Acute and Chronic Lithium Treatments Influence Agonist and Depolarization-Stimulated Inositol Phospholipid Hydrolysis in Rat Cerebral Cortex

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ABSTRACT
The ability of acute and chronic Li treatment to influence agonist and depolarization-induced phosphoinositide metabolism was examined in rat cerebral cortex slices. After acute treatment (6.75 mEq/kg, 18 hr), [3H]inositol phosphates accumulating in the presence of 100 μM carbachol (muscarinic), 31 mM K+, 300 μM histamine (H1) and 300 μM 5-hydroxytryptamine (5-hydroxytryptamine) were reduced significantly even after preincubation of slices with 2.5 mM myo-inositol. However, the response to noradrenaline (100 μM (α-1) was unaffected. In the absence of a drug-free period, chronic Li (2 weeks) maintained the reduced phosphoinositide response to receptor agonists and K+, and now even noradrenaline responses were reduced significantly. Dose-response curves revealed that reduction in the response to carbachol was due to a fall in maximal response and not in EC50. When rats were withdrawn from chronic treatment for 18 hr, the responses to carbachol were enhanced significantly with respect to untreated controls. Neither acute nor chronic Li treatments altered significantly the overall incorporation of [3H]inositol into phospholipids. Furthermore, Li treatment did not influence the activity of phospholipase C assayed in crude homogenates of cerebral cortex. In conclusion, acute and chronic Li treatments producing < 1 mM in cerebral tissue, severely disrupts phosphoinositide metabolism. Although such effects may well be secondary to inhibition of inositol monophosphatase, they are not reversed by inositol and therefore do not appear to result from depleted phosphoinositides.

Li salts have been used widely as an effective treatment for manic depressive disorder for many years although their mechanism of action is unknown. The possibility of interactions with inositol phospholipids was, however, suggested by the results of Allison and Stewart (1971) who reported that Li treatment reduced rat brain inositol levels and this was later seen to be accompanied by an increase in inositol-1-phosphate (Allison et al., 1976). This effect is due to a potent inhibition of inositol-1-phosphatase (Hallacher and Sherman, 1980), the enzyme which dephosphorylates inositol-1-phosphate derived from inositol phospholipid breakdown and inositol-1-phosphate from the de novo synthesis route for inositol from glucose. Indeed, this property of Li has been exploited in the development of a sensitive assay for phospholipase C-mediated inositol phospholipid hydrolysis in which the water soluble products of [3H]inositol prelabeled phospholipids accumulate predominantly as [3H]inositol-1-phosphate in the presence of LiCl (Berridge et al., 1982).

Because the therapeutic effectiveness of Li is observed with serum concentrations (0.5–1 mM) that effectively inhibit inositol monophosphatase, the suggestion has been made that the therapeutic effects of Li might be related to changes in the phosphoinositide cycle leading to alterations in the production of potential second messengers, such as inositol 1,4,5-trisphosphate and diacylglycerol (Berridge et al., 1982; Berridge, 1984). Therefore, we have examined the effects of acute and chronic administration of Li salts to rats on agonist and depolarization-evoked inositol phospholipid hydrolysis in cerebral cortical slices in vitro.

Materials and Methods

Drug administration. In acute experiments, male Sprague-Dawley rats (~200 g initial b.wt.) were injected i.p. with 6.75 mEq/kg of LiCl and sacrificed 16 to 18 hr later. For longer term treatments the rats were fed a diet consisting of 2.3 g of Li2CO3, 1.5 kg of rat food and 2 liters of water, presented as a mash. Water and normal saline were available ad libitum. In some experiments the Li diet was replaced by a normal diet 16 to 18 hr before death. The Li fed animals gained weight more slowly than the corresponding controls, final body weights being about 75% of controls after 14 days feeding.

Li+ levels. Whole cerebral cortices from Li-fed rats were homogenized in 5% TCA, centrifuged (1000 × g, 15 min) and Li in the

ABBREVIATIONS: TCA, trichloroacetic acid; IP, inositol phosphates; PI, phosphatidylinositol; s-HT, 5-hydroxytryptamine.
supernatant measured using an Instrumentation Laboratories (Lexington, MA) flame photometer.

\[^{[3]H}]IP\] accumulation. The methods used were essentially those described by Brown et al. (1984); 350 \times 350 \mu \text{m} of cross-chopped slices were prepared from whole cerebral cortex and incubated with three changes of medium for 60 min in a Krebs-bicarbonate buffer, pH 7.5, equilibrated with 95% O\textsubscript{2}-5% CO\textsubscript{2} at 37°C in a shaking water bath. In some experiments 2.5 mM myo-inositol were included for the initial 30 min of the incubation, followed by 30 min in normal Krebs'. This was followed by a 30-min incubation with 0.3 \mu\text{m}\[^{[3]H}]\text{inositol} and 5 mM LiCl followed by 45 min with agonists. For measurement of total \[^{[3]H}]IP\] incubations were terminated by the addition of chloroform-methanol (1:2 v/v). Further aliquots of chloroform and then water were added (Brown et al., 1984) and \[^{[3]H}]IP\] separated by ion exchange chromatography using Dowex anion exchange resin in the formate form. After addition of 10 \mu\text{M} of concentrated HCl, \[^{[3]H}\] labeled phospholipids were separated from the chloroform phase by evaporation overnight at room temperature. For estimation of individual \[^{[3]H}]IP\] production incubations were terminated by the addition of 300 \mu\text{L} of 1.0 M TCA. Samples were vortexed and centrifuged (3500 \times g, 20 min.) and aliquots of the supernatant washed 5 times with 2 volumes of water-saturated diethyl ether. The sample pH was adjusted to between 7 and 8 by addition of 5 mM NaHCO\textsubscript{3} and the \[^{[3]H}\] phosphates separated by anion exchange chromatography, as described by Batt and Nahorski (1988).

Phospholipase C activity. A crude phospholipase C preparation was prepared by homogenization of two whole rat brains in 12 ml of ice-cold 0.32 M sucrose. The brains were taken from either untreated rats or from rats treated 16 to 18 hr previously with 6.75 mM LiCl. The homogenate was centrifuged at 100 \times g for 90 min and 10-\mu\text{L} aliquots of the supernatant were incubated with 100 \mu\text{M} \[^{[3]}\text{H}]\text{IP}\) in a buffer consisting of 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 1 mg/ml of deoxycholate, 0.2 mM CaCl\textsubscript{2} and 100 mM NaCl, in a total volume of 300 \mu\text{L}. After various times the incubations were terminated by the addition of 0.94 ml of chloroform-methanol-concentrated HCl (200:100:2.61 v/v). The total \[^{[3]}\text{H}\) accumulating in the aqueous phase after centrifugation of the samples (comprising \[^{[3]}\text{H}]\text{IP}\) and \[^{[3]}\text{H}\] inositol) was taken as an index of the activity of phospholipase C in the preparation.

Materials. \[^{[3]}\text{H}]\text{myo-inositol}(15 Ci/mm) and \[^{[3]}\text{H}]\text{PI}\) were purchased from New England Nuclear (Boston, MA). All other drugs and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) or Pisons Ltd. (Leicestershire, UK).

Results

The effects of acute Li treatment on the agonist-stimulated accumulation of \[^{[3]}\text{H}]\text{IP}\) in cortical slices are shown in figure 1A. The responses to the muscarinic agonist carbachol and to elevated K\textsuperscript{+} and 5-HT\textsuperscript{+} were reduced significantly whereas the accumulation in the presence of noradrenaline and histamine were unaffected. Inasmuch as Li treatment has been shown to reduce brain levels of inositol (Allison and Stewart, 1971), a higher specific activity of \[^{[3]}\text{H}\] labeled endogenous inositol pools in the slices from treated animals could mask effects on \[^{[3]}\text{H}\] IP. Therefore, attempts were made to replete those pools by preincubating slices with 2.5 mM unlabeled inositol before the incorporation period with \[^{[3]}\text{H}\]inositol. This preincubation produced an apparent enhancement of the effects of Li treatment (figure 1B) with larger reductions evident in the responses to carbachol, elevated K\textsuperscript{+}, histamine and 5-HT\textsuperscript{+}. However, the response to noradrenaline was resistant to the Li treatment. Separation of the \[^{[3]}\text{H}\] labeled phosphates stimulated by 1 mM carbachol demonstrated that acute Li treatment caused a reduction in all of the \[^{[3]}\text{H}]\text{IP}\) fractions (table 1).

At least some of the effects of Li appear to be maintained on prolonged treatment as accumulation of \[^{[3]}\text{H}]\text{IP}\) in the presence of carbachol was also reduced after 14 days feeding with the Li diet (fig. 2A). In this case, the effect was not enhanced significantly by preincubating the slices with unlabeled inositol. However, now a small reduction in the response to noradrenaline was evident only after inositol preincubation. When the Li-containing diet was replaced by a normal diet 16 hr before death, the subsequent responses to both carbachol and noradrenaline were enhanced. Also, in this instance there was no significant effect of inositol preincubation (fig. 2B).

The effect of Li appeared to be on the maximal responsiveness to carbachol, as judged by the absence of change in the EC\textsubscript{50} values for the agonist after chronic treatment and withdrawal (fig. 3).

The fall in \[^{[3]}\text{H}]\text{IP}\) accumulation in the presence of agonists...
would not appear to be due to a Li-induced reduction of \([^3H]\) inositol incorporation as there were no significant differences in the amounts of \(^3H\) associated with total inositol phospholipid after acute or chronic treatment (table 2), although, as expected, preincubation with unlabeled inositol reduced greatly lipid labeling in both control and treated slices.

Acute Li treatment did not affect the activity of phospholipase C assayed subsequently in vitro. Crude enzyme preparations were made from the whole brains of untreated rats and from rats treated 16 hr previously with LiCl. Upon incubation with \([^3H]PI\), the rates of formation of the water-soluble products of \([^3H]PI\) hydrolysis were the same for each enzyme preparation (fig. 4). The addition of 5 mM exogenous LiCl to the incubations with the treated enzyme preparation did not influence this result (data not shown).

Fig. 2. Effect of 14-day Li administration and withdrawal on agonist-stimulated \([^3H]\)IP accumulation in rat cerebral cortex slices. Rats were fed a diet containing \(\text{LiCO}_3\) for 14 days as described under "Materials and Methods." Cortical Li\(^+\) concentration after this treatment was 0.4 ± 0.04 mEq/kg (wet wt.) (n = 10). A, rats had access to the Li-containing diet until immediately before death. Slices were prepared as described under "Materials and Methods" and were preincubated (hatched bars) or not preincubated (open bars) with 2.5 mM myo-inositol as described in the legend to figure 1. Ordinate, total \([^3H]\)IP accumulating in slices from Li-treated rats in the presence of carbachol or noradrenaline expressed as a percentage of that accumulating in slices from untreated rats under the same conditions. *P < .05 compared with untreated. Inositol preincubation had no significant effect. B, the Li-containing diet was replaced by standard diet 16 to 18 hr before death. The cortical Li\(^+\) concentration under these conditions was 0.2 ± 0.02 mEq/kg (wet wt.) (n = 8). The axes are as described in A. *P < .01 compared with untreated. Inositol preincubation had no significant effect. Four independent experiments were performed in duplicate. Basal and stimulated \([^3H]\)IP in untreated rats was very similar to figure 1.

Discussion

The therapeutically useful properties of Li in the treatment of manic depressive disorders has prompted intensive research into its mechanism of action. For example, various authors have reported Li-induced changes in radioligand binding to a number of central nervous system receptors (e.g. Maggi and Enna, 1980; Lerer and Stanley, 1985) as well as the ways in which binding can be modified upon chronic exposure to agonists and antagonists (Levy et al., 1982). Li treatment also has been shown to reduce brain adenylyl cyclase activity (Forn and Valdecasas, 1971; Palmer et al., 1972) and, in the case of the noradrenaline-sensitive cyclase, the inhibition is maintained during chronic treatment with Li (Ebstein et al., 1980). More recently, inhibitory effects on agonist-stimulated cyclic GMP in murine neuroblastoma cells also have been reported (Kanba et al., 1986), but as with almost all previous studies of this kind, effective concentrations of this monovalent ion are greatly in excess of those serum levels that are beneficial therapeutically. The present study was prompted by the finding that Li potently inhibits the enzyme inositol-1-phosphate phosphatase (Hallcher and Sherman, 1980), thereby causing a re-

Fig. 3. Effect of increasing carbachol concentrations on total \([^3H]\)IP accumulation in cerebral cortex slices after chronic treatment and withdrawal from chronic treatment with \(\text{LiCO}_3\). Slices were prepared and preincubated with 2.5 mM myo-inositol as described under "Materials and Methods." Results are expressed as total counts per minute of \([^3H]\) IP accumulating after 45 min with carbachol. The E\(50\) values for the curves are: control, 14 mM; treated, 22 mM; and withdrawn, 15 mM. The figure represents the mean of three independent experiments each conducted in triplicate with S.E.M. < 15% of the means. Basal \([^3H]\)IP was in the range 300 to 400 cpm.

TABLE 2
The effect of lithium treatments on incorporation of \([^3H]\)inositol into phospholipids

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No Inositol Preincubation</th>
<th>Preincubated with 2.5 mM myo-inositol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(cpm in 200 (\mu) CHCl(_3) phase)</td>
<td>(cpm in 200 (\mu) CHCl(_3))</td>
</tr>
<tr>
<td>Acute control</td>
<td>4717 ± 560</td>
<td>2266 ± 301</td>
</tr>
<tr>
<td>Li, 18 hr</td>
<td>4509 ± 377</td>
<td>1950 ± 155</td>
</tr>
<tr>
<td>Chronic control</td>
<td>5089 ± 256</td>
<td>2196 ± 191</td>
</tr>
<tr>
<td>Li, 2 week</td>
<td>4611 ± 171</td>
<td>1960 ± 145</td>
</tr>
<tr>
<td>Li, 2 week (withdrawn)</td>
<td>5982 ± 334</td>
<td>2353 ± 172</td>
</tr>
</tbody>
</table>
duction in central myo-inositol levels and an accumulation of inositol-1-phosphate (Sherman et al., 1981). In rat cortical slices, Li enhances carbachol-induced inositol mono- and bisphosphates with EC_{50} values of 0.5 and 4 mM, respectively (Batty and Nahorski, 1985). Although enhanced agonist-induced accumulation of inositol trisphosphate has also been reported in some cells (Drummond et al., 1984; Thomas et al., 1984), prolonged muscarinic receptor stimulation of cerebral cortical slices in the presence of Li suppresses inositol tris- and particularly tetrakisphosphate production (Batty and Nahorski, 1985; L. Batty and S. R. Nahorski, in preparation). These data suggest that Li can interfere with inositol phosphate metabolism at the relatively low concentrations observed in cerebral tissue of rats treated with this ion.

Acute treatment of rats with Li inhibited [3H]IP accumulation in cortical slices stimulated with the muscarinic agonist carbachol, histamine, 5-HT and elevated K^+ concentrations, although the full extent of the inhibition could only be seen after a preincubation with unlabeled inositol. It is likely that after Li treatment, a reduced inositol pool may result in a higher specific radioactivity of the lipids and phosphates than that seen in controls and this should be reversed by preincubation with cold inositol. The much smaller effect of inositol preincubation on the consequences of prolonged Li administration might be related to a less marked depletion of endogenous inositol due to the lower brain Li levels achieved by the feeding regime. It is important to note that these results suggest that the effects of in vivo Li administration do not simply appear to relate to inositol and phosphoinositide depletion and hence reduction in receptor responses. In that case, one would anticipate that inositol incubations would reverse the effects of Li and this was not seen. This contrasts with recent results in the parotid gland (Downes and Stone, 1986), and suggests other persistent actions of Li treatment that may or may not be secondary to the action of this ion on inositol phosphates. The fact that noradrenaline-stimulated [3H]IP accumulation was relatively resistant to Li exposure suggests that the ion's effects are not related to a direct nonspecific action on the phospholipid breakdown mechanism. Certainly, the total activity of phospholipase C itself did not seem to be affected, at least by acute Li exposure. Furthermore, the relative proportions of separate [3H]IP were not affected significantly by in vivo Li exposure, indicating that the activities of the inositol tria- and bisphosphatases were probably not altered persistently. The relative insensitivity of the noradrenaline-alpha-1 receptor responses may indicate that these sites are on cells that are somewhat resistant to Li. This could be simply an accessibility difference or, more intriguingly, indicate that different cells or receptors may be associated with different routes of IP metabolism, perhaps with variable sensitivity to Li (see Michell, 1986).

Inasmuch as the [3H]IP accumulation due to K^+-induced depolarization was also inhibited, it is tempting to speculate that Li's effects are distal to membrane recognition sites as the action of K^+ appears to be mediated, in part, by the activation of voltage-sensitive Ca^{2+} channels (Kendall and Nahorski, 1985) and perhaps a direct effect of Ca^{2+} on phosphoinositides (Nahorski et al., 1986; Rooney and Nahorski, 1986). A postreceptor mechanism of action is also supported by the finding that the reduction in carbachol-mediated [3H]IP accumulation is maintained upon prolonged Li exposure under conditions in which cortical muscarinic receptor binding is unchanged (Maggi and Enna, 1980; D. A. Kendall, unpublished observations).

The reduction in [3H]IP accumulation does not appear to have been due to a reduced incorporation of [3H]inositol into the phospholipids as there were no substantial changes in total 3H-labeling in Li-treated rats. However, it must be emphasized that this could be complicated by problems of specific activity as discussed above, and it is probable that only a fraction of the cerebral phosphoinositides are associated with receptors linked to phospholipase C. On the other hand, in GH3 cells, Drummond and Raeburn (1984) have shown that prolonged exposure to Li reduces the cellular content of both PI and PI-4-phosphate whereas PI-4,5-bisphosphate, probably the immediate substrate for phospholipase C attack (Berriedge, 1984), was maintained. Similarly, Downes and Stone (1986) demonstrated that Li in vitro reduced the rate of PI-4-phosphate but not PI-4,5-bisphosphate synthesis in carbachol-treated parotid gland. Furthermore, preincubation with inositol was shown to reverse the effects of the monovalent ion. The possibility remains that in brain, Li treatment in vivo may lead to a more severe disruption of polyphosphoinositide synthesis and this is not easily reversed by inositol preincubation in vitro. The reported enhancement by Li of agonist-induced diacylglycerol production in GH3 pituitary cells (Drummond and Raeburn, 1984) may, however, provide the best clue to the site of action of Li in the present experiments. Phorbol esters like diacylglycerol stimulate protein kinase C (Nishizuka, 1984) and have been reported to suppress carbachol-stimulated IP accumulation in hippocampal slices (Labarca et al., 1984) and astrocytoma cells (Orellana et al., 1985). Perhaps a protein kinase C-induced modification of phosphoinositide hydrolysis and/or inositol-1,4,5-trisphosphate metabolism underlies both the acute and chronic Li effects whereas during withdrawal the supersensitive responses reflect the enhanced effects of compensatory mechanisms associated with impaired receptor-effector coupling. Further experimentation is clearly required.

From the evidence presented thus far it seems unlikely that Li influences inositol phospholipid hydrolysis at the level of the recognition site, or the activity of phospholipase C itself. One obvious target that remains is the mechanism coupling...
receptors with phospholipase C. It now seems clear that one or more guanine nucleotide binding proteins, analogous to G_1 and G_2, are involved in the control of phosphoinositide hydrolysis (e.g. Berridge and Irvine, 1984) and an intriguing possibility for future investigation is that the effects of Li (perhaps via diacylglycerol-induced protein kinase C-mediated phosphorylation) could, in part, be on such a regulatory protein.

It is clear from the results presented that acute, chronic and withdrawal from Li treatment severely disrupts inositol phospholipid hydrolysis in the cortical slice preparation. We do not feel that these effects seen ex vivo simply reflect depletion of appropriate pools of phosphoinositides required for receptor activity (Berridge et al., 1982) as the effects are not reversed by inositol incubation. However, it still remains probable that in vivo the most likely site of action of Li is at inositol phosphatases. This then could induce indirectly persistent alteration of receptor-G-protein-phospholipase C coupling, perhaps via diacylglycerol and protein kinase C. Recent studies (Balla et al., 1984) have shown that Li suppresses angiotensin II (but not adrenocorticotropic hormone)-induced aldosterone production in adrenal glomerulosa cells. Because angiotensin also stimulates phosphoinositide hydrolysis in these cells, it was argued that Li's action may be exerted on inositol phosphatases. However, just as in the present study, incubation with inositol does not overcome the action of Li on adrenal cells (Balla et al., 1984).

In conclusion, the present studies have revealed that acute and chronic administration and withdrawal from Li severely disrupts cerebral phosphoinositide metabolism. These effects are not reversed by inositol incubation and show some selectivity for muscarinic receptors over alpha-1 adrenergceptor responses. It is tempting to suggest that these actions of Li underlie, in part, the therapeutic effects of this monovalent ion.

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References


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