Effects of Lithium Administration on Central and Peripheral Nervous System in Rats. Biochemical and Morphological Findings


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Summary
The effect of 4 weeks' treatment with lithium chloride on the central and peripheral nervous system of Wistar albino rats was studied. Normal activity values of some brain enzymes related to energy transduction (LDH, MDH, COX, NADH-cytochrome) and neurotransmission (AChE), evaluated both in the homogenate in toto and in the crude mitochondrial fraction, were obtained. Fine changes in mitochondrial organelles and nerve processes of neurocytes were ultrastructurally observed.

The peripheral nerve studies revealed in some treated rats a slight motor nerve conduction velocity impairment by electrophysiological methods, but no significant alterations in the sciatic nerve specimens examined by electron microscopy.

Wirkungen der Verabreichung von Lithium auf das Zentralnervensystem und das periphere Nervensystem von Ratten - Biochemische und morphologische Befunde

The Verfasser untersuchten die Wirkung einer vierwöchigen Behandlung mit Lithiumchlorid auf das zentrale und periphere Nervensystem von Wistar-Albinoratten.

Es wurden normale Aktivitätswerte einiger Gehirnenzyme hinsichtlich der Energieumwandlung (LDH, MDH, COX, NADH-cytochrome) und der Neurotransmission (AChE) erhalten; diese wurden sowohl im Homogenat in toto als auch in der groben Mitochondrienfraktion ausgewertet.

Feinveränderungen in den Mitochondrien-Organellen und den Nervenprozessen der Neurozyten wurden ultrastrukturrell beobachtet.

Die Untersuchungen der peripheren Nerven zeigten in einigen Ratten eine leichte Verringerung der Nervenleitungs geschwindigkeit bei den motorischen Nerven, wobei elektrophysiologische Methoden angewendet wurden; es zeigten sich jedoch keine signifikanten Veränderungen in den elektroneuronmikroskopisch untersuchten Ischiasnerven.

Since their introduction in psychiatric treatment, extensive studies have been performed on the mechanism of action of lithium salts on nervous tissues.

Neurotoxic effects of the experimental lithium treatment have been reported. Lithium is known to influence some brain enzyme activities in rats, with decrease of acid phosphatase and ary-sulphatase in neural tissues (Bera and Chatterjee, 1976) and increase of succinic dehydrogenase and fumarase (Abreu and Abreu, 1972; 1974); an inhibition of Na/K ATPase followed by activation of Mg and Mg/Ca ATPase in the synaptosomal preparations was also observed (Hesketh, 1977).

In short-term investigations, electron microscopy of brain tissues revealed some changes of the neurocyte membrane system (Roizin et al., 1970) and degenerative alterations of nerve processes of dissociated brain cells in culture (Janka et al., 1979).

In the present experiments, the effects on peripheral and central nervous system of 4 weeks' administration of lithium chloride to Wistar rats were studied. The activity of some enzymes related to energy transduction and neurotransmission was evaluated in the rat brain; combined electrophysiological and morphological studies were performed on the peripheral nerves. Morphological studies on cerebral and cerebellar areas were also carried out.

Materials and Methods
Seventy adult female albino Wistar rats weighing 230–275 g were purchased from Morini Company (Italy) and kept on Randoxin and Causeret pellets and water ad libitum. The animals were divided into two groups: the first group (30 rats) was used as control; the second group (40 rats) was injected daily with lithium chloride, i.p. at the dose of 3 meq lithium/kg body weight for 4 weeks.

Blood samples were taken for Li measurements during various steps of the investigation, and the serum Li concentrations were determined by a Corning 435 flame photometer.

Electrophysiological studies were performed on 10 control and 10 treated rats 3 and 4 weeks after the beginning of the experiment. The motor and sensory nerve response latency was determined along 7 cm of the tail on animals anesthetized with ethyl ether according to the methods proposed by Miyoshi and Goto (1973), Faschi et al. (1977), Moglia et al. (1981). Body temperature was maintained by radiant heat from a lamp.

Biochemical studies were performed on 8 controls and 8 treated animals at the end of the experiment (4 weeks). All animals used for the analytical evaluations were killed between 9:30 and 10:00 a.m. At the set time, the rats were sacrificed by decapitation and their brains were removed from the skull within 15 sec, and transferred to a precooled box at -5 °C. The 0.32 M sucrose washed and weighed brains were homogenized in 0.32 M sucrose for 30 sec, (precooled Potter-Braun homogenizer) with a teflon pestle. The homogenate obtained was diluted with 0.32 M sucrose (10 w/v) and an aliquot of each sample was taken for the assay of enzymatic activities. The remaining homogenate was submitted to fractioning for isolating the crude mitochondrial fraction. Three centrifugations (Sorvall RC-5 Supercentrifuge) at 900 x g were performed
to remove nuclei and contaminating materials. The crude mitochondrial fraction was obtained with two centrifugations at 14,000 x g. Mitochondrial pellets were resuspended in 0.32 M sucrose. On both the homogenate and the mitochondrial preparation samples, protein content was evaluated and the following enzyme activities were measured: malate dehydrogenase (MDH), total NADH-cytochrome c reductase (NADH-cyRT), cytochrome oxidase (COX). The activities of lactate dehydrogenase (LDH) and acetylcholine esterase (ACHE), were evaluated only in homogenate samples, while that of citrate synthase (CS) was measured only in the mitochondrial preparation samples. Enzymatic activities were recorded (Beckmann 25 Spectrophotometer Recorder) and calculated using the straight portion of the reaction curves. Results were expressed as specific activities (amoles · min⁻¹ · mg protein⁻¹) and statistically analyzed with the analysis of variance (Anova).

Morphological studies. Complete necropsies were done on all animals at the end of the experiment. For histology, the tissues of 10 treated and 10 untreated animals were fixed in neutral 10 % formalin or Carnoy's solution. All sections were stained with haematoxylin and eosin; selected tissues were in addition stained with PAS, van Gieson, Malloy trichrome. The central and peripheral nervous tissues were stained with haematoxylin and eosin, Luxol fast blue and Heidenhain's haematoxylin.

For electron microscopy studies, specimens from brain cortex, white matter, basal ganglia, cerebellum and sciatic nerve of 22 treated and 12 untreated rats were obtained following perfusion with 4 % glutaraldehyde in 0.1 M cacodylate buffer pH 7.4, with 5 % sucrose. The specimens were also processed according to standard procedures and examined with a Zeiss EM9 electron microscope.

Peripheral sciatic nerve transverse semithin sections were stained with toluidine blue and the frequency distribution of myelinated fibers and their number per square millimeter were determined on micrographs enlarged to a final magnification of 1,000 X.

Results

The animals were observed daily and showed no adverse effects from the intraperitoneal injection of Lithium or saline. No peritonitis or haemorrhage occurred as complications. At sacrifice, the mean weights of treated and untreated animals were not statistically different. The animals were also examined daily for signs of neuromuscular dysfunction; there were no differences between control and experimental rats with respect to position, ability to extend the hind limbs and responses to painful stimuli.

Table 1 reports the distribution of Lithium in serum with time, after a single i.p. injection. The serum Li levels declined slowly in an exponential fashion.

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>meq Lithium/l serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.60 ± 0.12</td>
</tr>
<tr>
<td>4</td>
<td>1.28 ± 0.10</td>
</tr>
<tr>
<td>6</td>
<td>1.00 ± 0.08</td>
</tr>
<tr>
<td>8</td>
<td>0.78 ± 0.06</td>
</tr>
<tr>
<td>12</td>
<td>0.50 ± 0.08</td>
</tr>
<tr>
<td>18</td>
<td>0.28 ± 0.04</td>
</tr>
</tbody>
</table>

Electrophysiological results. The mean value of the latency of the evoked motor response in control rats was 2.9 msec. (S.D. 0.11); that of the latency of the evoked sensory response was 2.72 msec. (S.D. 0.12). After three weeks of treatment, the mean value of motor latency was 3.01 msec. (S.D. 0.47) and that of sensory latency 2.67 msec. (S.D. 0.46). After 4 weeks of treatment the mean value of motor latency was increased to 3.30 msec. (S.D. 0.24), with a significant difference as compared to controls (P < 0.001, Student's t test). The mean value of sensory latency was 2.5 msec (S.D. 0.37). The individual values of motor and sensory latencies obtained in treated rats are reported in Fig. 1 vs. the mean values and S.D. of control rats.

As shown in Fig. 1 after 3 weeks of treatment 2 rats had a motor latency value exceeding the mean value + 2 standard deviations of controls. At the 4th week, there were 4 animals showing a motor latency value exceeding the above mentioned limit. No abnormalities of the sensory nerve latency were seen in treated rats.

Biochemical results. Table 2 reports the data relative to the cerebral enzymatic activities studied in the homogenate in toto and in the crude mitochondrial fraction from rats treated for 4 weeks and from controls of the same age. Lithium failed to affect the activities studied.

Morphological results. Central nervous system. Histological studies on routinely stained brain tissues did not reveal any significant changes both in the 10 treated and in the 10 untreated rats selected for this investigation.

Electron microscopy studies performed in particular on the brain areas in which the highest concentrations of lithium had been previously demonstrated (Ebadi et al., 1974) did not reveal any significant alterations of the neuronal cell bodies and processes, of the Golgi complex, of the synaptic vesicles and of the myelinated structures.

An evident increase in the number of mitochondria was ob-
served in 6% of the treated animals in different areas: polymorphous mitochondria, with fine changes of the membrane and with cristal dysmorphism were seen. There was no evidence of intracrystral inclusions.

**Sciatic nerve.** Light microscopy investigations on semithick sections of the sciatic nerve did not show any significant changes of the myelinated large and small nerve fibers, of the interstitial connective tissue and of the blood vessels. The frequency distribution of myelinated fibers and their number per square millimeter in treated and untreated animals did not show any significant differences.

Ultrastructural investigations on unmyelinated and myelinated fibers did not reveal any significant alterations.

**Discussion**

In previous experiments carried out in depressed patients treated with lithium, a slowing down of max MCV concurrent with a longer-lasting evoked muscular response had been reported (Pinelli et al., 1972; Zerbi et al., 1975; Girke et al., 1975). These findings had been correlated with possible functional changes of neuromuscular transmission, due to lithium-induced electrolytic variations, as is for example observed in the conditions marked by an excessive storage of Mg and Ca in the extracellular environment.

Several authors have found thyroid hypofunction caused by lithium (James et al., 1976; Boettcher, 1976; Brownlie et al., 1976; Lindstedt et al., 1977). In hypothyroidism, in addition to the many reports of an involvement of muscular fibers (Astrom et al., 1961; Kissel et al., 1965; Norris and Panner, 1966; Bergogignan et al., 1967; Roger et al., 1973; Nappi et al., 1975) numerous results also point to modifications of peripheral nerves (Nickel et al., 1961; Scarpalezos et al., 1973).

Our experimental data show a significant increase of the mean value of motor latency in lithium treated animals only at a late stage (4th week of treatment). The increase of this parameter is fairly great (exceeding the mean value ± 2 S.D. of controls) in a certain number of animals: 2 out of 10 in the 3rd week of treatment and 4 out of 10 in the 4th week.

These modifications are not easy to interpret in connection with the above mentioned pathology, especially when considering that the results of our histological and ultrastructural studies of muscles and nerves were negative.

Such electrophysiological findings do not necessarily imply the concept of peripheral neuropathy, even though it must be remembered that there are some polynueuritic conditions showing no related modifications that can be detected by histological methods.

Previous ultrastructural observations on cerebral tissue in experimental acute lithium intoxication revealed fine structural changes of mitochondria, Golgi complex and synaptic vesicles (Roizin, 1971). More recent electron microscopy investigations on dissociated cultures obtained from chick embryo cerebral hemispheres exposed to 5 and 10 mM Li chloride revealed a marked reduction of neuronal cell bodies and of the neuronal process length, with degenerative changes of the nerve processes (Janka et al., 1979).

This study, performed on brain areas in which the highest lithium concentrations had been experimentally found (Ebadi et al., 1974) demonstrated an increase in the number of mitochondria and fine structural alterations of these organelles in 6% of treated rats, but no significant changes in the other cerebral structures.

It can be suggested that, at toxic concentrations, lithium might interfere with cerebral biochemical processes (probably with some sodium-dependent processes) (Janka et al., 1979) and cause occasional fine structural alterations of brain tissue mitochondria.

These findings are in agreement with the unspecific morphological alterations of the human brain tissues found in two cases of iatrogenic lithium poisoning observed by us (Scelsi et al., 1980; unpublished data) and in other previously described cases (Chapman et al., 1972).

**References**


**Table 2** Specific enzymatic activities (μmoles · min⁻¹ · mg protein⁻¹) of brain of control rats and of rats treated with lithium chloride (3 meq Li per kg, i.p.)

<table>
<thead>
<tr>
<th>Animals</th>
<th>LDH</th>
<th>MDH</th>
<th>Homogenate</th>
<th>ACHe</th>
<th>COX</th>
<th>NADH-cRT</th>
<th>MDH</th>
<th>COX</th>
<th>NADH-cRT</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.795</td>
<td>1.507</td>
<td>0.117</td>
<td>0.329</td>
<td>0.050</td>
<td>1.885</td>
<td>1.103</td>
<td>0.157</td>
<td>0.108</td>
<td></td>
</tr>
<tr>
<td>±0.018</td>
<td>±0.022</td>
<td>±0.001</td>
<td>±0.021</td>
<td>±0.003</td>
<td>±0.003</td>
<td>±0.053</td>
<td>±0.064</td>
<td>±0.004</td>
<td>±0.003</td>
<td></td>
</tr>
<tr>
<td>Treated with</td>
<td>0.818</td>
<td>1.564</td>
<td>0.113</td>
<td>0.359</td>
<td>0.051</td>
<td>1.823</td>
<td>1.160</td>
<td>0.153</td>
<td>0.100</td>
<td></td>
</tr>
<tr>
<td>lithium</td>
<td>±0.015</td>
<td>±0.020</td>
<td>±0.003</td>
<td>±0.014</td>
<td>±0.003</td>
<td>±0.038</td>
<td>±0.032</td>
<td>±0.007</td>
<td>±0.002</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± S.E.M. of 8 experiments, each performed on a different animal.
**References**


Zerbi, F., L. Fenoglio, P. Tosca: Plasma and erythrocyte concentrations of lithium. Psychiatria clin. 8 (1975) 236–242

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