STABILIZATION OF MICROTUBULES BY LITHIUM ION

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SUMMARY: Lithium ion, in concentrations of 0.2 - 1.0 mM, promotes tubulin polymerization, provided small concentrations of Mg^{++} ion are present. This effect of lithium on polymerization, at suboptimal concentration of magnesium, is additive for the two metal ions. Both Li^{+} and Mg^{++} protect microtubules against the depolymerizing effects of colchicine or vinblastine.

INTRODUCTION

In the thyroid gland and kidney Li^{+} decreases or abolishes a hormone-stimulated secretion or urine concentration (1-4). Although in both tissues adenylate cyclase is inhibited by Li^{+} (5,6), it has been shown that Li^{+} also blocks the cAMP-mediated translocation of thyroid hormone or water (2,7). These effects of Li^{+} resemble those produced by colchicine in these tissues, and suggest an action of Li^{+} on microtubules or tubulin. Initial attempts to see whether Li^{+} depolymerized microtubules, as does colchicine, were unsuccessful. Neurite integrety is thought to be maintained by microtubules. Lithium chloride in concentrations up to 10 mM had no discernable effect on the number or nature of neurites extending from neuroblastoma cells (8). However, these experiments led to the discovery by Reiser *et al.* (9), that 10 mM lithium delayed the retraction of processes in glioma cells or glioma-neuroblastoma hybrids caused by colcemid.

Since we had shown that Li^{+} ion, at concentrations that block secretion of thyroid hormone, has no effect on colchicine binding to tubulin (8), we have tested the alternative hypothesis, that Li^{+} might block secretory phenomena by excessive stabilization of microtubules. Agents such as D_2O or hexylene glycol, that induce such stabilization, are known to interfere in

many (12-15) but not all (16) secretory processes. In the present study we show that Li^+ can indeed stabilize *in vitro* polymerized microtubules against the disaggregating effects of colchicine.

MATERIALS AND METHODS

For polymerization experiments, tubulin was purified from rat brain extracts by three cycles of polymerization and depolymerization, according to the method of Shelanski *et al.* (17) using buffer containing 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES) (pH 6.4), 1 mM ethylene glycol-bis (β-aminoethyl ether)-N,N'-tetraacetic acid (EGTA), 1 mM GTP, and 0.5 mM $MgCl_2$. After the final cycle of polymerization of rat brain tubulin, the protein was depolymerized in the same polymerization buffer but omitting $MgCl_2$.

The kinetics of tubule assembly were studied by turbidimetric measurements at 400 nm as described by Gaskin *et al.* (18) in a temperature-controlled chamber of a Cary spectrophotometer (Model 14) at 37°. Colchicine binding was determined by the DEAE-filter paper method (13).

The concentration of protein was determined by the method of Lowry *et al.* (19), with crystalline bovine albumin as a standard.

RESULTS AND DISCUSSION

It was first necessary to investigate the role of Mg^{++} ion in tubulin polymerization as carried out under our conditions. The importance of this had already been pointed out by Olmsted and Borisy (20). Added Mg^{++} increased both the *rate* and the *extent* of polymerization of rat brain tubulin (Fig. 1A). Although not shown in this figure, the full extent of polymerization was not attained in the absence of added $MgCl_2$ even after one hour of incubation. Nevertheless, substantial polymerization occurred in the absence of *added* $MgCl_2$. That this was probably due to Mg^{++} in the tubulin preparation is shown in Fig. 1B wherein EDTA completely abolished polymerization. This could be reversed by addition of Mg^{++}. Since EGTA did not abolish polymerization, it seems likely that the cation involved is Mg^{++}. Large concentrations of $MgCl_2$ inhibited polymerization. Addition of LiCl in the presence of EDTA did not result in any polymerization.

As shown in Fig. 1C, low concentrations of LiCl increased the rate of tubulin polymerization but not its final extent. Apparently, the Mg^{++} concentration in the tubulin preparation was enough to permit a partial

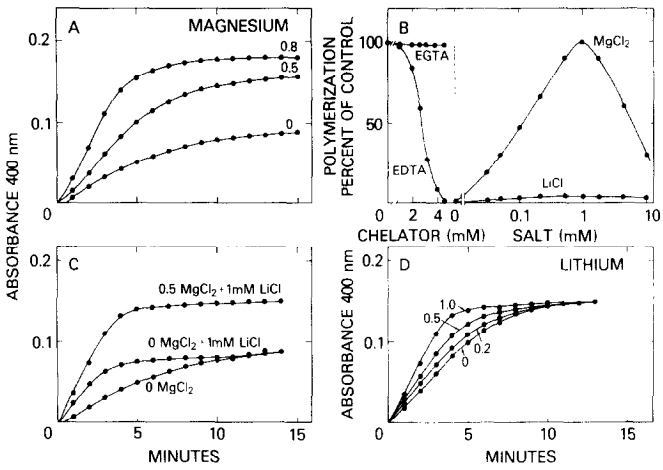

Figure 1. Effect of metal ions on tubulin polymerization.
A) Effect of magnesium concentration on the time course of tubulin polymerization. The added magnesium concentration in mM is indicated above the appropriate curve. The tubulin concentration was 2.4 mg/ml.
B) (Left panel): Effect of EDTA and EGTA on tubulin polymerization. Aliquots of tubulin 3.8 mg/ml in polymerization buffer were mixed with different concentrations of chelator immediately before incubation at 37°. Data are expressed as the maximum plateau level reached without chelator as 100% (Right panel): The samples were made to 5 mM EDTA, and polymerization followed at 37° in the presence of various added concentrations of $MgCl_2$ and LiCl.
C) The additive effect of magnesium and lithium on the time course of tubulin polymerization. The concentration of added salts is indicated in mM. The tubulin concentration was 2.6 mg/ml.
D) Effect of lithium concentration on the time course of tubulin polymerization. The added lithium concentration, in mM, is indicated on the appropriate curve. The tubulin concentration was 2.6 mg/ml.

polymerization by LiCl. The results of Fig. 1B also suggested that some Mg^{++} had to be present to demonstrate a Li^{+} effect on tubulin polymerization. The Li^{+} effect appeared to be maximal at a Mg^{++} concentration of ∿0.5 mM. Increasing Li^{+} concentrations in the presence of suboptimal amounts of Mg^{++} ion led to increasing rates of polymerization over a concentration range of 0.2 to 2.0 mM LiCl (Fig. 1D). These concentrations are well within the tissue level attained *in vivo* (1-4).

Large concentrations of LiCl inhibited polymerization of tubulin as shown in Fig. 2A. This was similar to the effect of excess Mg^{++} ion shown in Fig. 1B. The effect of Li^{+} ion was to displace the maximum of the rate toward lower Mg^{++}

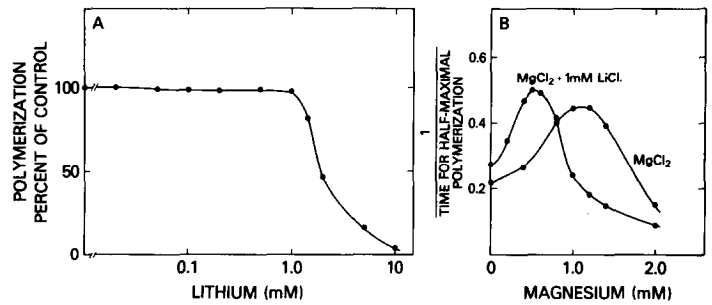

Figure 2. Effects of metal ions on the extent and rate of polymerization. A) Aliquots of tubulin (2.6 mg/ml) in polymerization buffer omitting $MgCl_2$ were mixed with different concentrations of LiCl immediately before incubation at 37°. Data are expressed as the maximal plateau level reached without lithium as 100%.

B) Aliquots of tubulin (2.4 mg/ml) in polymerization buffer omitting $MgCl_2$ were mixed with different concentrations of added $MgCl_2$ or both $MgCl_2$ and LiCl immediately before incubation at 37°. Data are expressed as the reciprocal of time required for half-maximal polymerization in each sample against the added magnesium concentration.

concentration, suggesting that these two cations acted in an additive manner (Fig. 2B).

Colchicine is well known for its ability to disrupt microtubules in many cells and this property can be demonstrated with in vitro polymerized microtubules (21,22). We therefore tested the possibility that the aggregating effects of LiCl on tubulin might be reflected as a stabilization of microtubules against the effects of colchicine. As shown in Fig. 3A, addition of Li^+ to microtubule suspensions did indeed protect these structures against the disaggregating effects of colchicine. With 2 mM LiCl this amounted to a 2.5-3 fold increase in the concentration of colchicine required to depolymerize microtubules by 50%.

We have recently shown (23) that tubulin contains two binding sites for vinblastine: one of high affinity and one of low affinity. These sites correspond, respectively, to the disaggregating and aggregating properties of this alkaloid. Although these sites differ from the colchicine binding site (23,24), Li^+ was also able to protect by a factor of $\sim$2.5 against the disag-

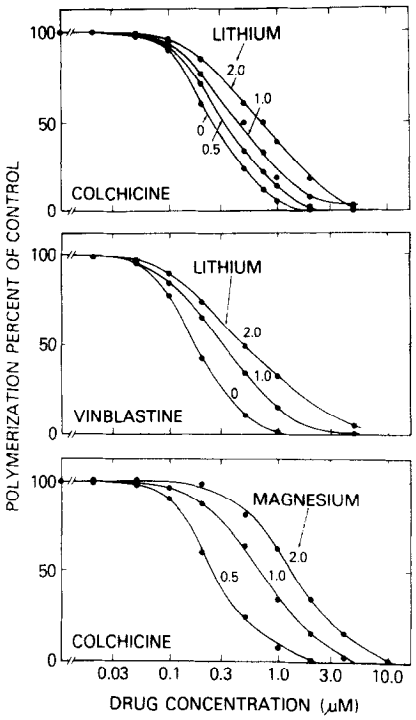

Figure 3. Effect of metal-ions on antimitotic drug-induced inhibition of tubulin polymerization. Aliquots of tubulin (2.4 mg/ml) in polymerization buffer omitting $MgCl_2$ were mixed with different concentrations of drugs and metal ions immediately before incubation at 37°. The metal ion concentrations are mM. Note, at 2 mM, both lithium (Fig. 2A) and magnesium (1B) inhibit the extent of polymerization but still enhance the stability of microtubules toward colchicine and vinblastine. In all cases data are expressed as the maximal plateau level reached without drug but with appropriate metal ion concentration as 100%.

gregation of microtubules produced by low concentrations of vinblastine (Fig. 3B).

Protection against colchicine could also be elicited by addition of $MgCl_2$ to the solutions. Total Mg^{++} was approximately twice as potent as Li^{+} (Fig. 3C). Although microtubules are characteristically depolymerized in the cold (11,20,21), Li^{+} had no effect on this type of depolymerization (data not shown).

It was possible that lithium ion merely blocked colchicine binding to tubulin. That this was not the case could be shown by direct binding studies using [^{3}H]-labeled colchicine. Colchicine binding activity was determined by

a DEAE filter disc method (13). Samples were incubated in 50 μM [^{3}H]colchicine with and without LiCl for 1 hour at 37°. The moles of colchicine bound per mole of tubulin were 0.53, 0.54 and 0.55 for controls, 1 mM LiCl, and 100 mM LiCl, respectively. Preincubation with LiCl for 30 min did not alter the above findings. A similar lack of Li^+ on colchicine binding has been reported previously (8,25). It appears probable, therefore, that the effect of Li^+ ion is not directly mediated by an action on the colchicine-binding site.

We had shown previously (5) that a probable site for the action of Li^+ on adenylate cyclase was by interaction with a Mg^{++}-requiring site on that enzyme. This probably occurred on the basis of the similarity in ionic radii of the two ions (0.60 Å for Li^+ and 0.65 Å for Mg^{++}). Since Mg^{++} has profound effects on the state of aggregation of tubulin (26), since Li^+ and Mg^{++} are additive over a limited concentration range in promoting polymerization (Fig. 2B), and since higher Mg^{++} concentrations abolish the Li^+ effect (Fig. 2B), it seems probable that considerations of ion size apply to tubulin stabilization as well. However, other factors also play a role, since Ga^{+++}, with an ionic radius of 0.62 Å, had no effect on tubulin polymerization (data not shown). Whatever the mechanism, Li^+ apparently stabilizes microtubules through some effect related to Mg^{++} that influences the equilibrium: tubulin $\rightleftharpoons$ microtubules in favor of the polymerized state and protects against drug-induced reversal by an action that does not involve displacement of the drug from their binding sites. If the *in vivo* equilibrium involves effects resembling those produced by antimitotic drugs or ions *in vitro*, then Li^+ can influence this equilibrium and hence the stability of microtubules.

Synaptic transmission may be viewed as a secretory phenomenon in which neurotransmitter is released by rapid exocytosis into the synaptic cleft upon stimulation (27,28). Moreover, synaptosomes are filled with microtubules that are associated with synaptic vesicles (29), and membrane-bound tubulin can be demonstrated in membranes derived from synaptosomes (30,31). Colchicine

and vinblastine inhibit the release of stored catecholamines, dopamine-β-hydroxylase or serotonin from various neural and related tissues (15,27), and 2.4 mM LiCl decreases the stimulated release of norepinephrine and serotonin from brain slices (32). There are thus some striking analogies between events at the synapse and the secretion of various hormones. While other effects on neurotransmitter turnover are in no way ruled out, the results of the present study suggest that one possible mode of action of Li^+ ion is to alter neurotransmitter or hormone release through increased stabilization of microtubules.

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