamine) agonists of the serotonin receptors 5-HT<sub>2A</sub> and the receptor agonists 5-HT<sub>1A</sub> (8-OH-DPAT, buspirone). It is also thought to be brought about by lithium chloride administration in rats and mice [4,9,18]. The results of these studies suggest that lithium may increase the transduction in neurons of the serotonergic system by increasing the release of serotonin from the nerve endings.

Our study showed that the lithium chloride, when administered alone at different doses, does not cause head twitching in these animals. However, lithium, depending on the dose, increased the number of head twitches brought about by 5-HTP (150 mg/kg) administered at a dose causing head twitching, and did not cause head twitching in mice given 5-HTP (50 mg/kg) in a dose not provoking head twitching in these animals. These results indicate that the effect of lithium may depend on the concentration of serotonin synthesized in the nerve endings from 5-HTP and released into the synaptic cleft.

Swann *et al.* [16], Berggren [2] and Herrero *et al.* [8] have shown that lithium increases the transport of tryptophan into the brain, thereby increasing its concentration in the serotonergic endings and inducing the synthesis of serotonin released into the synaptic cleft.

In order to increase the concentration of serotonin in the brain and in the serotonergic endings, experiments were performed in which animals were treated with 5-HTP with the peripheral decarboxylase inhibitor – carbidopa, which increases the concentration of 5-HTP in the brain and the synthesis of serotonin in the serotonergic endings [7]. The results of research conducted in this study showed that lithium induced head twitching in mice which were given 5-HTP at a dose not causing head twitching when administered along with carbidopa. These results indicate that the head twitches induced by lithium and 5-HTP administered at a dose not causing head twitching, but with co-administration of carbidopa, may be provoked by the intensity induced by the lithium of 5-HTP transport into the brain, the increase of the level of serotonin in the nerve endings and the increase of serotonin release into the synaptic cleft. This has been confirmed by the results of Treiser *et al.* [17] and Blier and Montigny [3], who showed that lithium increases

the transport of 5-HTP in the brain and increases the release of serotonin from serotonergic terminals.

In order to assess the effect of lithium on serotonergic nerve endings, serotonergic compounds reducing serotonergic transduction (reserpine, *p-chlorophenylalanine* 5.7-dihydroxytryptamine and ritanserin) were administered. Reserpine reduces serotonergic transduction by bringing about a significant reduction in the level of serotonin in the brain [11]. It also inhibits the storage of serotonin in the deep synaptic vesicles without affecting serotonin levels in the shallow synaptic vesicles from which newly synthesized serotonin is released. In order to increase the level of newly synthesized serotonin in the nerve endings, in our work, mice were treated with a reserpine precursor of serotonin – 5-HTP, applied in two doses. The result of this work demonstrated that lithium given at different doses, with 5-HTP applied in doses not causing head twitching, did not induce head twitching in non-*reserpinised* mice, whereas it did in mice which were *reserpinised*. These results are consistent with that obtained by Yamada and Furukawa [18], who revealed that lithium increases the number of episodes of head twitching provoked by 5-HTP in *reserpinised* mice.

In attempting to answer the question as to whether lithium increases the release of serotonin from the nerve endings, we conducted experiments with compounds that inhibit the synthesis of serotonin in the nerve endings (- *p-chlorophenylalanine*) or induced neurodegeneration of the serotonergic terminals (- 5.7 - dihydroxytryptamine). In this work, we found that *P-chlorophenylalanine* administration significantly reduced the number of head twitches induced by the administration of lithium with 5-HTP. This effect indicates that lithium and 5-HTP provokes head twitching in mice, if the synthesis of serotonin in nerve terminals is maintained, however, 5.7-dihydroxytryptamine (5.7-DHT) administered intracerebrally selectively destroys 70-80% of the nerve endings of the serotonergic neurons. The results of the studies in mice have demonstrated that the destruction of the 5.7-DHT of serotonergic nerve terminals completely inhibits head twitching provoked by the combination of lithium administration with 5-HTP as used in the doses bringing about head twitches. These results suggest that lithium enhances serotonin release from nerve endings [5].

It was also shown that increased head twitch response, enhanced by lithium and induced by 5-HTP, was blocked by the selective antagonist of the serotonin receptors 5-HT<sub>2</sub>-ritanserin. Similar results were obtained by Matsumoto *et al.* [13] in the model of head twitching in mice.

In conclusion, the results obtained in this study indicate that lithium enhances serotonin transduction in the central nervous system, by increasing serotonin release from the nerve terminals into the synaptic cleft, where the serotonin synthesis is maintained.

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