Lithium Blocks a Phosphoinositide-Mediated Cholinergic Response in Hippocampal Slices

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The effects of lithium on inositol phosphate metabolism may account for the therapeutic actions of lithium in affective disorder. Muscarinic stimulation of the phosphoinositide system blocks synaptic inhibitory actions of adenosine in the hippocampal slice. At therapeutic concentrations, lithium diminished this muscarinic response, whereas rubidium, which does not affect phosphoinositide metabolism, had no effect. A dampening of phosphoinositide-mediated neurotransmission may explain the normalizing effects of lithium in treating both mania and depression.

THE DISCOVERY THAT THE PHOSphoinositide (PI) system is a prominent second messenger for neurotransmitters has led to the suggestion that the influence of lithium on inositol phosphate metabolism accounts for its psychotropic actions (*1*). According to this hypothesis, the blockade by lithium of the enzymatic hydrolysis of inositol monophosphate (*2*) alters PI signal transduction by limiting the regeneration of inositol, an essential precursor in PI synthesis. At present, however, there is little evidence that lithium affects PI-mediated neurotransmitter responses.

Muscarinic activation of the PI system in hippocampal slices, either by electrical stimulation of intrinsic cholinergic fibers or by bath application of muscarinic agonists, blocks the inhibitory action of adenosine on synaptic transmission (*3, 4*). The action of adenosine is blocked by oxotremorine-M, which strongly stimulates the PI cycle in the hippocampus, but not by oxotremorine, a weak partial muscarinic agonist of PI turnover (*5*). Instead, oxotremorine antagonizes oxotremorine-M stimulation of PI turnover (*5*) and blockade of adenosine (*3*). Cholinergic inhibition of adenosine appears to involve protein kinase C (PKC) activation, since phorbol esters, which stimulate PKC activity, reproduce the effect of muscarinic agonists (*3, 6*). We now show that therapeutic concentrations of lithium selectively impair muscarinic cholinergic responses in the hippocampus that are mediated by the PI cycle.

Since lithium's predicted effects on PI-mediated neurotransmission should depend on the level of stimulation of PI turnover, we assessed the effects of carbachol, a muscarinic agonist, on adenosine responses during prolonged application. Cholinergic stimulation of the PI cycle monitored biochemically in the hippocampus persists for more than 1 hour (*7*). Similarly, we found that the adenosine responses were blocked throughout 1 hour of carbachol application (Fig. 1). The action of carbachol is reversible, since adenosine's potency is restored when carbachol is washed away (*3*). Furthermore, reapplication of carbachol restored the blockade of adenosine during a second hour of incubation (Fig. 1). To test the actions of lithium on this PI-mediated response, we ensured that carbachol blocked adenosine for 1 hour, and then we added lithium to the buffer 1 hour before a second challenge with carbachol. Under these conditions, lithium impaired the ability of carbachol to block adenosine (Fig. 1). Similar results were obtained when oxotremorine-M was used instead of carbachol.

The effect of lithium was concentration-dependent, with a partial effect at 0.5 m*M* lithium and near maximal effects at 1 m*M* and 2 m*M* lithium (Fig. 2). The inhibition of carbachol by lithium was also time dependent, beginning after 10 to 15 minutes of incubation, with submaximal and maximal effects apparent at 30 and 60 minutes, respectively. To ascertain the ionic selectivity of lithium, we examined the effect of rubidium, which does not inhibit inositol monophosphate hydrolysis (*2*). At concentrations of rubidium up to 2 m*M*, no reversal of the carbachol effect was observed.

Because the actions of lithium are reversible, a general toxic effect on the slices is unlikely. After exposure to lithium and carbachol under standard conditions, the slices

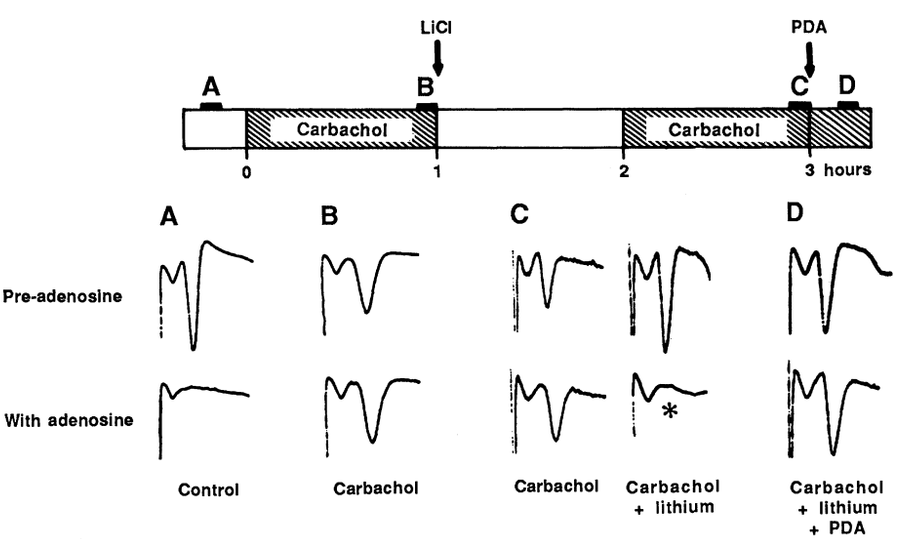

Fig. 1. Lithium blocks the PI-mediated response to carbachol. The protocol used to monitor carbachol's block of adenosine is shown. The sequence and duration of drug applications are indicated on the bar above tracings of the CA1 population spike (PS) elicited by stimulation of Schaffer collaterals (0.1 Hz), for which standard recording conditions were used (*3*). Letters above the bar indicate times at which tracings were taken. Traces in the top row were made before application of adenosine, those in bottom row during maximal response to adenosine at the end of a 5-minute application. (**A**) Application of adenosine (80 μ*M*) reversibly suppressed the PS. (**B**) Carbachol (50 μ*M*), applied for 1 hour, blocked adenosine's inhibitory action. Slices were then washed for 1 hour with control saline or saline containing lithium chloride (1 m*M*). (**C**) After a 1-hour reapplication of carbachol, the PS monitored in control saline remained resistant to adenosine, whereas the PS recorded in the presence of lithium chloride was reversibly suppressed by adenosine (*). (**D**) Subsequent addition of 2 μ*M* PDA to lithium-containing saline restored blockade of adenosine. Data shown are from one pair of representative experiments. Group data are presented in Fig. 2. As reported previously (*3*), application of carbachol transiently suppresses the PS, which returns to approximately two-thirds of initial amplitude as shown by the smaller PS in the top row of (B) and first column of (C) as compared to (A). Tracings shown in the second column of (C) and in (D) are taken from another slice, which was treated with lithium chloride (1 m*M*) after the first hour of carbachol administration.

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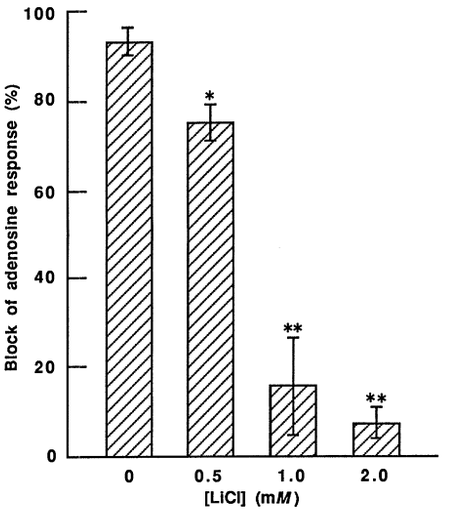

Fig. 2. Concentration dependence of lithium's reversal of the carbachol block of adenosine. The efficacy of the block of adenosine by carbachol was assessed after the second hour of carbachol application as described in Fig. 1 by comparing the PS amplitude before and after application of adenosine (80 μM). Adenosine completely suppressed the PS in the absence of carbachol but was minimally effective in the presence of carbachol (0 mM LiCl). Administration of 1 mM or 2 mM LiCl along with carbachol greatly reduced carbachol's block of adenosine. Data are the mean values of the number of determinations with bars indicating the standard error of the mean. Statistical analysis was performed by Student's t test. Significant differences shown are as follows: *, $P < 0.03$; **, $P < 0.003$ with Bonferroni correction for multiple comparisons. Number of determinations were as follows: for 0 mM LiCl, $n = 13$; for 0.5 mM LiCl, $n = 3$; for 1 mM LiCl, $n = 6$; and for 2 mM LiCl, $n = 11$.

were superfused for 60 minutes with control saline that did not contain lithium or carbachol. At the end of this period, the ability of carbachol to reverse adenosine's effect was restored and persisted throughout 60 minutes of application of carbachol, essentially as observed in fresh slices ($n = 4$).

Because cholinergic reversal of the effect of adenosine appears to involve PKC, we examined the influence of lithium on the effects of phorbol 12,13-diacetate (PDA) on adenosine. At a time when the blockade of adenosine by carbachol was abolished by lithium, PDA still reversed the effect of adenosine (Fig. 1), an indication that lithium acts in the PI cascade before PKC activation.

The cholinergic blockade by lithium appears to involve the PI cycle. In hippocampal slices, lithium elevates concentrations of inositol 1-phosphate in the presence of muscarinic agonists (*7, 8*). Moreover, lithium treatment of rats in vivo reduces muscarinic stimulation of PI turnover in brain slices, which has been measured biochemically (*9*). Rubidium, which does not inhibit inositol phosphatases, does not reproduce the effects of lithium. The requirement for prolonged stimulation with carbachol to demonstrate the lithium block fits well with the time required for the buildup of inositol phosphates and the presumed depletion of substrates for the PI cycle.

How might lithium block PI-mediated cholinergic responses in hippocampal slices? The cholinergic response we have observed appears to involve PKC since it is reproduced by phorbol esters. Since the response to phorbol esters in lithium-treated slices is normal, activation of PKC is apparently impaired by lithium treatment. This finding fits well with the inhibition of muscarinic-stimulated PI turnover by lithium (*9*).

We showed earlier that therapeutic concentrations of lithium retard the relaxation of smooth muscle elicited either by cholinergic or histamine stimulation acting through the PI cycle (*10*). This result may also reflect a diminished activation of PKC, since under the experimental conditions used phorbol esters relax smooth muscle (*11*).

Our study demonstrates that alterations in PI-associated central neurotransmission occur at therapeutic concentrations of lithium. These short-term effects of lithium might reflect changes leading to the delayed therapeutic actions of lithium in affective disorders. Numerous neurotransmitters act through the PI system. The dampening action of lithium on PI-associated neurotransmission could prevent excessive excursions of individual neurotransmitter systems from basal activity, with different PI-linked transmitters separately involved in manic and depressive episodes. This model would explain the normalizing action of lithium in providing symptomatic relief and serving as prophylaxis against episodes of both mania and depression (*12*).

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