Lithium Ion Does Not Protect Brain Against Transient Ischemia in Gerbils

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It has been proposed that lithium ion desensitizes neuronal receptors that function via the inositol phospholipid signaling mechanism. We examined the effects of lithium chloride on the morphologic outcome after 5 minutes of cerebral ischemia induced in gerbils by occluding both common carotid arteries under brief halothane anesthesia. In three treated groups of 10 gerbils each, 5 meq/kg i.p. lithium chloride was given 2 days, 1 day, and 2 hours before ischemia; 2 hours before ischemia; or immediately after the end of ischemia. Corresponding control groups of nine or 10 gerbils each received equivalent volumes of saline injected at comparable times. All gerbils were perfusion-fixed 1 week later, and neuronal density of the hippocampal CA1 pyramidal cells was determined. Lithium induced very mild intraischemic systemic hypothermia, but postischemic hyperthermia developed in both treated and control groups. Neuronal densities were equal in corresponding groups. The results indicate that our regimen of lithium administration provides no benefit in survival of hippocampal neurons, and intraischemic hypothermia of <0.8°C is not protective. Other strategies to inactivate the signal transduction system that is specific for excitatory neurotransmission should be evaluated. (Stroke 1991;22:84-89)

Pyramidal cells in the hippocampal CA1 subfield are selectively vulnerable to ischemia. In Mongolian gerbils (Meriones unguiculatus), for instance, most of the CA1 pyramidal cells ultimately die after exposure to as little as 5 minutes of ischemia, whereas no permanent damage occurs in neurons outside the hippocampus.1 Morphologic changes in the CA1 neurons develop slowly. This slowly evolving neuronal necrosis in the hippocampal CA1 region, termed "delayed neuronal death,"1 occurs in other species as well.2,3

Over the last several years, attention has been focused on the role of a brain glutamate system in the pathogenesis of brain injury caused by ischemia and seizures.4 Glutamate is an excitatory neurotransmitter in the mammalian central nervous system and produces neuronal firing through activation of postsynaptic receptors. Ionic events involved in glutamate-induced cellular damage have been identified in cultured hippocampal and neocortical neurons. Acute excitotoxic neuronal death is produced by the influx of Na+, Cl−, and water and by the eventual lysis of swollen cells,5 whereas delayed neuronal death is mediated by an elevated intracellular Ca2+ level.5 Thus, neurons may be killed in two different ways once they are bathed with a medium that contains high concentrations of glutamate. Evidence has grown that endogenous glutamate causes brain injury in the setting of in vivo ischemia in relation to selective neuronal necrosis in the hippocampus, where pyramidal cells are glutamatergic.6−8 One leading hypothesis concerning the mechanisms of delayed neuronal death in the hippocampus proposes that the extracellular accumulation of glutamate during ischemia induces an increase in free Ca2+ inside the cells and the subsequent activation of lipases and proteases during postischemic reperfusion.9

Lithium salts have been used widely in the treatment of bipolar affective disorders. Lithium ion is a powerful inhibitor of the enzyme inositol 1-phosphatase, producing an accumulation of inositol 1-monophosphate (IP1) and a reciprocal decrement of inositol in the brain.10 Accordingly, lithium reduces the supply of inositol necessary for the synthesis of inositol phospholipids. Hydrolytic cleavage of inositol phospholipids, particularly phosphatidylinositol 4,5-bisphosphate (PIP2), in the plasma membrane is an
integral part of the mechanism of cell surface receptors that function through Ca\(^{2+}\) and is responsible for releasing arachidonic acid. When this class of receptors is stimulated by agonists such as glutamate, acetylcholine, serotonin, and norepinephrine, PIP\(_2\) is cleaved into its two constituents diacylglycerol and inositol 1,4,5-trisphosphate (IP\(_3\)), both of which convey signals intracellularly.\(^{11}\) Following the completion of its role as a second messenger, IP\(_3\) is sequentially dephosphorylated to become IP\(_1\) and finally inositol.

An attractive hypothesis about its therapeutic effects on mania is that lithium acts by lowering the level of brain inositol, which leads indirectly to receptor desensitization by limiting the resynthesis of inositol phospholipids required for receptor-effector signal transduction.\(^{12}\)

We tested the hypothesis that lithium ion inhibits the increase in intracellular Ca\(^{2+}\) concentration and the activation of protein kinase C in response to ischemia-induced receptor activation, thereby contributing to neuronal survival.

**Materials and Methods**

We used 61 male Mongolian gerbils weighing 60–80 g. They had free access to food and water until the experiments began. Ischemia of both cerebral hemispheres was produced for 5 minutes by the application of an aneurysm clip (temporary clip, Sugita, Tokyo, Japan) to each common carotid artery, which was exposed through a ventral neck incision. The surgery to occlude the arteries was performed while the gerbils inhaled a gas mixture of 3–4% halothane, 40% O\(_2\), and a balance of N\(_2\). Arterial occlusion was achieved in <5 minutes, and then the halothane was discontinued. The gerbils in a cage were randomly assigned to receive injections of either LiCl solution (treated groups) or saline (control groups). LiCl (Kanto Chemical Co., Tokyo, Japan) was dissolved in distilled water to make a 0.75 M solution; a 0.75 M NaCl solution was also made. In the treated gerbils LiCl was administered at 5 meq/kg body wt i.p. 2 days, 1 day, and 2 hours before ischemia (chronic pretreatment), 2 hours before ischemia (acute pretreatment), or immediately after the end of ischemia (posttreatment). Each treated group had a corresponding control group that received equivalent volumes of saline injected at comparable times. A rectal temperature probe was inserted as soon as the gerbils were anesthetized, and rectal temperature was monitored thereafter. A decrease in rectal temperature during surgical preparation and ischemia was prevented by a heating lamp. When the gerbils had recovered following release of the carotid occlusion and had started to move around, they were returned to their cages and fed ad libitum. Gerbils that did not become comatose during carotid occlusion were discarded without perfusion-fixation.

One week following transient forebrain ischemia, the gerbils were fixed by transcardiac perfusion under deep halothane anesthesia; 120 ml of 10% formaldehyde in 0.1 M phosphate buffer was perfused at a constant pressure of 130 cm H\(_2\)O. The brains were removed 2 hours later and immersed in 10% formaldehyde solution for several days and then embedded in paraffin. Sections 5 \(\mu\)m thick located 0.5–1.0 mm posterior to the most rostral tip of the hippocampus were prepared and stained with cresyl echt violet. Since neuronal changes are similar throughout the rostrocaudal extent of the hippocampus, in one section from each gerbil the surviving neurons in the hippocampal CA1 subfield were counted. Neuronal density (i.e., the number of CA1 neurons per 1 mm length of the stratum pyramidale) was calculated for each cerebral hemisphere as described previously.\(^{13}\) Neuronal densities of the right and the left cerebral hemispheres were averaged for each animal and used for statistical evaluation.

Wilcoxon’s rank sum test was used to compare neuronal densities in the treated groups and their corresponding control groups because neuronal density did not show a Gaussian distribution.\(^{13}\) Rectal temperatures at the time of carotid occlusion and immediately after release of the carotid clamps in the treated groups and their corresponding control groups were compared by Student’s \(t\) test. Results are presented as mean±SEM.

**Results**

One treated and two control gerbils did not become comatose during carotid occlusion and were discarded. Table 1 lists neuronal densities in the treated and control groups. Chronic pretreatment, acute pretreatment, and posttreatment with LiCl produced no effect on neuronal density. In 111 hemispheres of 58 gerbils neuronal density ranged from 7 to 64 cells/mm. In the remaining five hemispheres from five animals (one in the LiCl chronic pretreatment group, one in the control acute pretreatment group, one in the LiCl acute pretreatment group, and two in the control posttreatment group), we found an exceptionally high neuronal density (262–265 cells/mm) not different from that in non-ischemic gerbils.\(^{13,14}\) We assumed that the unilateral hippocampus of these five gerbils had collaterals through the posterior circulation sufficient to withstand ischemia. These high values elevated the mean neuronal density of the concerned groups and its SEM. Nevertheless, even after these aberrant values were excluded from statistical analysis, no significant differences were found between the treated groups.

### Table 1. Neuronal Density of Gerbil Hippocampal CA1 Subfield After 5-Minute Occlusion of Both Common Carotid Arteries

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NaCl Density</th>
<th>LiCl Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic pretreatment</td>
<td>19±2</td>
<td>32±13</td>
</tr>
<tr>
<td>Acute pretreatment</td>
<td>30±14</td>
<td>30±13</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>52±19</td>
<td>35±5</td>
</tr>
</tbody>
</table>

Values are mean±SEM cells per 1 mm length.
Table 2. Rectal Temperature at and Immediately After Carotid Artery Occlusion in Gerbils

<table>
<thead>
<tr>
<th>Timing</th>
<th>NaCl</th>
<th>LiCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Occlusion</td>
</tr>
<tr>
<td>Chronic pretreatment</td>
<td>10</td>
<td>37.0±0.2</td>
</tr>
<tr>
<td>Acute pretreatment</td>
<td>9</td>
<td>37.0±0.2</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>9</td>
<td>37.3±0.3</td>
</tr>
</tbody>
</table>

Values are mean±SEM °C. *p<0.01, **p<0.05, respectively, different from corresponding controls by Student's t test.

Rectal temperature is presented in Table 2. Temperature at the onset of ischemia was 0.7–0.8°C lower in the LiCl chronic and acute pretreatment groups than in their corresponding control groups (p<0.01), whereas temperature at the onset of ischemia was similar in the two posttreatment groups. Rectal temperature did not change during ischemia within a group. Rectal temperature was closely monitored in three or four gerbils of each group not only during ischemia but also during recirculation (Figures 1–3). Rectal temperature rose above the preischemic level in all 20 animals examined and exceeded 39°C during the early period of recirculation in nine.

Discussion

Systemically administered lithium salts cause a dose-dependent increase in the brain level of lithium ion.10 When a single dose of 2–17 meq/kg s.c. LiCl is given, the increase in IP$_1$ concentration and decrease in inositol concentration in the brain tissue plateau at doses of ≥9 meq/kg. The effect of lithium on cerebral phosphoinositide metabolism is evident at 1.5 hours following administration, becomes maximal at 24 hours, and is still present at 72 hours. These data are derived from rats, and we selected the dose of 5 meq/kg LiCl to ensure the effect as well as the safety of repeated drug administration.

Cerebral ischemia results in a rapid decrease in the concentrations of cerebral inositol phospholipids, particularly polyphosphoinositides (PIP$_2$ and phosphatidylinositol 4-monophosphate [PIP]), and an increase in the concentrations of diacylglycerol and free fatty acids, all of which are rich in arachidonic acid.
Acid and stearic acid. This suggests that the hydrolysis of polyphosphoinositides mediated by phospholipase C occurs during ischemia, with production of diacylglycerol and inositol phosphates and subsequent deacylation of diacylglycerol by lipases. Similar changes in the concentrations of lipids take place in the ischemic hippocampus of gerbils. It has also been shown that protein kinase C in brain tissue is activated at the onset of ischemia. It has also been shown that protein kinase C in brain tissue is activated at the onset of ischemia. It has also been shown that protein kinase C in brain tissue is activated at the onset of ischemia. It has also been shown that protein kinase C in brain tissue is activated at the onset of ischemia.

The ischemia-induced decrease in the concentrations of polyphosphoinositides may be partly due to reduced energy-requiring synthetic activity; however, in all likelihood receptor-linked catabolism occurs as well. Thus glutamate, the extracellular concentration of which increases markedly in the hippocampus during ischemia, has been shown to enhance the hydrolysis of membrane inositol phospholipids in hippocampal slice preparations. This finding is consistent with the view that glutamate released during ischemia stimulates postsynaptic receptors, which in turn activate phospholipase C in neurons. Furthermore, free arachidonic acid is known to increase the eflux of glutamate from synaptosomes and to inhibit its uptake by glia and neurons. Decaylation of arachidonic acid would, therefore, reinforce the excitatory effect of this neurotransmitter. Strosznajder and coworkers found that pretreatment with 3 meq/kg i.p. lithium 5 hours prior to ischemia attenuates the decrease in cerebral polyphosphoinositide levels for up to 10 minutes of ischemia in gerbils. During recirculation after 5 minutes of ischemia in gerbils, the levels of polyphosphoinositides do not recover and the level of free arachidonic acid remains elevated in the hippocampus, whereas the changes in lipid levels are reversed in the less vulnerable parietal cortex. Evidence exists, nevertheless, that the CA1 neurons are alive for the first day of reflow. The adenosine triphosphate level is normal, and electrical activity exhibits seizure-like paroxysmal discharges. Furthermore, pentobarbital and indomethacin given immediately after the end of ischemia increase the survival of CA1 pyramidal cells. Pentobarbital may suppress the electrical hyperactivity. Indomethacin inhibits prostaglandin biosynthesis, which releases oxygen radicals. Both drugs probably prevent the posts ischemic hyperthermia (Figures 1–3) that may be detrimental to the brain. Whatever the mechanisms of drug action, it is apparent that most CA1 neurons are amenable to therapy during the initial period of reflow.

It is conceivable that posts ischemic neuronal hyperactivity directly causes delayed neuronal death. Sustained seizures induced by bicuculline result in the alteration of brain lipids, the pattern of which resembles that occurring during ischemia. Of interest, the administration of cholinergic agents to lithium-treated rats produces sustained limbic seizures and widespread brain damage, while the cholinergic agents or lithium alone have no such effects. If the events occurring in the posts ischemic hippocampus are essentially the same as those occurring during cholinomimetic-induced seizures, our lithium treatment would worsen the outcome. This assumption seems to be contrary to that expected from the receptor-desensitization hypothesis of lithium. To our knowledge, there is no information about the effects of lithium on posts ischemic lipid metabolism.

Our results indicate that our regimen of lithium administration has no effect on the survival of CA1 pyramidal neurons in the hippocampus after ischemia and that intracellular hypothermia of <0.8°C provides no protection for the neurons. Three explanations for the failure of lithium to ameliorate neuronal necrosis are possible. First, the glutamate-induced hydrolysis of inositol phospholipids might have nothing to do with delayed neuronal death despite evidence for the excitotoxic hypothesis in ischemic brain damage. Nonetheless, our results suggest that the voltage-dependent influx rather than the agonist-dependent influx or Ca2+ mobilized by IP3 is primarily responsible for the deleterious increase in the cytoplasmic Ca2+ level. Second, the hypothesis proposed by Berridge and coworkers, that upon stimulation in the presence of lithium a pathway that functions via the inositol phospholipid signaling mechanism suffers a reduction in the amount of free inositol sufficient to interfere with the resynthesis of inositol phospholipids and thereby leads to receptor

FIGURE 3. Graph of changes in rectal temperature during and after cerebral ischemia in gerbils in posttreatment groups. A, treated with LiCl; Δ, treated with NaCl.
desensitization, may be incorrect. Indeed, it was recently reported that acute and chronic lithium treatment does not lower the levels of individual inositol phospholipids in the brain. However, the control levels of polyphosphoinositides in those experiments were low, PIP, and PIP levels being 40% and 60% of our previously reported control values, respectively. This raises the suspicion that the rapidly metabolizing pools might be lost during handling of the brain tissue, making the interpretation of those data difficult. In addition, since receptor-linked pools may represent only 10–20% of the total inositol phospholipids, the effect of lithium would not be detected by measurement of their static contents. More dynamic assessment is necessary to settle the argument. Third, other neurotransmitters such as acetylcholine, norepinephrine, and serotonin that are released from nerve terminals during ischemia also stimulate the hydrolysis of inositol phospholipids; norepinephrine in particular has been demonstrated to be neuroprotective. Since lithium nonspecifically blocks the signal transduction involving the phosphatidylinositol cycle, the net effect could be small or null. We think that this is the most likely explanation. Strategies to manipulate the second messenger system for a specific agonist need to be developed. Our experiments, however, cannot rule out the possibility that the repeated administration of lithium during longer periods of reperfusion would reduce brain damage due to transient ischemia.

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