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Modifications by Lithium of Behavioral Responses to Methamphetamine and Tetrabenazine

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Abstract. Different groups of mice were injected s.c. daily with lithium chloride in three doses (0.52, 1.58 and 4.72 meq/kg) or with saline for a period of 3 weeks. Lithium administered acutely or chronically did not affect spontaneous locomotor activities. However, methamphetamine-induced hyper-locomotor activities were inhibited in the lithium groups as compared with those in the saline group, while the hyper-locomotor activities induced by tetrabenazine in the nialamide-pretreated animals were reduced to some extent but not significantly by lithium. Tetrabenazine brought about an initial transient increase followed by a decrease of spontaneous locomotor activities in the lithium groups, whereas it induced only a decrease of the activities in the saline group. In addition, jumping and vertical jumping behaviors, which were not observed in the saline group, occurred 30–60 min after tetrabenazine in the lithium groups. These effects of lithium tended to increase with an increase of the doses administered and with a prolongation of its daily administration. The results demonstrate that lithium modifies behavioral responses to methamphetamine and tetrabenazine.

Key words: Lithium – Methamphetamine – Tetrabenazine – Behavior – Interaction.

Although it has been proposed that lithium salts have valuable therapeutic and prophylactic effects in manic-depressive psychosis, their mode of action in affective illness remains obscure and knowledge about their pharmacological action is far from complete. The need to clarify behavioral effects of lithium has, during the past few years, stimulated considerable research using infrahuman animal subjects, which has produced certain consistent results. It has been reported that after repeated administrations of lithium no sedation was observed in mice (Carroll and Sharp, 1971), nor reduction of spontaneous motor activity in rats (Perez-Cruet *et al.*, 1971). Lithium reduced rearing frequency in rats, while there was no discernible effect upon horizontal locomotor activity (Johnson, 1972). Rats administered lithium were less active than those treated with saline (Syme and Syme, 1973), and lithium decreased the voluntary activity of rats without affecting their reactivity or muscle strength (Smith and Smith, 1973). No alteration in the rate of self-stimulatory behavior was found (Ramsey *et al.*, 1972). Lithium had an antagonistic effect on motor hyperactivity induced by the combined use of desmethylimipramine and benzoquinolizine (Ro. 4-1284) (Matussek and Linsmayer, 1968) and that elicited with combined use of dexamethasone and chlordiazepoxide (Cox *et al.*, 1971) in rats. In mice, the behavioral activation caused by morphine was antagonized by lithium (Carroll and Sharp, 1971).

Paralleling such studies have been suggestions about neurochemical mechanisms underlying the affective disorders. The catecholamine hypothesis proposes that some, if not all, depressions are associated with an absolute or relative decrease in catecholamines available at central adrenergic receptors sites, while elation, conversely, may be associated with an excess of such amines (Shildkaut, 1965). Associated with this hypothesis has been the proposal that actions of lithium on brain monoamine metabolism may be the mechanism by which it produces its effects (Kopin, 1969).

The aim of the present investigation was to study effects of lithium on behavioral responses induced by methamphetamine and tetrabenazine, which are alleged to act on metabolism of endogenous brain catecholamines.

Materials and Methods

The 240 animals serving as subjects in the experiment were healthy ddY male albino mice obtained from Kuroda Animal

Laboratory (Kumamoto, Japan). They were caged in groups of 10 for a week before as well as throughout the experiment, and were always placed with their cagemates after injection or between behavioral test trials. The body weights of mice were 15–17 g at arrival and 18–21 g at the beginning of lithium injection. The food consisted of MF, Oriental Yeast Ltd. The animals were permitted food and water *ad libitum* except during drug trials. All trials and breedings were carried out at an environmental temperature 24 ± 1 °C and moisture 50 ± 10%.

The mice were divided randomly into 4 groups of 10 animals. Each received a daily injection of saline as control, or of lithium chloride in doses of 22 mg/kg (0.52 meq/kg), 67 mg/kg (1.58 meq/kg), or 200 mg/kg) (4.72 meq/kg). They were given one subcutaneous injection each day for 21 days, administrations being between 9:40 AM and 10:20 AM. Different groups were used for each item of the experiment. Number of mice used for each experiment are shown in the explanatory description for each Table and Figure.

Three measures of behavior were recorded. The first used the test situation in which to observe spontaneous motor activity. The open-field chamber was 60 cm in diameter and 50 cm in height; the floor was divided into 19 blocks. A mouse was placed on the center of the floor and observed for 1 min. Two preliminary training trials were given at 30 min intervals in order to establish a relatively stable baseline prior to the test trials, which began 30 min later. The short period for each test trial was chosen so as to measure drug effects at short time intervals after administration. Locomotor activity, ambulation, was expressed in terms of the number of blocks traversed during 1 min.

The observation of the second measure of behavior employed what has been referred to as a "passive avoidance" situation. Each mouse was placed at the center of an octagonal platform made with non-transparent plastic, 35 cm high and 32 cm in diameter. Whether or not it jumped off the platform during 1-min period was recorded. This observation was repeated three times at an interval of 10 min. The jumping response was then calculated in terms of the percentage of animals in each group which jumped during the 3 trials.

Preliminary observations had suggested a third behavioral measure, vertical jumping. Each mouse was placed at the center of the floor of a cylinder-shaped open chamber constructed of transparent plastic, 7 cm in height and 20 cm in diameter. The behavior observed during each 1 min trial was whether or not a subject jumped vertically in such a way as to reach 7 cm or more above the floor of the chamber. This observation was repeated three times at the intervals of 10 min. The vertical jumping response was then expressed in terms of the percentage of animals in each group which jumped vertically during the 3 trials.

The drugs used were lithium chloride (Kishida Chemical), methamphetamine hydrochloride (Dainippon Pharmaceutical) tetrabenazine hydrochloride (F. Hoffman-La Roche) and nialamide hydrochloride (Pfizer-Taito). Lithium chloride was dissolved as 1.12, 3.35 and 10% solution with distilled water, sterilized, and administered subcutaneously within a volume of 0.1 ml/animal, using 0.25 ml syringe. The other drugs were also dissolved with distilled water and injected subcutaneously within the volume of 0.1 ml/10 g. Methamphetamine, 5 mg/kg, was administered 1 hr and tetrabenazine, 5 mg/kg, 2 hrs after lithium. In the nialamide pretreated animals, nialamide was injected, in a dose of 10 mg/kg, 24 hrs before tetrabenazine.

The various behaviors were measured at intervals after injections which are indicated in the figures and tables which follow. All statistical tests of differences between experimental treatments were carried out using Student's t test ($P < 0.05$).

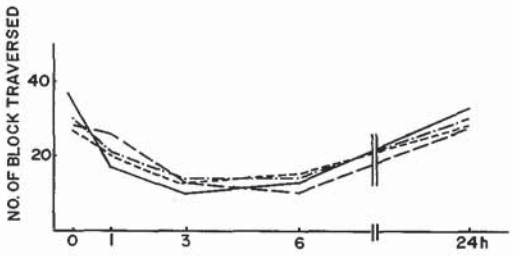

Fig. 1. Acute effects of lithium on spontaneous locomotor activity. —— saline ($N = 10$); ------- lithium chloride 22 mg/kg (0.52 meq/kg) ($N = 10$); – – – – lithium chloride 67 mg/kg (1.58 meq/kg) ($N = 10$); -·-·-·- lithium chloride 200 mg/kg (4.72 meq/kg) ($N = 10$)

Results

1. Effects of Lithium on Spontaneous Locomotor Activity

Spontaneous locomotor activity, ambulation, was measured at several time intervals after a single administration of lithium or saline. As Fig. 1 shows, all treatments were followed by decreases in ambulation which were maximal at 3 hrs and lasted for more than 6 hrs. Decreases following administration of the various doses of lithium were not significantly different from those of the saline group.

During chronic treatment, *i.e.*, daily administrations of lithium or saline for 3 weeks, body weight increased similarly in the lithium-treated animals and saline-treated animals. Ambulation was measured at 23.5 hrs after the 1st, 7th, 14th and 21st administrations of lithium or saline. Results of these tests are reported in Table 1. Again, no significant differences in ambulation were observed when the measures for the various treatments were compared.

2. Effects of Lithium on Methamphetamine-Induced Hyper-Locomotor Activity

As shown in Fig. 2, methamphetamine, 5 mg/kg, injected 1 hr after the 1st, 7th, 14th and 21st daily administrations of lithium or saline induced increases in ambulation which were maximal 30 min later and lasted over 3 hrs. The methamphetamine-induced increases were significantly less in the lithium than in the saline groups. This effect tended to be more pronounced as the dose of lithium and the period of its daily administration increased.

When methamphetamine was used at dose levels of 2 mg/kg similar results were obtained.

Table 1. Effects of lithium administered daily on spontaneous locomotor activity. All values show the mean number of blocks traversed ± standard errors in 10 mice. The activity was measured 23.5 hrs after 1st, 7th, 14th and 21st injections of lithium or saline

	Control	1st	7th	14th	21st
Saline	28.3 ± 3.4	28.5 ± 4.9	29.2 ± 4.2	29.0 ± 4.3	34.0 ± 5.2
LiCl 22 mg/kg	29.5 ± 3.5	23.4 ± 3.3	35.1 ± 3.2	32.6 ± 4.0	33.1 ± 3.5
67 mg/kg	23.3 ± 3.2	25.0 ± 3.7	27.1 ± 4.7	31.8 ± 5.9	24.5 ± 4.3
200 mg/kg	24.5 ± 2.5	24.5 ± 1.9	26.2 ± 5.3	23.4 ± 4.2	30.0 ± 5.7

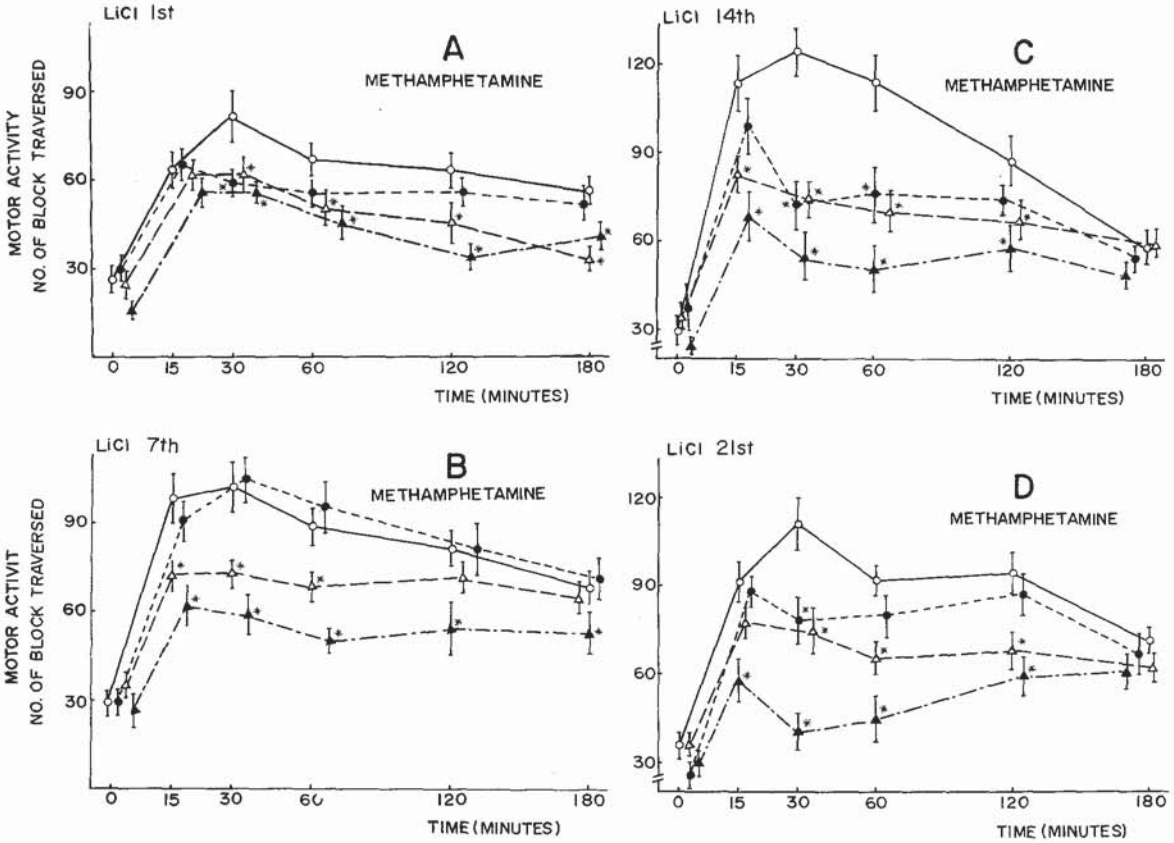

Fig. 2A–D. Effects of lithium administered daily on the methamphetamine-induced hyper-locomotor activity. A pannel shows 1st administration of lithium, B pannel 7th, C pannel 14th and D pannel 21st. Methamphetamine, 5 mg/kg, was administered subcutaneously 1 hr after lithium. Each point shows mean value ± S.E.M. * Mark denotes a significant change from saline control according to t test ($P < 0.05$). ○——○ saline ($N = 10$); ●-----● lithium chloride 22 mg/kg (0.52 meq/kg) ($N = 10$); △–––△ lithium chloride 67 mg/kg (1.58 meq/kg) $N = 10$); ▲-·-·-▲ lithium chloride 200 mg/kg (4.72 meq/kg) ($N = 10$)

3. Effects of Lithium on the Hyper-Locomotor Response to Tetrabenazine Administered after Nialamide

Tetrabenazine, 5 mg/kg, administered 24 hrs after pretreatment with nialamide and 1 hr after the 1st, 7th, 14th and 21st daily administrations of lithium or saline brought about similar hyper-locomotor activities which were maximal 30 min later and lasted for 2 hrs. Fig. 3 summarizes the results. The maximal response to tetrabenazine was reduced to some extent in the lithium group, but differences between the lithium and saline groups were not statistically significant.

4. Effects of Lithium on Behavioral Responses to Tetrabenazine

In the saline groups, tetrabenazine injected 1 hr after the 1st, 7th, 14th and 21st administrations of lithium or saline induced monotonic decreases in ambulation lasting for more than 3 hrs. It also elicited decreases

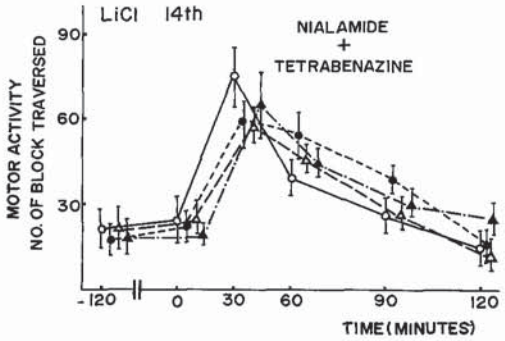

Fig. 3. Effects of lithium administered daily on the hyper-locomotor response to tetrabenazine administered after nialamide. Tetrabenazine, 5 mg/kg, was administered subcutaneously 2 hrs after 14th injections of lithium, and nialamide (10 mg/kg) 24 hrs before tetrabenazine. Further explanations and number of mice used as Fig. 2

in ambulation in the lithium groups, but this was preceded by temporary increases which were maximal at about 5 min and disappeared within 10 min after the injection. Recovery from the tetrabenazine-induced decrement in ambulation tended to be more rapid with higher doses and with prolonged administration of lithium. Fig. 4 summarizes the results.

The jumping (passive avoidance) and vertical jumping behaviors occurred in the lithium groups during the 30–60 min after injection of tetrabenazine despite their depressed locomotor activity. These behaviors were not observed in the saline group. Jumping behavior was observed even at the smaller doses of lithium, but vertical jumping behavior was seen only at the larger dose levels. These results are summarized in Table 2.

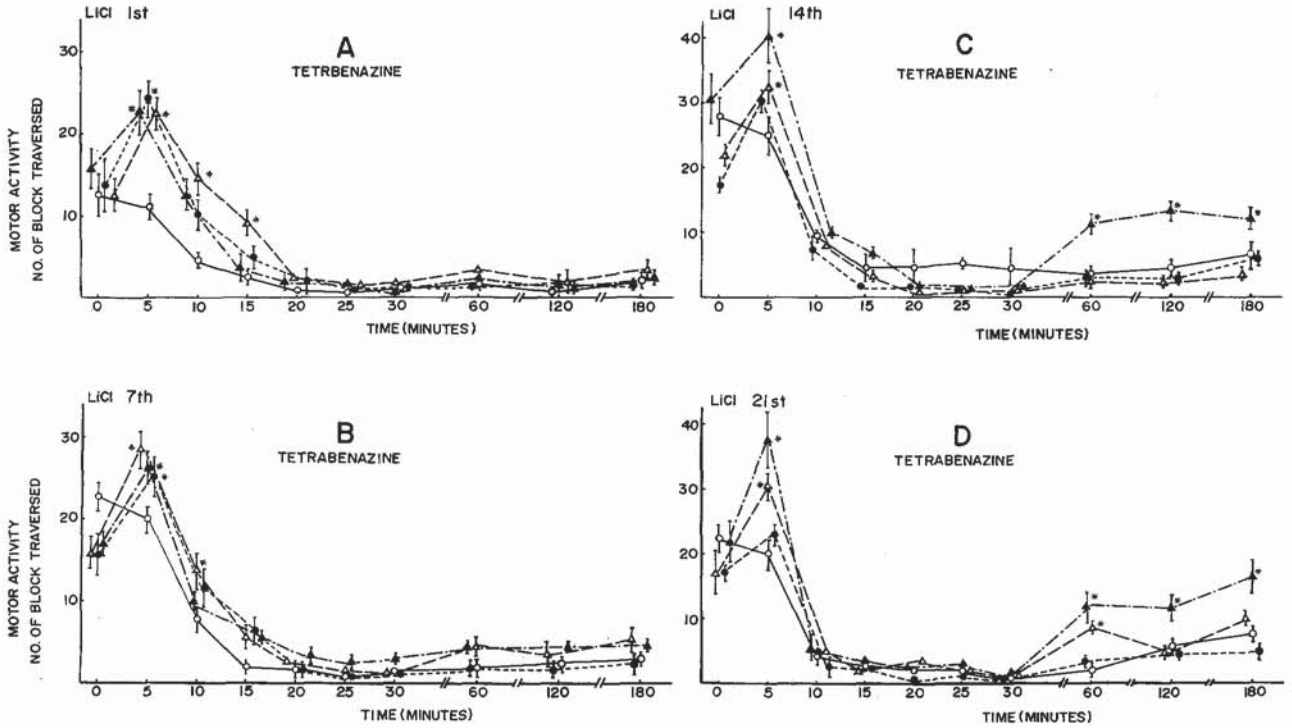

Fig. 4A–D. Effects of lithium administered daily on the tetrabenazine-induced hyper-locomotor activity. Tetrabenazine, 5 mg/kg, was administered subcutaneously 2 hrs after lithium. Further explanations and number of mice used as Fig. 2

Table 2. Jumping and vertical jumping behaviors observed after tetrabenazine. Each value show the number of mouse in which the behavior can be positively observed among 10 mice. Tetrabenazine, 5 mg/kg, was administered subcutaneously 2 hrs after 1st, 7th, 14th and 21st administrations of lithium or saline, and the behaviors were observed 1 hr after tetrabenazine

Behavior	1st		7th		14th		21st	
	Jump.	v. Jump.	Jump.	v. Jump.	Jump.	v. Jump.	Jump.	v. Jump.
Saline	0	0	0	0	0	0	0	0
LiCl 22 mg/kg	5	1	4	0	3	0	3	0
67 mg/kg	2	0	2	0	2	1	2	1
200 mg/kg	7	0	5	4	8	2	5	4

Discussion

As the safety margin in dosage of lithium has been thought to be narrow, if administration of lithium becomes toxic, its observed effects on behavior may be due to its non-specific rather than specific action. Two observations suggest that such toxicity was not involved in the present experiment. Firstly, body weight, determined throughout the experiment was not significantly different for saline and lithium groups. Secondly, lithium administered acutely or chronically in the dosages given, did not, in fact, affect spontaneous locomotor activity. It appears reasonable, therefore, to interpret behavioral effects observed in the present investigation in terms of specific actions and interactions of lithium.

As regards the interaction of lithium with amphetamine, related closely to methamphetamine in chemical structure and pharmacological actions, Lal and Sourkes (1972) reported that lithium did not affect amphetamine-induced stereotyped behavior, and Matussed and Linsmayer (1968) that lithium treatment of the rats for several days could not prevent an amphetamine excitation but prolonged the amphetamine-induced stereotyped behavior. In the present study, administration of lithium inhibited the methamphetamine-induced hyper-locomotor activity in mice in a relatively dose-dependent manner. It has been reported that lithium and amphetamine have opposite effects on the threshold of intracranial reinforcement, lithium raising and amphetamine lowering the threshold (Cassens and Mills, 1973). Opposing actions may contribute to the counteractive effect of lithium on methamphetamine.

From the viewpoint of brain amine metabolism, amphetamine releases amines, reduces amine uptake, and inhibits monoamine oxidase activity in large doses (Farnebo, 1971; Glowinski and Axelrod, 1965; Rutledge, 1970; Svensson, 1971) thereby increasing the availability of amines to receptor sites and inducing central excitation. On the other hand, lithium: induces a depletion of brain amines when given together with a tyrosine or a tryptophan hydroxylase inhibitor (Corrodi *et al.*, 1967, 1969); inhibits monoamines release (Katz *et al.*, 1968; Bindler *et al.*, 1971); acts to increase amines uptake (Colburn *et al.*, 1967; Komiskey and Buckner, 1974); activates brain monoamine oxidase activity (Kiseleva, 1972); and, alters amines catabolism toward deamination (Schildkraut *et al.*, 1969; Schanberg *et al.*, 1967), thereby decreasing availability of amines to receptor sites. In addition, lithium probably blocks brain receptors for amines since lithium inhibits brain adenyl cyclase activation (Forn and Valdecasas, 1971) and cyclic-AMP content increase (Berndt, 1973) stimulated by amines. Therefore, lithium and methamphetamine are thought to cause opposite changes in brain amines metabolism. In fact, it was proposed that lithium inhibited the methamphetamine-induced changes in brain norepinephrine metabolism (Nozu *et al.*, 1973). Thus, the biochemical effects on amines metabolism offer a plausible explanation for the antagonistic effect of lithium on methamphetamine. However, although reserpine or tetrabenazine administered after monoamine oxidase inhibitor was proposed to elicite hyperactivity because more endogenous amines were released as active form by inhibition of the enzyme activity, lithium reduced but did not affect significantly this hyperactivity.

As concerns the interaction between lithium and tetrabenazine, it was reported that the tetrabenazine-induced behavioral inhibition tended to be diminished in smaller doses of lithium whereas the inhibition tended to be potentiated in larger doses of lithium (Perkinson *et al.*, 1969). In the present study, lithium affected behavioral responses to tetrabenazine in a complicated manner: after tetrabenazine in the lithium-treated mice, an initial transient hyperactivity occured, duration of inhibitory effect was shortened in some degree, and jumping and vertical jumping behavior appeared.

From the viewpoints of brain amine metabolism, reserpine, related closely to tetrabenazine, depletes amines, activates monoamine oxidase activity, releases amines as inactivated form, and inhibits amines uptake (Izumi *et al.*, 1969; Iversen, 1967; Glowinski and Axelrod, 1965), thereby causing less active amines available to the receptor sites and central sedation. Segawa and Nakano (1974) have reported that lithium lessens the depletion rate of brain 5-HT by reserpine, and have proposed that lithium decreases the release of 5-HT by changing the properties of synaptic vesicular membrane and interfering with the releasing mechanism of reserpine at synaptic vesicles. Although it can be presumed that this counteractive effect of lithium on the rauwolfia alkaloid-induced depletion rate of brain amines may be involved in the behavioral interaction of lithium with tetrabenazine, there are still difficulties in elucidating the complicated behavioral interaction of both drugs.

As the removal of morphine or administration of morphine antagonist in rats or mice physically dependent upon morphine cause a withdrawal syndrome characterized by an uncontrollable urge to jump (Way *et al.*, 1969; Francis and Schneider, 1971; Saelens *et al.*, 1971), the jumping behavior has been thought to be specially related to morphine withdrawal syndrome. However, it is thus demonstrated that this behavior is not always related to morphine withdrawal syndrome since the behavior can be

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