Is Competition between Li^+ and Mg^{2+} the Underlying Theme in the Proposed Mechanisms for the Pharmacological Action of Lithium Salts in Bipolar Disorder?

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Received November 28, 2005

ABSTRACT

Lithium salts have been in use for the treatment of bipolar disorder for more than 50 years, but their pharmacological mode of action remains a matter of conjecture. Li^+ and Mg^{2+} share many physicochemical properties. Not surprisingly, many reported cellular targets for Li^+ action involve Mg^{2+}-activated enzymes, which are inhibited by Li^+. In this Account, we describe results from our and other laboratories that suggest that a competition mechanism between Li^+ and Mg^{2+} ions for Mg^{2+}-binding sites in cellular components is the underlying theme in putative mechanisms of Li^+ action.

Introduction

Bipolar disorder, formerly known as manic depression, is a serious mental illness characterized by disabling mood swings. It has an incidence rate of greater than one percent in the population and can result in significant loss of productivity and, occasionally, in patient suicide. Lithium, mostly in the form of carbonate and citrate salts, remains the "gold standard" for the treatment of acute episodes and the prevention of relapses in patients suffering from bipolar illness.[1] Lithium salts have also been proposed for or used in the treatment of a variety of other psychiatric and nonpsychiatric conditions, including depression, ischemic stroke, and viral infections.[2,3] Unlike most drugs, the doses used in lithium treatment (0.5−1.2 mM) are not far from toxic levels (≥2.0 mM). Therefore, a clearer understanding of the pharmacological mode for lithium action at the protein and cellular levels may provide leads for the development of less toxic drugs and to new insights into this devastating illness.

Li^+ inhibits several Mg^{2+}-activated enzymes involved in signal transduction pathways,[4−8] such as glycogen synthase kinase-3β, inositol monophosphatase, inositol polyphosphatase, and adenylyl cyclase, as well as guanine nucleotide-binding proteins (Figure 1). Li^+ also modulates the activity of other Mg^{2+}-dependent enzymes, such as pyruvate kinase, thus regulating several metabolic pathways.[2,9] Because Li^+ has an ionic radius similar to that of Mg^{2+}, these two ions exhibit similar physicochemical properties. Hence, it is plausible for the competition between these two ions for Mg^{2+}-binding sites in biomolecules to be the underlying theme in most, if not all, putative mechanisms for Li^+ action. In this Account, we describe some of the spectroscopic, kinetic, and structural evidence obtained by our and other laboratories supporting the hypothesis that Li^+/Mg^{2+} competition for bound Mg^{2+} occurs in intact cells, in Mg^{2+}-activated proteins, and for the phosphate groups of second messengers and substrates of signaling macromolecules.

Inorganic Chemistry of Li^+

There is a similarity, in some aspects of their chemistry, between lithium (the first member of group IA) and magnesium (the second element in group IIA).[10] This "diagonal relationship" is a consequence of their similar electronegativities and Pauling ionic radii (r) of their cations (0.60 Å for Li^+ and 0.65 Å for Mg^{2+}). Because of the low polarizabilities of both cations, their binding to hard donor atoms is generally well-described by the ionic model. Despite their similar radii, the different ionic charges (z) lead to chemical differences, such as their preferred coordination numbers (4 versus 6), geometries (tetrahedral versus octahedral), ligand preferences, and binding affinities. The polarizing power (z/r^2) of Mg^{2+} (4.73) is almost double of Li^+ (2.78), increasing the degree of covalency for binding of Mg^{2+} to softer donor atoms such as nitrogen. In aqueous solution, the hydration enthalpy and entropy values for all pretransition-metal cations are accurately determined by (z^2/r_{eff}), where the effective radius is $r_{eff} = r + 0.8$ Å for cations and $r_{eff} = r$ for anions. The similar r values for Li^+ and Mg^{2+} and larger z of the latter result in larger hydrated radii (about 3.3 and 4.6 Å, respectively) and much larger negative hydration enthalpy and entropy values for Mg^{2+} relative to Li^+. The water ligands are highly labile in all hydrated pretransition-metal cations, with exchange rates of 4×10^8 and 1.5×10^7 s^{-1} for $Li^+_{(aq)}$ and $Mg^{2+}_{(aq)}$, respectively.[11]

The chemical similarities of Li^+ and Mg^{2+} appear in the stabilities of their complexes in aqueous solution. The free energy of complexation, resulting from the replacement

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10.1021/ar030197a CCC: $33.50
Published on Web 03/11/2006

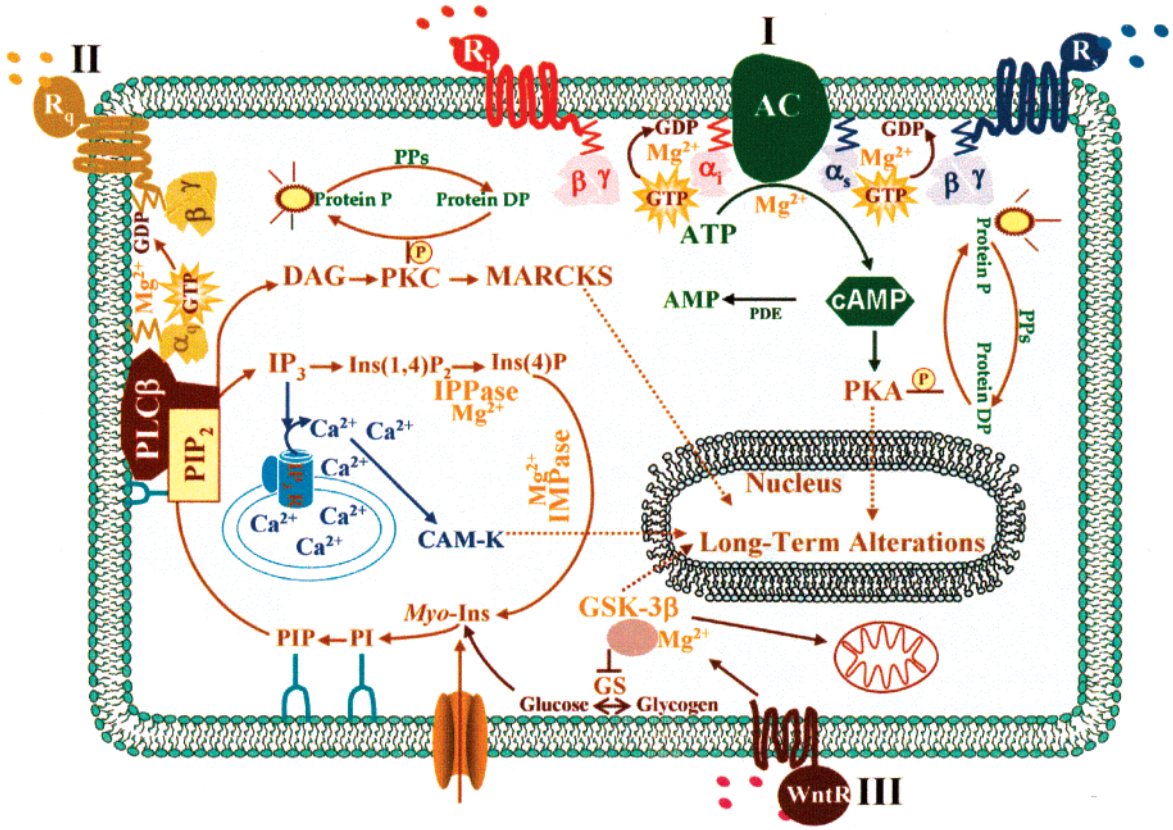

FIGURE 1. Adenylyl cyclase (AC), inositol phosphatases (IMPase and IPPase), guanine nucleotide-binding (G) proteins comprised of α, β, and γ subunits, and glycogen synthase kinase-3β (GSK-3β) are the main targets for Li^+ action. (I) Membrane receptors (R_s and R_i), coupled to G proteins (G_s and G_i), activate or inhibit AC-modulating cyclic AMP (cAMP) production, which, in turn, regulates protein kinase A (PKA) that is able to phosphorylate many substrate proteins. Protein phosphatases (PPs) convert the phosphoproteins back to the dephospho forms, whereas phosphodiesterase (PDE) degrades cAMP to AMP. (II) Activation of G_q induces phospholipase C (PLCβ)-mediated hydrolysis of phosphoinositide-4,5-bisphosphate (PIP_2) to diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP_3). DAG activates protein kinase C (PKC), an enzyme that phosphorylates many other proteins, including the myristoylated alanine-rich C kinase substrate (MARCKS). IP_3-induced release of intracellular calcium initiates the activation of calmodulin (CAM) and calmodulin-dependent protein kinase (CAM-K). IP_3 is recycled back to PIP_2 via steps catalyzed by IPPase and IMPase. (III) Binding of the wingless signal to its receptor (WntR) regulates GSK-3β, which affects cytoskeletal proteins and glycogen synthase (GS) activity.

of the coordinated water molecules by ligand-coordinating atoms L, can be described through the ionic model by $\Delta G_{comp} = \Delta G_{ML} - \Delta G_{hyd} = -B/(r_{eff} + \alpha) + A/r_{eff}$, where A and B are proportional to the coordinating capacity of H_2O and L, respectively, and $\alpha = r_L - r_O(H_2O)$.[12] Both Li^+ and Mg^{2+} have very low affinity for weakly coordinating ligands, such as strong acid anions (e.g., Cl^-, ClO_4^-, and NO_3^-), because the positive term dominates ΔG_{comp} ($B < A$) and the relative complex stability order, $Cs^+ > Rb^+ > K^+ > Na^+ >$ **Li^+** and $Ba^{2+} > Sr^{2+} > Ca^{2+} >$**$Mg^{2+}$**, is the opposite of (z^2/r_{eff}). However, both Li^+ and Mg^{2+} strongly bind weak acid anions, such as phosphate, pyrophosphate, and ATP, for which $B \gg A$ leads to negative ΔG_{comp} values and the relative complex stability order is reversed, **Li^+** $> Na^+ > K^+ > Rb^+ > Cs^+$ and **Mg^{2+}** $> Ca^{2+} > Sr^{2+} > Ba^{2+}$, now being proportional to (z^2/r_{eff}). For some ligands, where $B \geq A$, the two terms have similar and variable relative values along the cation series and a maximum stability is reached somewhere in the middle of the series. This is the case for $EDTA^{4-}$, for which the relative complex stability order is **Li^+** $> Na^+ > K^+ > Rb^+ > Cs^+$ but $Ca^{2+} >$ **Mg^{2+}** $\cong Sr^{2+} > Ba^{2+}$, because of an unfavorable enthalpy term in B for Mg^{2+} relative to the others as a result of steric hindrance in the coordination sphere of the small Mg^{2+} in the $Mg(EDTA)^{2-}$ complex.

In summary, Li^+ and Mg^{2+} have similar complexation ligand selectivities, both preferring weak acid anionic ligands considerably more than other IA or IIA cations. The complexes of Mg^{2+} are stronger than those of Li^+, which are usually closer to those of Ba^{2+} in stability. However, the degree of metal ion competition can be enhanced by taking advantage of the subtle differences in ligation preferences of Li^+ and Mg^{2+}, as demonstrated for small inorganic ligands such as uramildiacetate and *o*-carboxyphenyliminodiacetate, which contain a 3 oxygen, 1 nitrogen coordination site.[10] Morever, high ligand denticity favors Li^+ versus Mg^{2+}. For example, the ratio of stability constants for the 1:1 complexes of Mg^{2+} and Li^+ decreases from 91 for phosphate, through 58 for pyrophosphate, to 30 for triphosphate,[13] thus increasing the capacity of Li^+/ Mg^{2+} competition for the phosphate ligands of higher denticity. This Li^+/Mg^{2+} competition for a given ligand in solution allows Li^+ to interfere with Mg^{2+}-dependent biological processes.[2,9]

Proposed Mechanisms for Li^+ Action

Li^+ inhibits several Mg^{2+}-dependent enzymes by competing with bound Mg^{2+}; however, only a few are significantly inhibited at therapeutic serum lithium concentrations (0.5−1.2 mM), as it is described in Figure 1.

GSK-3β is an Mg^{2+}-activated enzyme that is involved in glycogen metabolism and also has an apoptotic function. Because the inhibition of GSK-3β by Li^+ confers neuroprotection, this system has received considerable attention as a possible target for Li^+ action in bipolar disorder.[3,4,14]

The observations of inhibition of both IMPase and IPPase by Li^+ have led to the inositol depletion hypothesis,[5,6] which proposes that Li^+ exerts its mood-stabilizing effect by decreasing inositol levels and thus the amount of phosphoinositide-4,5-bisphosphate available for signaling cascades that rely upon the phosphatidyl inositol pathway, including G_q-mediated signaling.

Li^+ affects cAMP levels; the molecular mechanisms involved are still unclear, but Li^+/Mg^{2+} competition seems to participate in vitro in the inhibitory direct effect of Li^+ on AC activity.[7] In the rat brain, Li^+ reduces the production of cAMP that is stimulated postreceptor, for example, forskolin,[7] and decreases both receptor-mediated stimulation and inhibition of AC activity.[15] Moreover, Li^+ alters the activity of G proteins regulating the activity of AC,[16] and the addition of excess Mg^{2+} to Li^+-treated membranes from rat cortex reverses the inhibition by Li^+ of G proteins,[17] suggesting that competition between Li^+ and bound Mg^{2+} in G proteins is present. G_s and G_i, with stimulatory and inhibitory AC-signaling properties, respectively, may account for both the antimanic and antidepressant Li^+ therapeutic effects.[16,18] Findings of high $G_{s\alpha}$ levels[19,20] and increased forskolin-stimulated cAMP formation[19] in cerebral cortical regions of *postmortem* brains from bipolar untreated patients support the hypothesis that an hyperactive G protein-coupled cAMP-signaling system may be involved in the pathophysiology of this disease.[21] It was later proposed that, in addition to G proteins,[16] a bimodal model for Li^+ action also applies to the IMPase and GSK-3β mechanisms.[18]

There are pros and cons in favor and against each of the postulated mechanisms of Li^+ action. For example, the uncompetitive behavior of the inhibition of IMPase by Li^+ implies that the accumulation of more substrate molecules, i.e., inositol monophosphates, cannot outcompete with the inhibitor, Li^+, and this effect may explain why Li^+ therapy becomes more effective with the length of treatment.[5] However, inositol is ubiquitous in the brain, and no solid evidence exists for detectable changes in inositol levels.[2] Unlike IMPase[22] and G proteins,[16] which are inhibited by Li^+ concentrations within its therapeutic range, GSK-3β has a K_i value of 2.0 mM,[14] making it a less likely candidate as a site for Li^+ action.

If Li^+/Mg^{2+} competition is crucial for Li^+ action, the extent of ionic competition for distinct Mg^{2+}-dependent systems is likely to be different and will not contribute equally toward the mode of action of this drug. Several of the mechanisms for Li^+ action have been questioned, which may be associated with the inefficiency of metal ion competition in some of these biological systems. The smaller charge of Li^+ relative to Mg^{2+} suggests that Li^+ can compete only in part for low-affinity Mg^{2+}-binding sites in metabolites and proteins, as observed by us for ATP[23] and for the G protein transducin−GDP complex.[24]

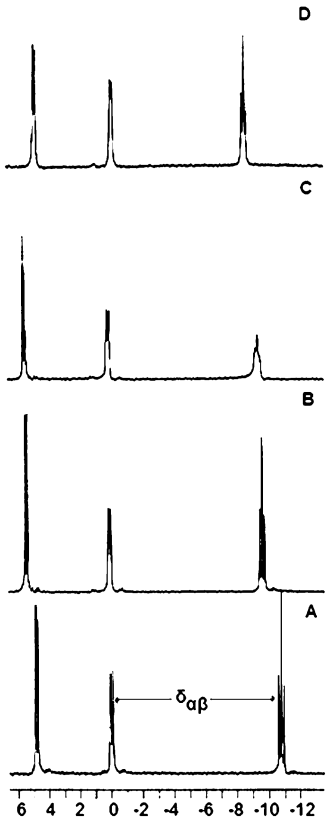

FIGURE 2. ^{31}P NMR spectra of (A) 5.0 mM Tris-ATP alone, (B) 5.0 mM Tris-ATP with 150 mM LiCl, (C) 5.0 mM Tris-ATP with 0.5 mM $MgCl_2$ and 150 mM LiCl, and (D) 5.0 mM Tris-ATP with 50 mM $MgCl_2$. Trimethyl phosphate was the external reference, and the ionic strength, pH, and temperature were 0.15 M, 7.4, and 37 °C, respectively, in all samples.

Evidence for Competition between Li^+ and Bound Mg^{2+} in Biological Systems

Small Molecule Studies. To investigate Li^+/Mg^{2+} competition, one requires a method(s) that is able to differentiate between free and bound forms of the metal ions. Whereas atomic absorption spectrophotometry is able to measure total concentrations of these ions, NMR and fluorescence spectroscopic methods can be used for monitoring the equilibrium between the free and bound species. This methodology is particularly important for the investigation of bipolar disorder because intracellular Li^+ or Mg^{2+} ions exist in free and bound states and the distribution between these two forms may vary with the presence or absence of a mood disturbance.

Figure 2 depicts ^{31}P NMR spectra of ATP-containing solutions in the absence and presence of Li^+ and/or Mg^{2+}. The chemical-shift separation between the α- and β-phosphate resonances of ATP ($\delta_{\alpha\beta}$), which is an indicator of

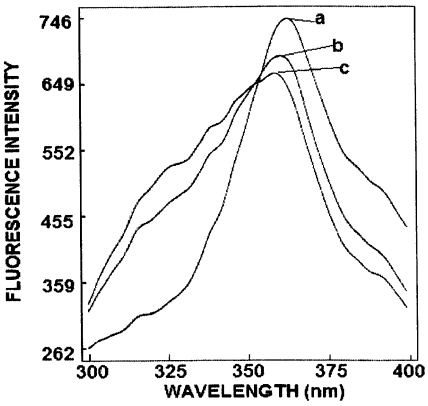

FIGURE 3. Fluorescence excitation spectra of 2 μM furaptra in solutions containing 5.0 mM NaATP and 150 mM Tris-Cl (pH 7.4) with (a) 100 mM LiCl, (b) 2.5 mM $MgCl_2$ and 100 mM LiCl, and (c) 2.5 mM $MgCl_2$.

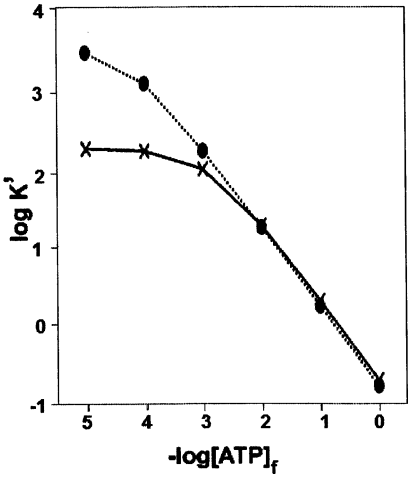

FIGURE 4. Logarithmic plots of the Mg^{2+} (●) and Li^+ (×) conditional binding constants to the RBC membrane as a function of the free ATP concentration.

metal ion complexation by ATP, decreased as saturating amounts of Li^+ and/or Mg^{2+} were added. The $\delta_{\alpha\beta}$ value changed from 10.82 ppm in metal-free ATP (Figure 2A) to 9.80 ppm in Li^+-saturated ATP (Figure 2B) and 8.43 ppm in Mg^{2+}-saturated ATP (Figure 2D); the mixture of Li^+ and Mg^{2+} salts with ATP (Figure 2C) gave an intermediate $\delta_{\alpha\beta}$ value, 9.43 ppm, indicating that metal ion competition for ATP-phosphate binding sites occurs.[25] Evidence for Li^+/Mg^{2+} competition in ATP solutions was also obtained using furaptra, a Mg^{2+}-sensitive fluorescent sensor that has a weak specificity for Li^+ (Figure 3). The λ_{max} values for furaptra in Mg^{2+}-depleted and Mg^{2+}-saturated media are 370 and 335 nm, respectively.[26] The fluorescence intensity observed at 335 nm for Li^+-containing ATP solutions (no Mg^{2+} present; spectrum a in Figure 3) was considerably lower than that observed in Li^+/Mg^{2+} mixtures (spectrum b in Figure 3), which in turn was lower than that obtained for Mg^{2+}-containing ATP solutions (spectrum c in Figure 3).[23] Complementary evidence was also obtained for Li^+/Mg^{2+} competition for binding sites in ATP by using ^{7}Li NMR spectroscopy.[23,25] Li^+ ions in Li^+-containing solutions of nucleotides are in fast exchange on the ^{7}Li NMR time scale, and thus, the observed ^{7}Li chemical shift and the spin−lattice (T_1) and spin−spin (T_2) relaxation time values represent weighted averages of bound and free Li^+ ions.[27] Because the ^{7}Li nucleus has a narrow chemical-shift range,[27] ^{7}Li NMR chemical shifts are not sensitive to Li^+ binding to biomolecules. However, free Li^+ affords large relaxation values, whereas Li^+ that is tightly bound has relatively smaller values.[27] We used ^{7}Li T_1 relaxation time measurements to probe Li^+ binding to ATP and found that an increase in the Mg^{2+} concentration caused an increase in ^{7}Li T_1 values for ATP solutions containing fixed Li^+ concentrations, whereas, for a fixed Mg^{2+} concentration, an increase in the Li^+ concentration caused an increase in ^{7}Li T_1 values.[23,25]

Conditional binding constants are more useful than stoichiometric binding constants to understand competitive ion binding for biological ligands.[10] Most ligands that bind Li^+, including ATP, will also bind Mg^{2+} and Ca^{2+} with even higher affinity. When ligands with high Mg^{2+} affinity, such as the red blood cell (RBC) membrane, are also present, the absolute values of the stoichiometric binding constants do not necessarily reflect the tendency of Li^+ to bind preferentially to ATP. The free intracellular Li^+ and Mg^{2+} concentrations are of the same order of magnitude in tissues of bipolar patients undergoing lithium therapy; the free intracellular Ca^{2+} concentrations are, however, 4−5 orders of magnitude smaller than those of Li^+, implying that Ca^{2+} does not compete appreciably with Li^+ for binding to biological ligands. The free intracellular Na^+ and K^+ concentrations are approximately 1−2 orders of magnitude larger than those of Li^+; any ligand with conditional binding constants that are 2 log units smaller for Na^+ and K^+ than for Li^+ will preferentially bind Li^+. Most ligands meet this criterion, and therefore, competition between Li^+ and Mg^{2+} can take place in the presence of physiologically relevant intracellular Na^+, K^+, and Ca^{2+} concentrations.[10]

Figure 4 shows the calculated conditional binding constants for Mg^{2+} and Li^+ binding to the RBC membrane in the presence of ATP as a function of the free ATP concentration, $[ATP]_f$. For $[ATP]_f$ values larger than 1.0 mM, Li^+ binds as strongly as Mg^{2+} to the RBC membrane. A representative $[ATP]_f$ value in RBCs is of the order of 0.2 mM ($-\log[ATP]_f = 3.69$).[28] From Figure 4, we estimated that, for typical $[ATP]_f$ values in RBCs, the ratio of the conditional binding constants of Mg^{2+} to Li^+ to the RBC membrane was approximately 4. In contrast, in the absence of ATP, the ratio of the stoichiometric binding constants of Mg^{2+} to Li^+ to the RBC membrane is approximately 20.[23] Using the calculated stoichiometric and conditional binding constants[23,25] and representative total concentrations of intracellular ATP and ions ($[ATP]_t$ = 2.0 mM, $[Li^+]_t$ = 1.0 mM, and $[Mg^{2+}]_t$ = 2.4 mM), as well as the estimated value of 0.8 mM of membrane-binding sites,[23] we calculated the free metal ion concentrations in the absence of either ATP or membrane and in the presence of both. These calculations indicated that, for $[ATP]_f$ values in RBCs, Li^+ can compete with approximately 14−21% of the Mg^{2+}-binding sites in the RBC membrane, depending upon the relative values of $[Mg^{2+}]_f$

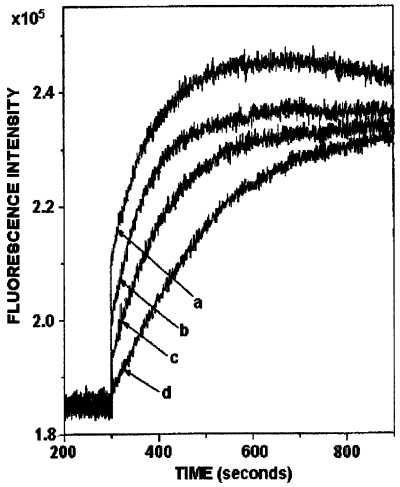

FIGURE 5. Time dependence of the fluorescence emission at 340 nm of 2.50 μM metal-depleted $rG_{i\alpha1}$, 2.50 μM GDP, 1.00 mM NaF, and 20.0 μM $AlCl_3$ in a solution containing 50.0 mM NaCl and 50.0 mM Tris-Cl at pH 7.9, upon the addition of 0.15 mM Mg^{2+} (trace a), a mixture of 0.15 mM Mg^{2+}/0.50 mM Li^+ (trace b), 0.15 mM Mg^{2+}/1.25 mM Li^+ (trace c), and 0.15 mM Mg^{2+}/2.0 mM Li^+ (trace d).

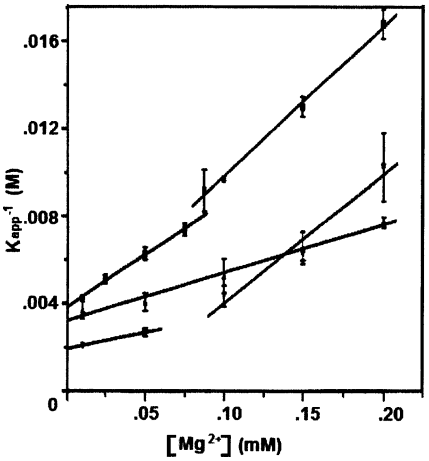

FIGURE 6. Variation of values of K_{app}^{-1} with $[Mg^{2+}]_t$ for SROS membranes with (▼) and without (▲) heterotrimeric G_t and for purified G_t alone (■), in the presence of varying concentrations of $MgCl_2$. The SROS membrane protein concentration was 9.0 ± 0.8 mg/mL, whereas that of G_t was 0.1 mM. The concentration range of LiCl varied from 1 to 30 mM. The reported, apparent Li^+-binding K_{app} values are the averages from two separately prepared samples, and the error bars represent the range of the values obtained.

and $[Li^+]_f$; however, in the absence of ATP, Li^+ would compete for less than 5% of the Mg^{2+} membrane-binding sites.[23] Thus, in the absence of the RBC membrane, competition between Li^+ and Mg^{2+} for ATP (in 1:1 stoichiometries) favors Mg^{2+} binding (equilibrium constant of 4.3×10^{-2}); however, coupling of the reaction between Li^+ and ATP with binding of the displaced Mg^{2+} to the RBC membrane becomes energetically more favorable (equilibrium constant of 0.86), with the extent of the coupled reaction depending upon the relative intracellular concentrations of Mg^{2+} and Li^+.

As we observed for ATP using fluorescence and ^{31}P and ^7Li NMR,[23,25] we also found evidence for Li^+/Mg^{2+} competition for phosphate groups in second messengers and in substrates of signaling macromolecules, such as IP_3, cAMP, GTP, GDP, and ADP.[25,29,30]

Protein Studies. Heterotrimeric G proteins are membrane-bound proteins that play obligatory roles in the transduction of extracellular signals to various intracellular effectors. Once an extracellular agonist binds to a specific receptor, the activated receptor induces a conformational change of the inactive GDP-bound G protein, which is comprised of a $\alpha\beta\gamma$ trimer. GTP then exchanges for GDP bound to its α subunit, and the $\beta\gamma$ complex dissociates. G proteins also exhibit slow GTPase activity, which is responsible for the hydrolysis of GTP into GDP and reassociation of the $\beta\gamma$ complex, recycling the G protein into its inactive conformation.

We studied the effect of Li^+ upon the Mg^{2+}-dependent activation of purified, metal-reconstituted recombinant $G_{i\alpha1}$ by monitoring its intrinsic tryptophan fluorescence (Figure 5).[31] The fluorescent method takes advantage of the fact that, upon activation with GTP or GTP analogues, such as GDP·AlF_4^-, the intrinsic tryptophan fluorescence of G proteins increases.[32,33] Because the standard fluorescence activity assay calls for an excess of Mg^{2+} (5.00 mM) in the storage buffer, Li^+ was unable to compete with Mg^{2+} under these conditions. However, physiological $[Mg^{2+}]_f$ and $[Mg^{2+}]_t$ values are approximately 0.2 and 2.0 mM, respectively.[28] Thus, our method varied from those of others in that we initially prepared apo G protein and then reconstituted by titration with concentrations of Mg^{2+} within the physiological range. The $rG_{i\alpha1}$ protein sample showed, upon Mg^{2+} activation, an increase in the intrinsic tryptophan fluorescence always greater than 25%, which was in agreement with the values reported by others[32,33] for fully active G_α subunits, indicating that proper protein folding occurred for these Mg^{2+}-reconstituted samples. Trace a in Figure 5 indicates that, after 5 min, the apoprotein titrated with Mg^{2+} was almost fully activated, with minimal increased activation continuing up to 15 min. It is apparent from this trace that the Mg^{2+}-dependent activation is biphasic; first there is an initial intensity jump in the activation of the protein, followed by a slower intensity increase over time. Whereas the addition of EDTA after 5 min caused a small but significant decrease in the fluorescence intensity (2.9 ± 0.3%), a significantly larger decrease of 4.4 ± 0.2% upon addition of EDTA was observed during the initial jump (at 10 s). Because the fluorescence decrease upon EDTA addition was significantly larger at 10 s than at 5 min, we conclude that Mg^{2+} populated the low-affinity sites before the high-affinity sites. Thus, the biphasic behavior suggested the existence of two distinct Mg^{2+}-binding sites.

In the presence of Li^+/Mg^{2+} mixtures (traces b−d in Figure 5), significantly lower maximal fluorescence intensities (for 10 min or longer) were obtained, indicating that Li^+ decreased the overall percent of Mg^{2+}-dependent activation. In contrast, the initial jump (within 10 s) observed for a Li^+/Mg^{2+} mixture containing a therapeutically relevant Li^+ concentration (0.5 mM) (trace b in Figure 5) was approximately half of that observed with the same Mg^{2+} concentration and no Li^+ (trace a in Figure 5),

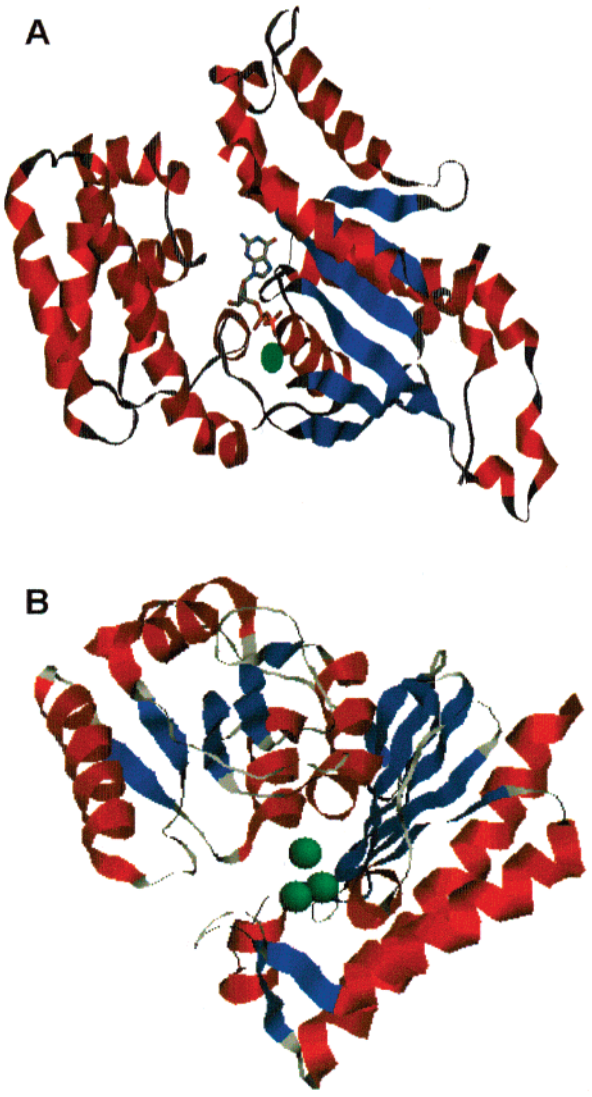

FIGURE 7. Crystal structures of (A) $rG_{i\alpha i}$ (PDB 1BOF) showing its single-bound Mg^{2+} in green and the bound GDP substrate with its phosphorus atoms in orange and (B) IMPase (PDB 2BJI) showing three Mg^{2+} in green; the Mg^{2+} ion sitting on top of the other two has been modeled as the Li^+-binding site.

indicating that Li^+ competed primarily and effectively with Mg^{2+} in $rG_{i\alpha 1}$. Because physiological and therapeutic levels of Mg^{2+} and Li^+, respectively, were used in these experiments, we conclude that therapeutic Li^+ levels inhibit the formation of the GDP·AlF_4^-·$rG_{i\alpha 1}$ complex,[31] lending support for the G protein hypothesis for Li^+ action.[16]

We have recently conducted a study on Li^+/Mg^{2+} competition for bound Mg^{2+} in heterotrimeric transducin (G_t), a G protein isolated from bovine eye retinas, in its inactive GDP-bound conformation.[24] Using ^7Li T_1 relaxation measurements and fluorescence spectroscopy with the Mg^{2+} fluorophore furaptra, we observed Li^+/Mg^{2+} competition in three preparations: purified G_t, stripped rod outer segment membranes (SROS), and SROS with purified G_t reattached (ROS-T). When purified ROS-T, SROS, or transducin were titrated with Li^+ in the presence of increasing amounts of Mg^{2+}, the apparent Li^+-binding constants decreased because of Li^+/Mg^{2+} competition (Figure 6). Whereas for SROS, the competition mechanism

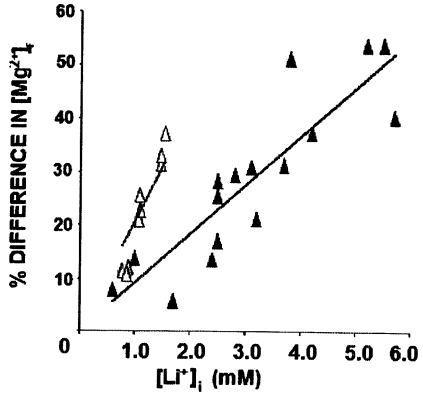

FIGURE 8. Percent difference in $[Mg^{2+}]_f$ per millimolar of $[Li^+]_i$ for cells loaded with Li^+ under chronic Li^+-loading conditions (after Li^+ reached a steady state) (△) and acute Li^+-loading conditions (prior to Li^+ reaching a steady state) (▲). Lines shown are lines of best fit for each loading condition in which the *x* and *y* intercepts to the axes were fixed to 0 and correlated with the data in both chronic ($R^2 = 0.97$) and acute ($R^2 = 0.95$) cases.

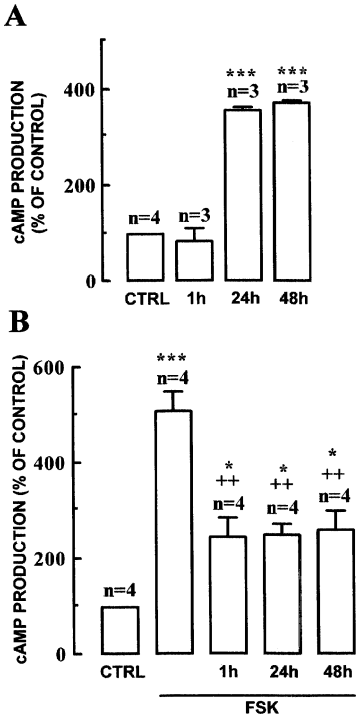

FIGURE 9. Effects of Li^+ on cAMP production by SH-SY5Y cells. (A) Cells were pretreated with 25 μM Ro-201724 (an inhibitor of phosphodiesterases), for 15 min, and then incubated or not (ctrl) with 1 mM Li^+, for the indicated periods of time. (B) Where indicated, the cells were stimulated with 10 μM forskolin (fsk), for 15 min, after treatment with Li^+. The cAMP levels were measured using a radioactive assay. Data are presented as a percentage relative to the control. Values are means ± SEM, for the indicated number of independent experiments. (*) $p < 0.05$, and (***) $p < 0.001$, significantly different from the control. (++) $p < 0.01$, significantly different from the fsk stimulation in the absence of Li^+.

was monophasic, for G_t, it was biphasic, suggesting that, in G_t, Li^+/Mg^{2+} competition occurred with different affinities for Mg^{2+} in the two types of Mg^{2+}-binding sites (Figure 6). G_t release from the ROS-T membrane, induced by trypsin digestion of SROS, was also inhibited by Li^+. In addition, as $[Li^+]$ increased, the fluorescence excitation

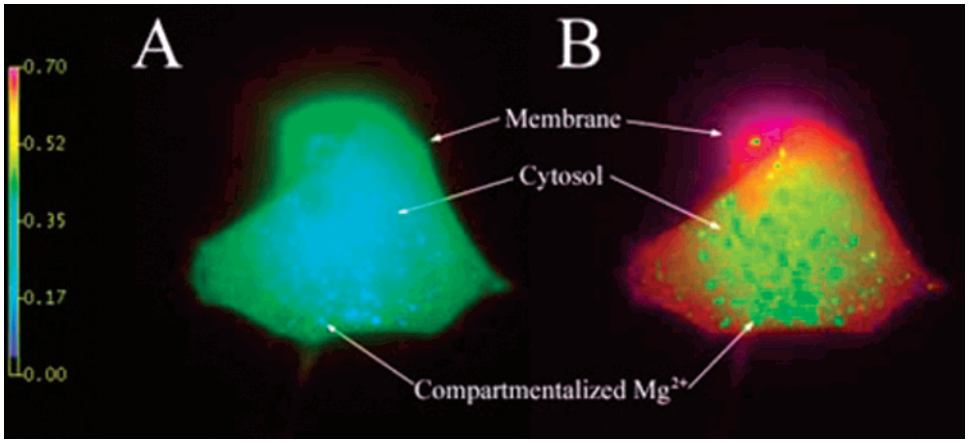

FIGURE 10. Scale on the left represents the fluorescence intensity ratio (R; I_{335}/I_{370}) in a single neuroblastoma SH-SY5Y cell loaded with the ester form of furaptra before (A) and after incubation with 5.0 mM LiCl (B). An increase in R corresponds to an increase in intracellular $[Mg^{2+}]_f$. In each image, the membrane, cytosol, and one region of compartmentalized Mg^{2+} are indicated by arrows.

spectra of both ROS-T and G_t were blue-shifted, as for ATP-containing solutions (Figure 3),[24] indicating an increase in $[Mg^{2+}]_f$ compatible with the displacement of Mg^{2+} from two low-affinity Mg^{2+}-binding sites of G_t. It is interesting that, when fluorescence spectroscopy was used, we observed the presence of two low-affinity Mg^{2+}-binding sites in G_t,[24] whereas, for IMPase,[34] only one low-affinity Mg^{2+}-binding site was proposed from modeling studies.

It is well-established from kinetic studies conducted by us[31] and others[32,33] that heterotrimeric G proteins have at least two types of Mg^{2+}-binding sites: a high-affinity Mg^{2+}-binding site (K_d value in the nanomolar range) that is needed for the hydrolysis of GTP and a low-affinity Mg^{2+}-binding site(s) (K_d value in the millimolar range) that is required for the hormone-catalyzed GDP/GTP exchange. In all X-ray crystallographic studies conducted for the wild-type and some mutants of G_i, G_s, and G_t proteins in different conformations, i.e., in the presence of GTPγS (a nonhydrolyzable GTP analogue), of the GDP$-AlF_4^-$ complex (a transition-state analogue) and of GDP alone,[35,36] only one divalent metal ion (Mg^{2+} or Ca^{2+}) was found coordinated to the α subunit. Mg^{2+} crystallographic titrations of $rG_{i\alpha1}$ showed evidence for conformational changes upon Mg^{2+} addition, despite the observation of only one Mg^{2+}-binding site (Figure 7A).[37] Unequivocal evidence from kinetic studies for the presence of high- and low-affinity Mg^{2+}-binding sites occurs not only for G proteins[31−33] but also for IMPase.[38] Crystallographic studies of IMPase showed the presence of three Mg^{2+}-binding sites; because one of these sites has long bond distances around Mg^{2+}, it was assigned as the low-affinity Mg^{2+}-binding site and modeled as the Li^+-binding site (Figure 7B).[34] Because Mg^{2+} must bind with relatively high affinity to be incorporated in a crystal, the fact that the low-affinity Mg^{2+}-binding site(s) was not observed in the X-ray diffraction studies of G proteins might suggest that the affinity of Mg^{2+} for its low-affinity site is weaker than that for the low-affinity Mg^{2+}-binding site of IMPase. If this is indeed the case, the low-affinity Mg^{2+}-binding site(s) of G proteins is presumably more susceptible to Li^+/Mg^{2+} competition and a more likely site for Li^+ action.

Intact Cell Studies. We have demonstrated, by using fluorescence spectroscopy and the Mg^{2+} indicator furaptra, that Li^+/Mg^{2+} competition occurred at therapeutically relevant Li^+ levels in human neuroblastoma SH-SY5Y cells, an appropriate neuronal model for the investigation of bipolar disorder (Figure 8).[39] The different behavior for percent differences in $[Mg^{2+}]_f$ per millimolar of $[Li^+]_i^+$ under chronic and acute Li^+ incubation conditions is a result of a slow equilibrium for Li^+/Mg^{2+} competition, which is achieved under chronic (△) but not under acute (▲) Li^+ conditions. We also found evidence for Li^+/Mg^{2+} competition in human CCRF-CEM lymphoblastoma cells,[40] human erythrocytes,[28,40] and bovine chromaffin cells.[41] The degree of Li^+/Mg^{2+} competition was shown to be cell-specific.[40] In these cell models, we found by ^{7}Li NMR spectroscopy or atomic absorption spectrophotometry that Li^+ uses Na^+ transport pathways, in particular the Na^+/Ca^{2+} and Na^+/Li^+ exchangers and Na^+ channels,[40−42] to equilibrate across cell membranes. For each cell model studied, the equilibrium intra- and extracellular $[Li^+]$ values were not the same and were dictated by the transmembrane ionic potential, which is unique to each cell type.

Exposure of SH-SY5Y cells to 1 mM Li^+ for 24 and 48 h increased basal cAMP levels (Figure 9A); however, preincubation with Li^+, at the same concentration, decreased cAMP production in response to forskolin, a known activator of AC (Figure 9B).[42] The increase of cAMP basal levels by Li^+ was attributed to the inhibition of G_i, which has been reported to be the G protein that is preferentially activated under basal conditions. This effect might involve Li^+/Mg^{2+} competition for Mg^{2+} bound to the α subunit of G_i.[42] Under nonstimulated conditions, cAMP levels at 24 and 48 h of Li^+ incubation were not significantly different from those obtained after forskolin stimulation for the same Li^+ incubation periods, thus suggesting a total Li^+ inhibitory effect on forskolin-stimulated AC.

Using a combination of ^{7}Li and ^{31}P NMR measurements, we proposed that the major Li^+-binding sites in

Table 1. Representative Total and Free Li^+ and Mg^{2+} Concentrations (in Millimolar) in Model Systems[39,40,44]

	ATP		RBCs		SH-SY5Y cells	
$[Li^+]_t$[a]	5.0	100	0	16	0	1.5
$[Li^+]_f$[b]	4.6	96	0	*c*	0	*c*
$[Mg^{2+}]_t$[a]	2.5	2.5	2.4	2.4	5.0	4.9
$[Mg^{2+}]_f$[d]	0.16	0.56	0.20	0.30	0.48	0.64

[a] Determined by atomic absorption; for cells, these values indicate intracellular concentrations. [b] Estimated from ^7Li NMR relaxation data. [c] Not determinable by current methodology. [d] Calculated from furaptra fluorescence except for RBCs, where ^{31}P NMR was used instead.

both human neuroblastoma SH-SY5Y cells[43] and erythrocytes[27] reside in membrane domains. Fluorescence microscopy and an Mg^{2+} sensor allowed us to monitor the Mg^{2+} concentration gradients in intact SH-SY5Y cells and confirm that, at the subcellular level, the highest degree of Li^+/Mg^{2+} competition occurs at the plasma membrane (Figure 10), where G proteins and AC are located.

Concluding Remarks

If Li^+/Mg^{2+} competition for bound Mg^{2+} in cells is indeed the underlying basis for Li^+ action, it is unlikely, from the point of view of inorganic chemistry, that Li^+ will compete specifically with only one Mg^{2+}-activated enzyme or protein involved in signal transduction or neuroprotection. It will therefore be of interest to conduct in the future a systematic comparison of the extent of Li^+/Mg^{2+} competition for the postulated Li^+ cellular targets described in this Account. Although evidence for Li^+/Mg^{2+} competition in cells[39] or for proteins[14,17,31] has been observed for therapeutically relevant Li^+ levels in some cases, progress in lithium research has been hampered by the unavailability of a sensitive and specific Li^+ spectroscopic tool. This problem is compounded by the fact that Li^+ only has two electrons, and thus, Li^+ bound to a protein cannot be detected by X-ray crystallography. Therefore, it would be useful to develop a sensitive Li^+ fluorophore that would be able to detect *directly* Li^+/Mg^{2+} competition at therapeutic Li^+ and Mg^{2+} concentrations (Table 1), rather than use the less sensitive and indirect fluorescence method based on an Mg^{2+} fluorophore.

Work in our laboratories is supported by grants from the NIH and NSF to D. M. deF. and from FCT and FEDER to M. M. C. A. C. and C. F. G. C. G. We thank Abde Abukhdeir, Eugene Tarasov, Christopher Malarkey, and Liliana Montezinho for help with the preparation of the figures. The authors apologize to those whose important work is not cited in this Account because of space restrictions.

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AR030197A