BRAIN SEROTONIN METABOLISM IN LITHIUM TREATED RATS

Tomio SEGAWA and Masumi NAKANO
Department of Pharmacology, Institute of Pharmaceutical Sciences
Hiroshima University School of Medicine, Hiroshima, Japan
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Abstract—Five days administration of Li (Li₂CO₃ 2.5 mEq/kg, i.p. twice daily for 4 days, followed by one injection on the 5th day) to rats did not significantly alter brain serotonin (5-HT) levels. Animals appeared somewhat sedated but showed considerable exploratory and rearing behavior in new circumstances. Turnover rate of brain 5-HT as measured from the accumulation after inhibiting monoamine oxidase was decreased by repeated administration of Li. Furthermore, Li lessened the depletion rate of brain 5-HT by reserpine. These results suggest that certain of the effects of Li on manic states can be attributed to the reduced functional activity of brain 5-HT neurons.

The clinical effectiveness of Lithium (Li) salts in the treatment of manic states was first reported by Cade et al. (1). The exact mechanism underlying the action is not fully understood; however, since Li is chemically related to Sodium it appears that the ion modulates the excitability of the nerves. If this is the case, a change in nervous activity in the brain can be expected, and would in turn result in a change in the rate of metabolism of and turnover rate of brain monoamines.

Schildkraut et al. (2, 3) observed an increase of norepinephrine (NE) turnover and deaminated metabolite as well as a decrease of normetanephrine in brain after administration of LiCl. Katz et al. (4) made the observation that electrically evoked release of NE from rat brain slices in vitro was inhibited by the addition of Li. Colburn et al. (5) and Kuriyama and Speken (6) reported an increase of NE-uptake into synaptosomes after Li injection. Conclusively speaking it may be stated that Li treatment decreases levels of NE available to central adrenergic receptors.

There is increased evidence that serotonin (5-HT) acts as a neurotransmitter at the synapse in the central nervous system. Many researchers have examined the effects of Li on the metabolism of 5-HT in brain and there is general acceptance that Li treatment decreases the evoked release of 5-HT from brain (4, 7, 8). On the other hand, however, there appear to be inconsistencies in literature regarding the effect of Li on the turnover of 5-HT. Thus, Schildkraut et al. (7), Corrodi et al. (8) and Ho et al. (9) found that Li administration led to a decrease in the turnover rate of 5-HT while Sheard and Aghajanian (10) and Perez-Cruet et al. (11) reported an increase when pretreatment of animals with Li was administered.

In the present study, therefore the following experiments were designed to investigate
the effect of Li treatment on brain 5-HT metabolism in rats: (1) brain levels of 5-HT (2) behavior and motor activity (3) turnover rate of 5-HT in brain (4) release of 5-HT from brain by reserpine.

MATERIALS AND METHODS

Male rats of Wistar strain, weighing from 150 to 180 g were used. The solution of Li$_2$CO$_3$ was neutralized to pH 7.4 with citric acid. In one series of experiments rats were given Li i.p. three times in doses of 2.5, 2.5 and 1.2 mEq/kg at intervals of 1 hr. In a second series 2.5 mEq/kg was injected i.p. twice daily for 4 days followed by one injection of the same dose on the 5th day. Control rats were given saline instead of Li$_2$CO$_3$ solution.

In a second series of experiments, measurement of spontaneous motor activity was made on groups of two rats each by means of the aninex activity meter (12). Recordings were made everyday for 5 min before the administration of Li. After 5 days administration of Li, rats were sacrificed by decapitation. Brains were removed, the cerebellum was discarded and the anterior portion of the brain was separated from posterior by cutting above the corpora quadrigemina. The whole brain excluding cerebellum, anterior and posterior portions were subjected to 5-HT analysis.

Turnover rate of 5-HT in brain was calculated according to Tozer et al. (13). The animals were injected with a dose of 3 mg/kg of pheniprazine (monoamine oxidase inhibitor, MAOI) i.p. and the rate of 5-HT formation was determined from the initial accumulation of 5-HT. Preliminary studies (unpublished) indicated that monoamine oxidase (MAO) is completely and immediately inhibited by pheniprazine at this dose level.

For the investigation of the effect of Li administration on reserpine-induced release of 5-HT, rats were injected with 2 mg/kg of reserpine i.p. 30 min after the final administration of Li and were sacrificed 1, 2, 4 and 6 hr after reserpine. Thereafter rats were sacrificed and both anterior and posterior portions of the brain were subjected to 5-HT analysis.

5-HT was assayed by the methods of Snyder et al. (14) and Curzon and Green (15).

RESULTS

1. Brain levels of 5-HT

The effect of Li administration for 5 days on brain levels of 5-HT in rats is shown in Fig. 1. Levels of 5-HT in Li-
treated animals appeared to be higher than control values, particularly in the posterior portion but the difference was statistically insignificant. Three injections of Li a day did not alter the concentration of 5-HT in any part of the brain.

2. Behavior and motor activity

During 5 days injection of Li, the animals appeared somewhat sedated, weakened and crouched in a corner of the cage. Pilo-erection was also observed. In order to measure spontaneous motor activity, two animals were placed together in a new cage and these showed considerable exploratory and rearing behavior which was almost indistinguishable from that observed in saline treated controls. Interestingly enough, animals appeared sedated again when they were put back into their own cage. Fig. 2 shows the activity counts measured by animex for 5 min. An injection of saline was not found to alter the activity but owing to usual adaptation to new circumstances the activity declined gradually day by day. The activity in Li-treated animals was somewhat lower than in controls but the difference was statistically insignificant and after 5 days treatment, animals still showed considerable counts of activity. Li administration for 5 days significantly influenced body weight as compared to saline-treated controls. Thus, from 2 to 4 days after injection there were almost no increase in body weight, while a significant decrease in body weight was found on the 5th day.

![Fig. 2. Spontaneous motor activity of rats after administration of Li. Markers on the bars represent the standard error of the mean.](image)

**Table 1. Turnover rate of brain 5-HT after 5 days administration of Li.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5-HT levels (ng/g ± S.E.)</th>
<th>Rate constant (k[hr]-1±S.E.)</th>
<th>Turnover rate (ng/g/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>376.33±12.85 (12)</td>
<td>0.75±0.01 (11)</td>
<td>282</td>
</tr>
<tr>
<td>Li</td>
<td>389.27±20.75 (11)</td>
<td>0.62±0.01 (11)</td>
<td>241</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate number of experiments.
3. Turnover rate of brain 5-HT

The results presented in Table 1 show that the administration of Li for 5 days decreased both rate constant and turnover rate of brain 5-HT when compared to the control group. MAOI, given to rats treated with Li as well as control rats did not result in any display of hyperactivity.

4. Release of 5-HT by reserpine

Reserpine, at a dose of 2 mg/kg i.p. caused a decrease of 5-HT content in rat brain. Figs. 3 and 4 show a time course of a decrease of 5-HT in anterior and posterior portions of the brain, respectively. In both parts of the brain, a rapid initial fall of 5-HT took place within 1 or 2 hr after reserpine injection. The 5-HT levels then decreased gradually until reaching approx. 55% of the initial values at 6 hr. Generally, pretreatment with Li for 5 days tended to retard a decrease rate of 5-HT in both parts of the brain. In the anterior portion at 1 hr after reserpine injection, 5-HT level was significantly higher in Li-treated rats than in controls treated with reserpine alone (p<0.001). On the other hand three injections of Li in one day did not alter the 5-HT depletion effect of reserpine in either part of the brain (Fig. 3, 4).

Reserpine, at a dose of 2 mg/kg i.p., induced a characteristic syndrome in rats. Shortly after injection, animals showed a startle response to auditory stimuli which lasted about 1 hr. At this dose ptosis was found about 30 min after injection while a hunchback posture was assumed about 2 hr after injection. Soft stools and diarrhea were also ob-

![Graph 3](#)

**Fig. 3.** Effect of pretreatment with Li on release of 5-HT from anterior portion of rat brain by reserpine.

*significantly different from control p<0.001.

Points represent values for 4-16 animals with the standard error of the mean.

![Graph 4](#)

**Fig. 4.** Effect of pretreatment with Li on release of 5-HT from posterior portion of rat brain by reserpine.

Points represent values for 3-12 animals with the standard error of the mean.
served from 30 min to 2.5 hr after reserpine. Pretreatment with Li three times in one
day delayed the onset of ptosis about 15 min and caused complete inhibition of increase
in responsiveness to auditory stimuli induced by reserpine. However, 1.5 hr after reserpine
administration there was somewhat of an increase in spontaneous motor activity together
with rearing and exploratory behavior which lasted about 2 hr. On the other hand re-
serpine-induced syndrome was modified greatly by a 5 day pretreatment with Li. Thus,
soft stools, diarrhea, hunchback posture and startle response to auditory stimuli were ab-
sent. Ptosis was observed, but it appeared only about 1 hr after reserpine. Within 1 hr
after reserpine, animals became hyperactive and showed an increase in food intake and
drinking, which lasted for about 1.5 hr.

DISCUSSION

There is evidence supporting the hypothesis that an abnormal metabolism of brain
5-HT could be the basis of affective disorder. There are also several psychotropic drugs
which affect the metabolism of 5-HT in brain. The results of our experiments indicate
that Li may exert its effect to some extent through modification of brain 5-HT metabolism.
There were no significant changes in brain 5-HT levels in Li-treated rats. This is in agree-
ment with the reports of Kuriyama and Speken (6), Corrodi et al. (8) and Bliss and Ailon
(16). Five days treatment with Li had a somewhat sedative effect on rats but was found
to be less effective in suppressing their exploratory and rearing behavior in new circum-
stances. It is therefore suggested that with regard to the sedative effect on animal be-
havior Li is slightly different from ordinary depressants. Further studies are necessary
to elucidate whether or not this is in any way related to its antimanic effect.

Our observation that 5 days treatment with Li decreased 5-HT turnover in rat brain
is in agreement with those of Schildkraut et al. (7), Corrodi et al. (8) and Ho, et al. (9).
Since the turnover of transmitter is thought to be directly related to nerve impulse activity
it is feasible that one of the effects of Li is to reduce functional activity in brain 5-HT neu-
rons.

When rats were treated with Li for 5 days, the depletion rate of 5-HT by reserpine in
the anterior portion of the brain was retarded significantly. This could indicate that Li
decreases the release of 5-HT by interfering with the releasing mechanism of reserpine
at synaptic vesicles. It is interesting to note that most authors have favoured the view
that Li treatment decreases the evoked release of 5-HT from the brain. Presumably Li
alters the properties of the synaptic vesicular membrane, thereby preventing the release
of 5-HT. Perez-Cruet et al. (11) suggested that a decreased accumulation of 5-HT in
Li-treated animals after MAOI simply reflects a decreased storage capacity in serotonergic
granules. However, present experiment results demonstrated clearly that Li-treatment
did not decrease storage capacity of 5-HT in synaptic vesicles but rather increased it.

In conclusion, pretreatment of rats with Li for 5 days decreased the rate of brain 5-
HT turnover as well as the release of 5-HT from synaptic vesicles, thereby decreasing the
amount of 5-HT reaching the central 5-HT receptors. In this connection it is interesting
to refer to Sheard and Aghajanian's report (10) that the great distractibility present in manic syndrome may in part represent a failure of habituation which is associated with increased release of 5-HT in the forebrain. It may be said, therefore, that certain of the effects of Li on manic states can be attributed to the reduced functional activity of brain 5-HT neurons.

REFERENCES