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MAGNESIUM REVERSAL OF LITHIUM INHIBITION OF β-ADRENERGIC AND MUSCARINIC RECEPTOR COUPLING TO G PROTEINS

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Abstract—Recently, lithium was found to inhibit the coupling of both muscarinic cholinergic and β-adrenergic receptors to pertussis toxin-sensitive and cholera toxin-sensitive G proteins respectively. These findings suggest that G proteins are the common site for both the antimanic and antidepressant therapeutic effects of lithium. Magnesium ions are crucial to the function of G proteins and interact with them at multiple sites. In the present study using rat cerebral cortex, we determined that magnesium can reverse the ability of lithium to inhibit isoprenaline- and carbamylcholine-induced increases in guanosine triphosphate (GTP) binding to G proteins. Lithium concentrations effective in attenuating G protein function were found to be hyperbolically dependent on free Mg^{2+} concentrations, suggesting multiple sites of competition between lithium and magnesium on G proteins. Free intracellular Mg^{2+} concentrations in rat cerebral cortex *in vivo* are known to be less than 1 mM. At such Mg^{2+} concentrations, therapeutically efficacious lithium concentrations (1 to 1.5 mM) were still able to alter G protein function, which supports the physiological and clinical relevance of lithium action on G proteins.

Lithium is used to treat the acute states of bipolar affective disorder and is known to prevent both its manic and depressive episodes. Two principal theories of lithium action have been suggested. The first theory concentrates on the inhibitory action of lithium on adenylate cyclase function [1–4], while the other suggests lithium inhibition of phosphatidylinositol (PI) turnover [5–7]. Recently, we described a direct interaction of lithium with G proteins, a core feature of both these signalling systems. Lithium inhibits the coupling of both muscarinic cholinergic receptors and β-adrenergic receptors to pertussis toxin-sensitive and cholera toxin-sensitive G proteins respectively [8, 9].

Magnesium ions are crucial to the function of G proteins as they interact with these proteins at multiple sites (for review, see Ref. 10). The interaction of magnesium with a low-affinity site is required for βγ-facilitated guanine nucleotide exchange. Neurotransmitters lower the concentration requirement for magnesium at this site [11–13]. Gilman [10] suggests that neurotransmitters "shift the dependency on magnesium from a concentration range where βγ stabilizes the binding of guanosine diphosphate (GDP) to a range where βγ facilitates guanine nucleotide exchange".

In the present study, we determined that the degree to which lithium attenuates β-adrenergic- and muscarinic-induced increases in guanine nucleotide binding to G proteins is inversely related to magnesium concentration. Lithium competition with magnesium ions on magnesium low-affinity sites essential for guanine nucleotide exchange on G proteins is thus suggested as the molecular site for the therapeutic action of lithium.

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MATERIALS AND METHODS

Membrane preparation. Male Sprague–Dawley rats (200–250 g) were decapitated, and the cerebral cortex was removed rapidly. The tissue was homogenized in buffer A [25 mM Tris, pH 7.4, 1 mM dithiothreitol (DTT)] containing 1 mM ethyleneglycol-bis-(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA). Membranes were obtained by centrifuging the preparations twice for 10 min, 10,000 *g*, at 4°.

[^{3}H]*GTP binding to rat cerebral cortex membranes.* Two hundred microliters of membranes (0.5 to 1.5 mg protein), suspended in 25 mM Tris (pH 7.4), 0.7 mM adenosine triphosphate (ATP), 2.2 mM Mg^{2+}, 1 mM EGTA, and 1 mM DTT, was pipetted into plastic microfuge tubes containing various concentrations of [^{3}H]GTP (0.5 to 4.0 μM), which approximate its dissociation constant (≤1 μM) found in the present study which is in accord with the K_m for GTP in purified systems [10]. The incubation was performed at room temperature for 5 min (equilibrium conditions), and the reaction was stopped by adding 5 vol. of ice-cold buffer A, followed by centrifugation at 10,000 *g* for 2 min. The pellets were washed rapidly three times with ice-cold buffer A by repeating centrifugation and were then resuspended in 200 μL of the same buffer. Bound radioactivity was measured by liquid scintillation spectrometry by adding a 150-μL aliquot dissolved in scintillation liquid. All assays were performed in triplicate, together with triplicate control samples containing 100 μM unlabeled guanyl-5'-yl imidodiphosphate [Gpp(NH)p], to determine

nonspecific binding. Isoprenaline or carbamylcholine was added at a final concentration of 50 μM and 100 μM respectively. These doses were shown previously to be maximally effective [8].

Adenosine diphosphate (ADP) ribosylation. Membranes were suspended in 1 mL buffer containing 25 mM Tris, pH 7.4, 10 mM nicotinamide adenine dinucleotide (NAD), 1 mM ATP, 1 mM EGTA, 100 μM GTP, 2 mM DTT, 5 mM $MgCl_2$, 10 mM thymidine, 20 mM creatinine phosphate, and 40 $\mu g \cdot mL^{-1}$ creatine phosphokinase. ADP ribosylation was carried out for 15 min at 30° by adding cholera toxin (50 $\mu g \cdot mL^{-1}$) preactivated for 10 min at 37° with 20 mM DTT, or pertussis toxin (25 $\mu g \cdot mL^{-1}$) preactivated for 10 min at 30° with 20 mM Tris. The reaction was stopped by adding 25 mL of ice-cold 25 mM Tris, pH 7.4, and 5 mM $MgCl_2$ immediately followed by centrifugation at 10,000 *g* for 10 min. [^{3}H]GTP binding was then carried out as described.

Estimation of "free" Mg^{2+} concentration. In the experiments described, Mg^{2+} is presumed to exist as the free ion and in complexes with EGTA, ATP, and GTP. An expression for the total Mg^{2+} concentration can be written as:

$$[Mg^{2+}]_{total} = [Mg^{2+}]_{free} + f_1[EGTA] + f_2[ATP] + f_3[GTP]$$

$$f_i = \frac{K_i[Mg^{2+}]_{free}}{1 + K_i[Mg^{2+}]_{free}}$$

where each K_i is the equilibrium constant for the particular binding reaction. The calculated affinity of EGTA for Mg^{2+} at pH 7.4 is 6.3 μM [14] and of ATP for Mg^{2+} 60 μM [11]. The contribution to the sum of f_3 [GTP] is negligible as GTP concentrations used in the binding experiments are in the micromolar range whereas magnesium concentrations are in the millimolar range. Thus, the only unknown in the above equation is the free metal concentration, which can be determined by this computation.

It should be noted that this calculation is a rough estimate for free Mg^{2+} concentrations, as the membrane preparations, although washed and centrifuged, may still contain endogenous metal. The membrane also contains components that may form complexes with magnesium.

RESULTS

β-Adrenergic and muscarinic receptor coupled G protein function was directly assessed by measuring isoprenaline- and carbamylcholine-induced increases in [^{3}H]GTP binding to membranes prepared from rat cerebral cortex. The β-adrenergic agonist effect was exerted through the stimulatory G protein (G_s) as cholera toxin-catalyzed ADP ribosylation totally abolished the isoprenaline effect, whereas ribosylation with pertussis toxin had no effect. Pertussis toxin-catalyzed ADP ribosylation partially abolished carbamylcholine-induced increases in [^{3}H]GTP binding capacity. This muscarinic agonist effect was unchanged by pretreatment with cholera toxin, thus supporting a muscarinic effect through G proteins other than G_s [i.e. inhibitory G proteins (G_i, G_0)]. These findings are in agreement with our previously reported results [8].

Lithium attenuates both β-adrenergic- and muscarinic agonist-induced increases in [^{3}H]GTP binding (Fig. 1) [8, 9] in a selective manner. Increasing the concentration of sodium ions up to 100 mM did not affect agonist-induced increases in GTP binding to G proteins. The ability of lithium to attenuate G protein function depends on magnesium concentration. Figure 1 shows that elevating the total magnesium concentration from 1 mM to 2.2 mM and to 5 mM reduced the effect of lithium (1 mM) on G protein function from totally inhibiting the effects of both isoprenaline and carbamylcholine to partially inhibiting (~50%) or nulling the effect respectively.

At each magnesium concentration, it was possible to titrate lithium concentrations that inhibited ~50% of the agonist effect (Fig. 2) is a representative example of 2.2 mM magnesium concentration). Lithium concentrations that inhibited ~50% of isoprenaline or carbamylcholine effects were hyperbolically dependent on free Mg^{2+} concentrations (Fig. 3).

DISCUSSION

Both β-adrenergic and muscarinic cholinergic agonists induced similar increases in the binding of [^{3}H]GTP to G proteins. The β-adrenergic agonist effect was totally blocked by pretreatment with cholera toxin, whereas the muscarinic agonist effect was partially inhibited by pertussis toxin. In brain, G_i and G_0 are present in much greater quantities than G_s, so one might expect carbamylcholine to have a greater effect than isoprenaline. However, in previous studies we showed that carbamylcholine-induced increases in GTP binding capacity are exerted through the M_1 subtype of muscarinic receptors, whereas M_2 muscarinic receptors fail to induce such increases [9]. Moreover, carbamylcholine inhibits β-adrenergic receptor-coupled G_s protein function through M_2-coupled, pertussis toxin-sensitive, non-G_s proteins [15]. These differences in carbamylcholine effect may stem from different coupling mechanisms of the various muscarinic receptor subtypes. Specifically, concerning PI turnover there are clearly discrepant observations on the effects of pertussis toxin on receptor-stimulated synthesis of inositol triphosphate (IP_3) (for review, see Ref. 10). The partial inhibition by pertussis toxin of the stimulation of GTP binding caused by carbamylcholine, found in the present study, may stem from the heterogeneity of G proteins, pertussis toxin sensitive and insensitive, involved in the carbamylcholine effect.

The present study shows that magnesium can reverse the ability of lithium to inhibit the coupling of β-adrenergic and muscarinic cholinergic receptors to cholera toxin-sensitive and pertussis toxin-sensitive G proteins respectively. Lithium concentrations necessary to attenuate both isoprenaline- and carbamylcholine-induced increases in GTP binding to G proteins in rat cerebral cortex were found to be hyperbolically dependent on the free magnesium concentrations. This finding suggests multiple sites of competition between lithium and magnesium on

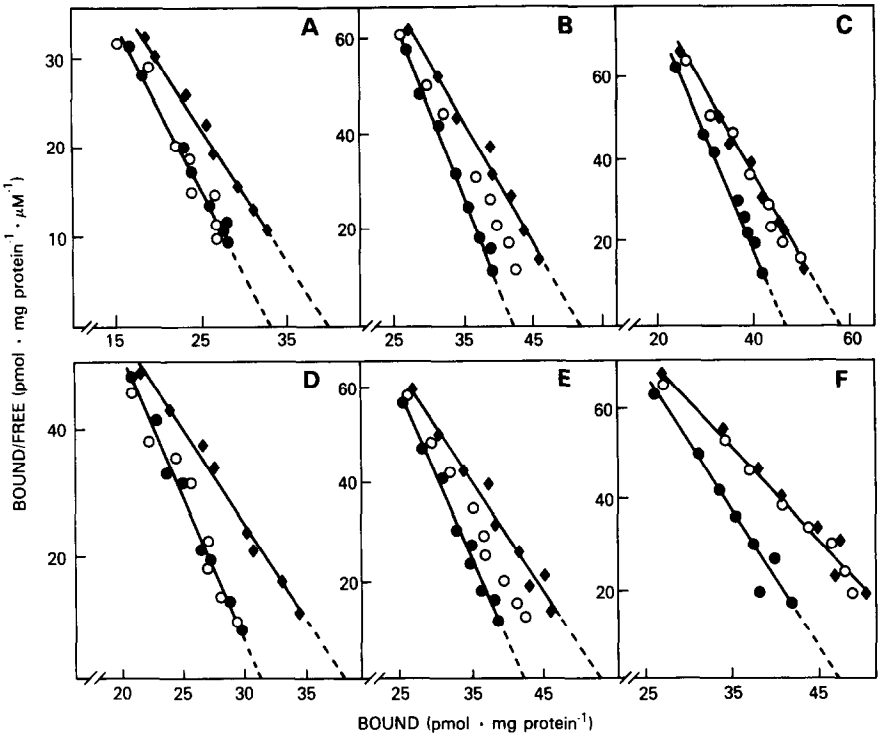

Fig. 1. Magnesium reversal of lithium inhibition of β-adrenergic and muscarinic receptor coupled G protein function (representative curves at 1 mM lithium). Upper panels: Effect of increasing the total magnesium concentration from 1 mM (A) to 2.2 mM (B) and to 5 mM (C) on lithium inhibition of isoprenaline (50 μM)-induced increases in [^{3}H]GTP binding to G_s. Lower panels: Effect of increasing total magnesium concentration from 1 mM (D) to 2.2 mM (E) and to 5 mM (F) on lithium inhibition of carbamylcholine (100 μM)-induced increases in [^{3}H]GTP binding to non-G_s proteins. Key: basal [^{3}H]GTP binding (●—●); agonist (isoprenaline or carbamylcholine)-induced [^{3}H]GTP binding (◆—◆); and agonist-induced [^{3}H]GTP binding in the presence of lithium (○—○).

G proteins. Indeed, multiple magnesium sites, essential to the function of G proteins, have been described (for review, see Ref. 10). An interaction of magnesium with a high-affinity site (in the nanomolar range) is required for the hydrolysis of GTP; an interaction of magnesium with a low-affinity site (in the millimolar range) is necessary for βγ-facilitated guanine nucleotide exchange [12, 13]. Neurotransmitters lower the concentration requirement for magnesium at this site (to the micromolar range) [11, 12]. The similar competitive behavior of lithium and magnesium ions on magnesium low-affinity sites on G_s and on non-G_s (e.g. G_i, G_o) proteins found in the present study may be explained by the similar mechanism of Mg^{2+} interaction with the various members of the G protein family [10]. Thus, we suggest that lithium competition with Mg^{2+} ions on magnesium low-affinity sites (essential for guanine nucleotide exchange on G proteins) represents the molecular site for the therapeutic action of lithium. Estimation of free intracellular Mg^{2+} concentration, measured *in vivo* in rat cerebral cortex using phosphorus magnetic resonance spectroscopy [16–18], is between 0.3 and 1 mM. Our present findings indicate that such Mg^{2+} levels can still show a definite lithium effect on G proteins at therapeutically efficacious concentrations (1 to 1.5 mM), thus supporting the physiological and clinical relevance of our findings.

Lithium and magnesium theoretically may also interact on Mg^{2+} high-affinity sites needed for GTPase activity. However, lithium ions at concentrations up to 150 mM were found not to inhibit agonist-induced GTPase activity in cardiac sarcolemma membranes [19, 20]. Rather, GTP hydrolysis was enhanced by these lithium concentrations in a non-selective manner similar to effects exerted by other monovalent cations [19, 20]. Also, considering free Mg^{2+} concentrations in the brain (~1 mM) [16–18], as well as therapeutically efficacious concentrations of lithium (~1 mM), a theoretically possible interaction between these ions on Mg^{2+} high-affinity (~nM) sites seems clinically irrelevant.

Magnesium and lithium share many similar characteristics, despite their difference in valence [21]. Previous studies have shown that lithium inhibition of adenylate cyclase activity, stimulated by hormones [22–25], forskolin [26, 27], or Gpp(NH)p [24], is competitive with magnesium ions. The β-adrenergic agonist and the guanine nucleotides clearly act through G proteins. The high-affinity site for forskolin is thought to be associated with the complex of the adenylate cyclase catalytic unit and

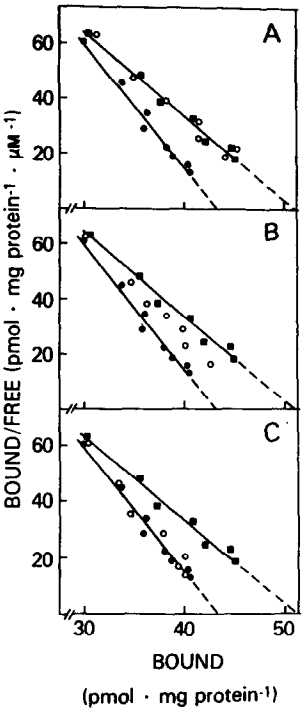

Fig. 2. Titrating lithium concentrations that inhibit ~50% of isoprenaline-induced [^{3}H]GTP binding (representative curves at 2.2 mM total magnesium concentration). Key: basal [^{3}H]GTP binding (●—●); agonist [isoprenaline (50 μM)]-induced [^{3}H]GTP binding (■—■); and agonist-induced [^{3}H]GTP binding in the presence of lithium (○—○). (A) [Li^+] = 0.6 mM: (B) [Li^+] = 1.0 mM; and (C) [Li^+] = 1.5 mM.

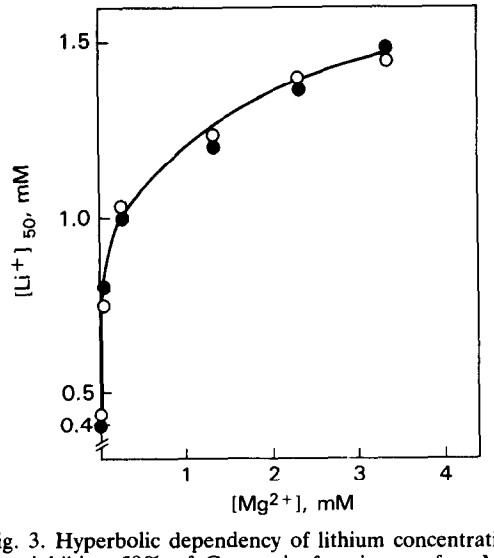

Fig. 3. Hyperbolic dependency of lithium concentrations that inhibit ~50% of G protein function on free Mg^{2+} concentrations. Lithium concentrations that inhibit ~50% of isoprenaline-induced [50 μM (●)] or carbamylcholine-induced [100 μM (○)] increases in GTP binding capacity at various magnesium concentrations are plotted as a function of free Mg^{2+} concentrations (calculated as described in Materials and Methods). Results are the averages of three separate experiments with SE of ±10%.

activated G proteins [28]. Thus, the ability of lithium to attenuate adenylate cyclase stimulation by these agents is competitive with magnesium and may be mediated through its effects on G proteins, which we also show in the present study to be inversely dependent on magnesium concentration.

Our present findings showing that increased magnesium ion concentrations can reverse lithium action may have important clinical implications. At present, no antidote is available for lithium toxicity. Our findings suggest that elevated magnesium concentrations may be of potential benefit in relieving lithium toxicity. Indeed, Worthley [29] reported that intravenous magnesium sulfate is helpful in the treatment of lithium toxicity. Moreover, one should note that changes in plasma magnesium have been found during lithium treatment [30]. However, a series of acute and chronic experiments in animals [31] failed to find a positive effect of magnesium on lithium-induced death.

Lithium is successful in ameliorating the manic-depressive condition in approximately 60% of the cases. One of the reasons that some patients do not respond to lithium treatment may be increased magnesium intracellular levels, shown here to reverse the effects of lithium on G proteins. Reducing magnesium levels in lithium-resistant patients may prove beneficial for these patients. In this regard, it is interesting to note that a large NIMH collaborative study showed elevated plasma magnesium levels in both depressed and manic patients as compared to controls [32]. However, the technical and theoretical difficulties in measuring free magnesium levels, the intracellular compartmentalization of magnesium, and the complex interrelation between intracellular and extracellular magnesium levels may limit the immediate clinical applications of our present findings.

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