Lithium is one of the most widely used drugs for treating bipolar (manic-depressive) disorder. Despite its efficacy, the molecular mechanism underlying its action has not been elucidated. One recent study has proposed that lithium inhibits glycogen synthase kinase-3 and thereby affects multiple cellular functions. Because glycogen synthase kinase-3 regulates the phosphorylation of tau (microtubule-binding protein that forms paired helical filaments in neurons of the Alzheimer’s disease brain), we hypothesized that lithium could affect tau phosphorylation by inhibiting glycogen synthase kinase-3. Using cultured human NT2N neurons, we demonstrated that lithium reduces the phosphorylation of tau, enhances the binding of tau to microtubules, and promotes microtubule assembly through direct and reversible inhibition of glycogen synthase kinase-3. These results provide new insights into how lithium mediates its effects in the central nervous system, and these findings could be exploited to develop a novel intervention for Alzheimer’s disease.

Paired helical filaments are the major building blocks of neurofibrillar lesions in Alzheimer’s disease brains. Paired helical filaments are composed of hyperphosphorylated central nervous system tau protein (1–3). Predominantly expressed in axons, tau is a group of microtubule-associated proteins that normally promote and stabilize the assembly of microtubules (4–6). Paired helical filament-tau differs from normal tau by its abnormal hyperphosphorylation, which results in the decreased (10) affinity of paired helical filament-tau for microtubules, coupled with the reduced amount of normal tau, may lead to the destabilization of microtubules and result in impairment of axonal transport, neuronal degeneration, and the aggregation of paired helical filaments. Therefore the hyperphosphorylation of tau is believed to be a key event in the pathogenesis of Alzheimer’s disease.

The phosphorylation of tau is regulated by kinases and phosphatases. We have shown previously that glycogen synthase kinase-3 (GSK-3),\(^1\) a serine/threonine protein kinase, plays an important role in the regulation of tau phosphorylation (9, 10). When overexpressed in cultured human neurons, GSK-3 induces an Alzheimer’s disease-like hyperphosphorylation of tau. When GSK-3 activity is down-regulated by insulin or insulin-like growth factor I, the phosphorylation state of tau is decreased (10).

GSK-3 was originally identified as a kinase that phosphorylates and inactivates glycogen synthase, which catalyzes a regulated step in insulin-mediated glycogen synthesis (11, 12). Insulin induces a phosphorylation-dependent down-regulation of GSK-3, which leads to the activation of glycogen synthase and therefore increased glycogen synthesis.

GSK-3 was later found to have many other cellular functions. For example, the homologs of GSK-3 play a central role in the development of diverse organisms including Drosophila, Dicystostelium, and Xenopus (13–18). During dorso-ventral axis formation in Xenopus embryos, inhibition of GSK-3 can lead to increased dorsal tissue (17). Interestingly, lithium, a drug that has been widely used for the treatment of bipolar (manic-depressive) disorder, induces similar effect on Xenopus development, i.e. causing an expansion of the dorsal mesoderm leading to duplication of the dorsal axis (19, 20). Indeed, a recent study showed that lithium directly inhibits GSK-3, suggesting that this may be a mechanism whereby lithium exerts its effect on cell fate determination as well as other cellular functions (21).

Additionally, lithium has been reported to activate glycogen synthase and stimulate glycogen synthesis in rat hepatocytes (22). This has also been proposed (21) to be a result of the inhibition of GSK-3 by lithium because the inhibition of GSK-3 abrogates the phosphorylation-dependent inactivation of glycogen synthase, which in turn increases glycogen synthesis.

Despite the remarkable efficacy of lithium in treating bipolar disorder, the molecular mechanisms underlying its therapeutic actions have not been fully elucidated. Previous studies have shown that lithium inhibits the phosphorylation of the mid-sized neurofilament subunit and alters cytoskeletal organization in growing chick sensory neurons (23, 24). These studies suggest that lithium may exert its effect on the central nervous system by affecting the neuronal cytoskeleton. As a neuronal microtubule-associated protein, tau is a substrate of GSK-3, and the phosphorylation of tau by GSK-3 could be directly inhibited by lithium and microtubule assembly thereby affected.

To test this hypothesis, we examined the effects of lithium on

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\(^1\) The abbreviations used are: GSK-3, glycogen synthase kinase-3; SFV, Semliki Forest virus; WT, wild type; DMEM-HG, Dulbecco’s modified Eagle’s medium with high glucose; Mes, 4-morpholineethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; Pipes, 1,4-piperazine-ethanesulfonic acid; MAb, monoclonal antibody.
lithium reduces tau phosphorylation by inhibition of GSK-3

EXPERIMENTAL PROCEDURES

Materials—[γ-32P]ATP and [35S]-labeled secondary antibodies were purchased from NEN Life Science Products and the Sambrook virus (SFV) gene expression system from Life Technologies, Inc. Taxol was obtained from Dr. V. Narayanan of NCI. Other chemicals were purchased from Sigma. GSK-3β S9A cDNA and the cDNA construct to make recombinant tau were obtained from Dr. M. Goedert. GSK-3β WT cDNA was from Drs. X. He and H. Varmus.

Cell Culture—To generate NT2N neurons, a human embryonal carcinoma cell line (NTera2/D1 or NT2) was grown and maintained as described (26). Briefly, NT2 cells were treated with retinoic acid for 5 weeks and then replated at reduced density. After 10 days, neuron-like NT2 cells were mechanically and enzymatically separated from the parent NT2 cells and replated at a density of 1 × 10^5/well on six-well plates previously coated with poly-l-lysine (10 μg/ml) and Matrigel. The NT2N neurons were maintained for up to 4–6 weeks in Dulbecco’s modified Eagle’s medium with high glucose (DMEM-HG), supplemented with 5% fetal bovine serum, penicillin/streptomycin, and mitotic inhibitors (1 μM cytosine arabinoside, 10 μM fluorodeoxyuridine, and 10 μM uridine). 2-4-week-old NT2N neurons were used for experiments, and each experiment was repeated at least three times.

Overexpression of GSK-3β in NT2N Neurons Using the SFV Gene Expression System—Two GSK-3β/SFV viral constructs were used. GSK-3β WT is the wild type GSK-3β; and GSK-3β S9A is a constitutively active form of GSK-3β, in which the serine 9 residue was mutated to alanine. A 10-amino-acid e-Myc tag (EQKLISETEDL) was introduced at the carboxyl terminus of both constructs expressing β-galactosidase (LacZ) was used as a control. To overexpress GSK-3β in NT2N neurons, cells were washed once with DMEM-HG without supplements, and viral stocks diluted in DMEM-HG (multiplicity of infection = 10) were applied to the cells. After 1 h, the medium containing viruses was replaced by DMEM-HG with 5% fetal bovine serum. The infected NT2N neurons were then incubated overnight (18 h). Cells were either used for lithium or calyculin A in infected cells, were treated with 10 mM LiCl for 2 h or with 0–50 nM calyculin A for 30 min after overnight infection.

Treatment of NT2N Neurons—To determine the effects of lithium on tau phosphorylation, NT2N neurons were treated with 0–25 mM LiCl for 2 h before being harvested. To examine the phosphorylation sites in tau, NT2N cells were either treated with 10 mM LiCl or treated with 10 mM LiCl with GSK-3β/SFV overnight or treated with 10 mM LiCl for 2 h. To test whether calyculin A blocks the effects of lithium on tau phosphorylation, cells were treated with or without 10 mM LiCl for 30 min in the presence of 0–50 nM calyculin A. To determine whether lithium reversibly decreases tau phosphorylation, NT2N neurons were treated with 10 mM LiCl for 2 h and subsequently washed three times with lithium-free medium and incubated for another 0.5 or 2 h. As controls, cells were treated with lithium for 2, 2.5, and 4 h without wash.

Immunoblot Analysis of Tau Phosphorylation—NT2N neurons were washed once with phosphate-buffered saline and lysed in ice-cold high salt RAB buffer (0.1 M NaCl, 0.5 mM MgSO4, 1 mM EGTA, 2 mM dithiothreitol, and 0.75 M NaCl, pH 6.8) supplemented with 0.1% Triton X-100 and a mixture of protease inhibitors. Cell lysates were incubated on ice for 10 min, sonicated, and centrifuged for 20 min at 50,000 × g, 4 °C. The supernatants were collected and protein concentrations determined using the bichoncinic acid method (Pierce). An equal amount of total protein was resolved on 10% SDS-PAGE. Immunoblotting was performed with a panel of site and phosphorylation-sensitive anti-tau antibodies. The blots were developed by the enhanced chemiluminescence or 3,3’-diaminobenzidine method. Alternatively, for the quantitation of the relative levels of tau protein, 125I-labeled goat anti-mouse IgG was used as secondary antibody, and the blots were exposed to PhosphorImager plates. 10 μg of total protein was loaded for the detection of tau on immunoblots using the antibodies T14/46, 25 μg for the antibodies T1, PHF1, T3P, and PHF13, and 50 μg for the antibodies AT8, AT270, PHF6, and 12E8.

RESULTS

Lithium Reduces Tau Phosphorylation in NT2N Neurons in a Dose-Dependent Manner—To examine the effects of lithium on tau phosphorylation in neuronal cells, NT2N neurons, which endogenously express tau, were treated with 0, 0.5, 1, 5, 10, or
immunoreactivity and are plotted in Fig. 1.

Increased, indicating a reduction of tau phosphorylation. This was accompanied by an increased electrophoretic mobility of tau, detected by T14/46. Panel B, the T1 and PHF1 immunoreactivities were quantitated, normalized to the levels of T14/46, and plotted. T1 increased and PHF1 decreased in a dose-dependent manner, indicating a reduction of tau phosphorylation (n = 3).

25 mM LiCl. After 2 h of treatment, cells were lysed and an equal amount of total protein was resolved on SDS-PAGE for immunoblot analysis with a panel of anti-tau antibodies. As shown in Fig. 1, with increasing concentrations of lithium, T1 immunoreactivity increased and PHF1 immunoreactivity decreased, indicating a reduction of tau phosphorylation. This was accompanied by an increase in electrophoretic mobility of tau, detected by T14/46. The quantitative data on T1 and PHF1 immunoreactivities were normalized to the levels of T14/46 immunoreactivity and are plotted in Fig. 1B. These results demonstrate that lithium decreases the phosphorylation of tau in a dose-dependent manner in the NT2N neurons.

**Lithium Reduces Tau Phosphorylation by Direct Inhibition of GSK-3 but Not through Phosphorylation-dependent Down-regulation of GSK-3**—To test whether the effect of lithium on tau phosphorylation is a result of the inhibition of GSK-3, we first demonstrated that lithium directly inhibits GSK-3 in NT2N neurons. We overexpressed WT and a constitutively active mutant (S9A) form of GSK-3β in NT2N neurons using the SFV gene expression system. Both GSK-3β/SFV constructs have a c-Myc tag at their COOH termini, and the overexpressed protein can be immunoprecipitated by a polyclonal anti-c-Myc antisera. In Fig. 2A, overexpressed GSK-3β S9A was immunoprecipitated, and a GSK-3 kinase assay was performed in the presence of 0–10 mM LiCl. GSK-3 activities were assayed by monitoring ^32P incorporation into human recombinant tau. The amount of GSK-3 present in each reaction was determined by immunoblotting with a MAb to GSK-3 and used to normalize the relative GSK-3 activities. As shown in Fig. 2A, lithium directly inhibited the activity of GSK-3β S9A with an IC_{50} of 3 mM. Similar results were obtained when GSK-3β WT was overexpressed in NT2N neurons (data not shown). Other monovalent cations (Na+, K+, Cs+, and NH_4+) did not have a similar effect (data not shown).

We also examined the in vivo effects of lithium on tau phosphorylation in NT2N neurons that overexpressed GSK-3β WT or GSK-3β S9A. As shown in Fig. 2B, overexpression of both GSK-3 constructs increased tau phosphorylation because T1 (dephosphorylated) immunoreactivity decreased and PHF1 (phosphorylated) immunoreactivity increased compared with control (LacZ). When treated with LiCl, the increased tau phosphorylation induced by overexpression of both GSK-3β WT and GSK-3β S9A was abrogated (WT+Li and S9A+Li), as the changes in T1 and PHF1 immunoreactivities were reversed (Fig. 2B).

These experiments indicate that lithium reduces tau phosphorylation by direct inhibition of GSK-3. The serine 9 residue in GSK-3β is required for the phosphorylation-dependent inactivation, and mutating this residue to alanine in GSK-3β S9A abolishes the down-regulation of its activity by insulin and other growth factors (10, 39–41). Because the effects of lithium on GSK-3 and tau phosphorylation are not affected by this mutation, the phosphorylation of serine 9 must not be involved in the inhibition of GSK-3 by lithium.

**Lithium and GSK-3 Have Opposite Effects on the Same Phosphorylation Sites of Tau in NT2N Neurons**—To examine the phosphorylation sites affected by lithium and GSK-3, we used a panel of site-specific phosphorylation-sensitive anti-tau antibodies. The results of a typical experiment are shown in Fig. 3A. The immunoreactivities of phosphorylated and nonphosphorylated tau were quantitated and normalized to the levels of T14/46 (total tau), as shown in Fig. 3B. With the overexpression of GSK-3, T1 (dephosphorylated residues 189–207) immunoreactivity decreased, whereas PHF1 (phosphorylated serine 396/404), T3P (phosphorylated serine 396), PHF13 (phosphorylated serine 396), AT8 (phosphorylated serine 202 and threonine 205), AT270 (phosphorylated threonine 181), and PHF6 (phosphorylated threonine 231) immunoreactivities increased. Lithium treatment had the opposite effect on the immunoreactivities of the same antibodies (Fig. 3, A and B). However, phosphorylation...
A stronger decrease in T1 immunoreactivity in the soluble fraction (bound to microtubules) was determined by immunoblotting analysis with AT14/46 and T1. As shown earlier, lithium inhibits the phosphorylation of tau after 2–4 h of incubation as indicated by an increase in T1 immunoreactivity. This effect was reversed rapidly by the removal of lithium, since T1 immunoreactivity resumed to its control levels (Fig. 5A). We also demonstrated in an in vitro kinase assay that the inhibition of GSK-3 activity by lithium is reversible (Fig. 5B). When the GSK-3 kinase assay was performed in the presence of LiCl for 20 min (LiCl+, Wash−), GSK-3 activity (represented by 32P incorporation into tau) was strongly reduced compared with the control (no lithium incubation, LiCl−, Wash−). When cells were washed extensively after 20 min of lithium incubation, GSK-3 activity was recovered (LiCl+, Wash+) (Fig. 5B). These results indicate that lithium reduces tau phosphorylation in the NT2N neurons by direct and reversible inhibition of GSK-3 and are in agreement with a recent in vitro study showing that lithium reversibly inhibits GSK-3 (47).

Lithium Increases Tau Binding to Microtubules and Affects Microtubule Assembly—The phosphorylation status of tau affects its affinity for microtubules, and this also may alter the stability of microtubules (7, 8). We therefore examined whether lithium treatment affects the microtubule binding of tau and microtubule assembly in NT2N neurons. After overnight treatment by 10 mM LiCl, NT2N neurons were lysed, and soluble (S) and pelletable cytoskeletal (P) fractions were obtained from the cell lysates. The tau protein in the cytoskeletal fraction (bound to microtubules) and in the soluble fraction (unbound to microtubules) was determined by immunoblotting analysis with AT14/46 (Fig. 6A). Shown in Fig. 6B, the ratio of microtubule-bound tau to soluble tau was increased significantly by lithium treatment. This promotion of tau binding to microtubules is likely a result of the lithium-induced decrease in tau phosphorylation.

We also examined the effects of lithium on microtubule assembly by immunoblotting with antibodies against α-tubulin, Tyr-tubulin, and Glu-tubulin. The assembly of microtubules by tubulin polymerization is a dynamic equilibrium (44). When α-tubulin is incorporated into microtubules, it undergoes post-translational modifications such as detyrosination to generate the more stable Glu-microtubules (45, 46). However, when Glu-microtubules are depolymerized, tyrosine residues are rapidly added back to generate Tyr-tubulin. Therefore, as shown in Fig. 6A (Control), in the normal postmitotic NT2N neurons, more Tyr-tubulins are found in the soluble fraction (S), whereas 90% of Glu-tubulins are present as Glu-microtubule polymers in the cytoskeletal fraction (P). The overall total α-tubulin subunits are also distributed with 70% as microtubule polymers (Fig. 6A, Control). After lithium treatment, the amounts of soluble α-tubulin and Tyr-tubulin decreased, but the α-tubulin and Tyr-microtubule polymers remained un-

The Effects of Lithium on GSK-3 and Tau Phosphorylation Are Reversible—To study further the mechanism of the inhibition of GSK-3 by lithium, we determined whether the effects of lithium on tau phosphorylation and GSK-3 are reversible. NT2N neurons were treated with 10 mM LiCl for 2 h and subsequently washed and incubated with lithium-free medium for another 0.5 or 2 h. As controls, the cells were treated with lithium for 2, 2.5, or 4 h. Tau phosphorylation was detected by immunoblot analysis with T14/46 and T1. As shown earlier, lithium inhibits the phosphorylation of tau after 2–4 h of incubation as indicated by an increase in T1 immunoreactivity. This effect was reversed rapidly by the removal of lithium, since T1 immunoreactivity resumed to its control levels (Fig. 5A). We also demonstrated in an in vitro kinase assay that the inhibition of GSK-3 activity by lithium is reversible (Fig. 5B). When the GSK-3 kinase assay was performed in the presence of LiCl for 20 min (LiCl+, Wash−), GSK-3 activity (represented by 32P incorporation into tau) was strongly reduced compared with the control (no lithium incubation, LiCl−, Wash−). When cells were washed extensively after 20 min of lithium incubation, GSK-3 activity was recovered (LiCl+, Wash+) (Fig. 5B). These results indicate that lithium reduces tau phosphorylation in the NT2N neurons by direct and reversible inhibition of GSK-3 and are in agreement with a recent in vitro study showing that lithium reversibly inhibits GSK-3 (47).

Lithium Reduces Tau Phosphorylation by Inhibition of GSK-3

We also examined the effects of lithium on microtubule assembly by immunoblotting with antibodies against α-tubulin, Tyr-tubulin, and Glu-tubulin. The assembly of microtubules by tubulin polymerization is a dynamic equilibrium (44). When α-tubulin is incorporated into microtubules, it undergoes post-translational modifications such as detyrosination to generate the more stable Glu-microtubules (45, 46). However, when Glu-microtubules are depolymerized, tyrosine residues are rapidly added back to generate Tyr-tubulin. Therefore, as shown in Fig. 6A (Control), in the normal postmitotic NT2N neurons, more Tyr-tubulins are found in the soluble fraction (S), whereas 90% of Glu-tubulins are present as Glu-microtubule polymers in the cytoskeletal fraction (P). The overall total α-tubulin subunits are also distributed with 70% as microtubule polymers (Fig. 6A, Control). After lithium treatment, the amounts of soluble α-tubulin and Tyr-tubulin decreased, but the α-tubulin and Tyr-microtubule polymers remained un-
Fig. 4. The lithium-induced decrease in tau phosphorylation is not blocked by the inhibition of phosphatases. Panel A, NT2N neurons were treated for 30 min with or without 10 mM LiCl in the presence of 0–50 nM calyculin A. T1 immunoreactivity was normalized to T14/46 and plotted (No LiCl, No Calyculin A = 1). Calyculin A alone induced an increase of tau phosphorylation (decrease in T1), but it did not block the dephosphorylation of tau induced by lithium (increase in T1). Panel B, NT2N neurons were infected with GSK-3/βSFV or LacZ/SFV overnight and treated with 0–50 nM calyculin A for 30 min. The increase of tau phosphorylation induced by overexpression of GSK-3 was potentiated by calyculin A (LacZ, No Calyculin A = 1).

Fig. 5. The effects of lithium on GSK-3 and tau phosphorylation are reversible. Panel A, NT2N neurons were treated with 10 mM LiCl for 2 h and subsequently washed with lithium-free medium and incubated for another 0.5 or 2 h. As controls, cells were treated with lithium without wash for 2, 2.5, or 4 h. Lithium-induced increase in T1 immunoreactivity was reversed rapidly after removal of lithium. Panel B, overexpressed GSK-3 was immunoprecipitated, incubated with or without 10 mM LiCl for 20 min (LiCl+ or -). GSK-3 kinase assay was performed in the presence of lithium (Wash+) or after lithium was removed by the wash (Wash-). Lithium inhibited GSK-3-mediated 32P incorporation into tau, but the effect was reversed by the removal of lithium.

Fig. 6. Lithium enhances tau binding to microtubules and affects microtubule assembly. NT2N neurons were treated with or without 10 mM LiCl overnight. Cell lysates were separated into cytoskeletal fraction (P) and soluble fraction (S). Panel A, tau, α-tubulin (α-tub), Tyr-tubulin (Tyr-tub), and Glu-tubulin (Glu-tub) present in each fraction were determined by immunoblot analysis. Panel B, the ratios of tau, α-tubulin, Tyr-tubulin, and Glu-tubulin in the cytoskeletal fraction versus those in the soluble fraction (P/S) were determined after quantitation and plotted. Lithium treatment significantly increased tau binding to microtubules and shifted the microtubule assembly equilibrium toward polymerization (n = 4, *p < 0.01). The distribution of Glu-tubulin was not altered.

DISCUSSION

Lithium has been used widely for decades to treat bipolar (manic-depressive) disorder. Despite its remarkable efficacy, the molecular mechanisms underlying its therapeutic actions have not been elucidated fully. It has been proposed that the inhibition of GSK-3 by lithium could explain its mechanism of action (21), but this has not been demonstrated in nerve cells. In the current study, we demonstrated in neuronal NT2N cells that lithium treatment prevents the phosphorylation of tau through direct and reversible inhibition of GSK-3. This provides another clue as to how lithium elicits its effects on the central nervous system.

One other recent study conducted in non-neuronal COS1 cells transiently transfected with GSK-3 and tau has shown that lithium treatment resulted in a decrease in tau immunoreactivity detected by the MAb AT8 (phosphorylated serine 202 and threonine 205) (47). However, this study did not examine other phosphorylation sites of tau, and the non-neuronal nature of COS1 cells limits its relevance to the neurons of the central nervous system. The NT2N neuronal culture system provides another clue as to how lithium elicits its effects on the central nervous system.

We showed previously that insulin and insulin-like growth factor I have effects similar to those of lithium on tau phosphorylation and the binding of tau to microtubules in NT2N neurons (10). These effects are also mediated through the inhibi-
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2 neurofibrillary lesions correlate reliably with the degree of dementia in Alzheimer’s disease patients (59–61). We have shown previously that overexpression of GSK-3 can induce an Alzheimer’s disease-like phosphorylation of tau in neurons (10). It is likely that GSK-3 plays an important role in the normal and abnormal regulation of tau phosphorylation. By inhibiting GSK-3 and causing a decrease in tau phosphorylation, it is possible that lithium could be used as a therapeutic agent to block the hyperphosphorylation of tau and slow the progression of Alzheimer’s disease. Future studies on the exact mechanism of this inhibition will facilitate the design of new GSK-3 inhibitors that could be used for the treatment of Alzheimer’s disease and bipolar disease.

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15. In all cases, lithium treatment blocks the initial neurite outgrowth. But, if neurites are allowed to begin extending before exposure to lithium, further outgrowth ceases, and neurites appear frozen at the length achieved before treatment (24, 48). Moreover, lithium treatment does not induce cell death, and the cytoskeletal alterations are completely reversible (24). Lithium has also been reported to induce rapid degradation of newly synthesized tubulin in neurons, resulting in a decrease in the fraction of tubulin present in the unassembled form. However the assembled microtubules are not affected by this degradation. The dynamic equilibrium of tubulin polymerization is actually shifted toward assembly, since the proportion of tubulin present in the assembled microtubule fraction is increased (49). Our results are consistent with these findings, and they suggest that the decreased tau phosphorylation and increased tau binding to microtubules may contribute to this stabilizing effect of lithium on assembled microtubules. Because neurite elongation is primarily a function of microtubule dynamics, this microtubule stabilizing effect, coupled with the increased degradation of nascent tubulin, may halt further neurite growth and in the meantime preserve the extant neurites.

One reason why the molecular mechanisms underlying the therapeutic effects of lithium have been difficult to characterize is that lithium could affect multiple cellular targets. It has been proposed that lithium targets key components of signal transduction pathways (50). One of the prevailing hypotheses is that lithium inhibits the inositol depletion hypothesis, which is based on the observation that lithium inhibits inositol monophosphatase (51, 52). As a result of this inhibition, the cellular source of inositol could be depleted, and cells could therefore become unable to generate inositol 1,4,5-trisphosphate. Thus, the inositol 1,4,5-trisphosphate-dependent responses to extracellular stimulation would be blocked. However, this hypothesis cannot fully explain the effect of lithium on glycogen synthesis or on cell fate and death, and the cytoskeletal alterations are completely reversible (24). Lithium has also been reported to induce rapid degradation of newly synthesized tubulin in neurons, resulting in a decrease in the fraction of tubulin present in the unassembled form. However the assembled microtubules are not affected by this degradation. The dynamic equilibrium of tubulin polymerization is actually shifted toward assembly, since the proportion of tubulin present in the assembled microtubule fraction is increased (49). Our results are consistent with these findings, and they suggest that the decreased tau phosphorylation and increased tau binding to microtubules may contribute to this stabilizing effect of lithium on assembled microtubules. Because neurite elongation is primarily a function of microtubule dynamics, this microtubule stabilizing effect, coupled with the increased degradation of nascent tubulin, may halt further neurite growth and in the meantime preserve the extant neurites.

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