Lithium dampens neurotransmitter response in smooth muscle: Relevance to action in affective illness

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Contributed by Solomon H. Snyder, January 3, 1986

**ABSTRACT** Lithium, by inhibiting inositol phosphate metabolism, interferes with the phosphatidylinositol ("phosphoinositol") cycle, which is stimulated by numerous hormones and neurotransmitters. To examine the relevance of this action to neurotransmission, we evaluated effects of lithium treatment on smooth muscle responses to transmitters. In lithium-pretreated tracheal muscle, the relaxation following carbachol or histamine contractions is retarded. Lithium does not affect relaxation following contractions elicited by treatment with KCl and phorbol 12,13-diacetate in combination, which bypasses receptor stimulation of the phosphatidylinositol cycle. Half-maximal effects of lithium occur at 1 mM, corresponding to therapeutic concentrations. Dampening of neurotransmitter responses by lithium treatment may explain the unique ability of lithium to relieve and prevent both mania and depression.

Lithium is a major therapeutic agent in affective disorder, exerting both prophylactic and acute therapeutic action in mania and in some forms of depression (1). Studies of the mechanism of action of lithium have focused upon its effects on biogenic amine neurotransmitters (2, 3), which are involved in actions of antidepressant drugs and may play a role in the pathophysiology of affective disorder (4). The ability of lithium to affect the phosphatidylinositol (PtdIns) system, which is stimulated by biogenic amine neurotransmitters, has led recently to suggestions that interference with this cycle may mediate lithium’s therapeutic action (5, 6).

The PtdIns cycle is a major second-messenger system mediating actions of numerous hormones and neurotransmitters (7–9). Receptor stimulation triggers the cleavage of phosphatidylinositol 4,5-bisphosphate, giving rise to diacylglycerol and inositol 1,4,5-trisphosphate (Fig. 1). Inositol trisphosphate mobilizes intracellular calcium, while diacylglycerol activates protein kinase C by enhancing its affinity for calcium which stimulates protein kinase C activity. Phosphatase enzymes sequentially remove phosphate groups from inositol trisphosphate, giving rise to free inositol which is then converted to phosphatidylinositol bisphosphate to reinitiate the PtdIns cycle.

Inhibition by lithium of phosphatase activity causes a buildup of various inositol phosphates (6). In principle, this action could slow down the PtdIns cycle and thus dampen cellular responses to neurotransmitters. However, the relevance of this effect of lithium to its therapeutic action is unclear, since in most systems employed to evaluate lithium’s action on phosphatidylinositol phosphates, 5–10 mM lithium is used, higher than the therapeutic serum levels of lithium, about 1 mM (1). Moreover, evidence is lacking that lithium’s effect on the PtdIns cycle actually alters cellular response to neurotransmitters.

**Fig. 1.** Schematic diagram of PtdIns cycle and effect of lithium. Receptor stimulation by agonist triggers hydrolysis of phosphatidylinositol 4,5-bisphosphate (PtdIns-P2) to diacylglycerol (AcylGro) and inositol 1,4,5-trisphosphate (Ins-P3). Diacylglycerol activates protein kinase C (PKC), an action mimicked by phorbol esters. Inositol trisphosphate induces release of calcium from intracellular stores. Activated protein kinase C (PKC*), and elevated levels of intracellular calcium act together in mediating physiological responses to receptor stimulation. Inositol phosphates and diacylglycerol are recycled to replenish phosphatidylinositol bisphosphate stores. Reutilization of inositol phosphate is blocked by lithium, so that lithium may alter PtdIns-M-mediated responses.

The PtdIns system mediates contractile responses to neurotransmitters in smooth muscle. In a wide variety of smooth muscle preparations, receptor-mediated stimulation of the PtdIns cycle by neurotransmitters and inositol trisphosphate-induced release of calcium from intracellular stores have been documented (7, 9–14). Also, we have demonstrated potent actions of phorbol esters, which activate protein kinase C (15, 16), on contractile responses in smooth muscle (17, 18). To test the hypothesis that lithium’s inhibition of inositol phosphate turnover alters the physiological response to transmitters, we sought a smooth muscle system in which the PtdIns cycle could be maximally stimulated and the contractile response maintained so that any slowdown in the cycle could be reflected in the contractile response. In smooth muscle preparations such as the guinea pig ileum and pulmonary artery, neurotransmitter-elicited

**Abbreviation:** PtdIns, phosphatidylinositol.

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contractions fade with persistent stimulation. However, sustained contractions elicited by muscarinic cholinergic stimulation are readily elicited in the guinea pig trachea. Since its contractile response to muscarinic stimulation is largely independent of extracellular calcium (19, 20), the trachea is particularly well-suited for investigating lithium’s effects on the PtdIns cycle which is thought to be involved in mediating contractile response to agonists in this tissue (12, 21). Using this preparation, we found that lithium in therapeutic concentrations dampens responses to neurotransmitter stimulation.

METHODS

Trachea from male guinea pigs (300–500 g) were dissected free of surrounding tissue and cut transversely into segments of 1–3 adjacent tracheal rings. Three segments were tied into a chain and hung in a 25-mL tissue bath containing 95 or 115 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, and 11.6 mM glucose. This buffer was kept at 37°C and continuously bubbled with 5% CO₂ in oxygen. One end of the chain was fixed, and the other was attached to a Grass force-displacement transducer for isometric measurements of tension. Initial resting tension was set at ~2 g. The tissue was allowed to equilibrate for at least 30 min before administration of drugs. Depolarizing buffer was prepared by replacement of NaCl with KCl on an equimolar basis. Phorbol 12,13-diacetate was obtained from LC Services (Woburn, MA). Other agents were obtained from standard commercial sources.

RESULTS

Maximal contractions of the trachea were elicited by incubation with 40 μM carbachol, a concentration that strongly stimulates the PtdIns cycle (11), for 2 hr in either the presence or the absence of 3 mM lithium. Tension during this incubation was not affected by lithium. However, at the end of 2 hr, the relaxation initiated by washing carbachol from the tissue with multiple, rapid changes of the incubation fluid was prolonged in tissues incubated with lithium. In the absence of lithium, relaxation to 50% of the tension occurred by about 5 min. In contrast, in lithium-treated samples, relaxation is markedly slowed, with a 50% decrease in tension at 12 min (Fig. 2A).

To determine whether the contractile response to carbachol stimulation is altered by this treatment, tissues were rechallenged with carbachol at 1 μM. Lithium has no effect on the amplitude or rate of rise of the carbachol response, indicating that lithium’s effect is selective for relaxation.

Relaxation following incubation with carbachol can also be initiated by atropine, a selective muscarinic antagonist. With this method, relaxation times are more rapid and reproducible than when carbachol is diluted by repeated changes of the incubation buffer. Even more striking prolongation of the relaxation phase is produced by lithium when atropine is used. After a 2-hr incubation of tissue with 40 μM carbachol, 4 μM atropine was added to initiate relaxation. Tissue incubated with both carbachol and 3 mM lithium required 3.4 times longer to relax by 90% than those exposed to carbachol alone (n = 4). This ratio increased to 4.8 with 40 μM atropine (n = 7; Fig. 3). Even at the higher concentrations, atropine is still a selective antagonist, as it does not affect contractions produced by prostaglandin F₂α.

To assess the dependence of this effect on the concentration of lithium, tissues were incubated with 40 μM carbachol and a range of lithium concentrations for 2 hr. Some prolon-
Table 1. Lithium inhibition of relaxation of guinea pig trachea

<table>
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<tr>
<th>Contractile stimulus</th>
<th>% increase in $t_{99}$</th>
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<tr>
<td>Carbachol (4 μM)</td>
<td>73 ± 9</td>
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<tr>
<td>Histamine (400 μM)</td>
<td>59 ± 10</td>
</tr>
<tr>
<td>KCl (59 mM) plus PAC_2 (4 μM)</td>
<td>−6 ± 7</td>
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Tracheal rings were incubated for 2 hr with each contractile stimulus, with and without 3 mM LiCl. Each of these stimuli produces comparable near-maximal contractions. At the end of 2 hr, relaxation was initiated by atropine (40 μM) or triprolidine (40 μM) for the carbachol- and histamine-induced contractions, respectively. For the KCl/phorbol 12,13-diacetate (PAC_2)-induced contractions, relaxation was started by switching back to nondepolarizing buffer without phorbol ester. The percent increase (mean ± SEM, n = 3 preparations) in the times required to achieve 90% relaxation ($t_{99}$) produced by lithium is shown for each contractile stimulus.

Relaxation rate of relaxation was observed at 0.5 mM lithium, and a half-maximal effect occurred at about 1 mM (Fig. 3). Prolonged relaxation is not restricted to the recovery from muscarinic cholinergic stimulation. We observed a similar effect of lithium on the relaxation following histamine-induced contractions (Table 1).

We wondered whether the effect of lithium is restricted to receptor-mediated responses or might occur with any type of contraction. Stimulation of protein kinase C with phorbol esters, when combined with potassium depolarization, also produces a large sustained contraction of the guinea pig trachea (18). Relaxation following a 2-hr maximal contraction produced by bypassing receptor stimulation in this manner was not affected by 3 mM lithium, indicating that lithium’s effect is selective for receptor-mediated events (Fig. 2B; Table 1).

In other experiments, we examined the time course for the actions of lithium (Fig. 4). The relaxation slowed slightly following exposure to carbachol alone for 30 min or longer. However, a 1-hr incubation with lithium and carbachol further prolonged relaxation. Maximal influence occurred at 2 hr and no further increase was observed with 4 hr of incubation. Incubation with lithium alone for 2 hr did not alter the relaxation following a brief exposure to carbachol. Addition of lithium just prior to atropine did not alter the relaxation rate. Thus, to ensure maximal effects of lithium, the incubation medium must include both carbachol and lithium during this period. Lithium’s effect on relaxation was most apparent when contraction was elicited by 40 μM carbachol, but qualitatively similar effects were produced by 4 μM carbachol (Table 1).

**DISCUSSION**

Might the effects of lithium on the PtdIns cycle underlie its therapeutic efficacy in affective illness (5, 6, 22)? This model of lithium’s action predicts that low concentrations of lithium alter the physiological response to neurotransmitters that stimulate the PtdIns cycle. Our findings that lithium at therapeutic concentrations dampens or prolongs neurotransmitter responses provide support for this hypothesis.

Several features of lithium’s effect on neurotransmitter responses indicate that its action is mediated by interference with the PtdIns cycle. Lithium affects responses to both muscarinic cholinergic and histamine H1 receptors, both of which are linked to the PtdIns cycle, whereas contractions elicited by depolarization, effectively bypassing receptor stimulation, are not affected by lithium. Further, nearly an hour of coinubcation with lithium and agonists that stimulate the PtdIns cycle is required to produce effects suggesting that a buildup of inositol phosphates is involved. In addition, the slowing of relaxation following incubation with 40 μM carbachol is greater than with 4 μM, consistent with biochemical data that the higher concentration of carbachol induces greater stimulation of the PtdIns cycle (11). Since a variety of inositol phosphates accumulate during lithium treatment (23-28), it is unclear whether inositol trisphosphate or other inositol phosphates are primarily involved.

Muscarinic stimulation inhibits relaxation of tracheal smooth muscle produced by activation of the adenylate cyclase system (29). We also found that incubation with carbachol prolonged relaxation in control tissues (Fig. 4). Therefore, one can view lithium’s action as a further enhancement of carbachol’s ability to retard relaxation.

Although these studies utilized smooth muscle, the similarity of neurotransmitter actions on the PtdIns cycle in the brain and peripheral tissues argues that lithium effects on neurotransmitter responses mediated by the PtdIns system occur also in brain, especially since lithium at therapeutic levels does cause accumulation of inositol phosphates in brain in vivo (5, 25, 30). In electrophysiological studies using the hippocampal slice preparation, we have found that low concentrations of lithium also alter neuronal cholinergic responses (unpublished observations). In contrast to other drugs that either stimulate or depress mood, the “normalizing” ability of lithium to relieve both mania and depression has been difficult to explain. Since lithium’s effect appears to depend on the level of activation of the PtdIns system, lithium should be most effective at sites where the PtdIns system is overactive, which may differ in mania and depression. Transmitters affected would include all those known to act via the PtdIns cycle, including norepinephrine, serotonin, acetylcholine, histamine, and several peptides. This “second-messenger” concept of the actions of lithium fits better with the normalizing effects of lithium than would influences on any single transmitter. If lithium’s interference with the PtdIns cycle accounts for its therapeutic action in affective disorder, we suggest that chemicals that potently and selectively inhibit inositol phosphate metabolism may also be therapeutically active.

We thank N. Bruce for expert secretarial assistance. This work was supported by U.S. Public Health Service Grants MH18501 and DA00266 and Research Scientist Award DA00074 (to S.H.S.). J.M.B. is a Lucille P. Markey Scholar, and this work was supported in part by a grant from the Lucille P. Markey Charitable Trust and by a grant of the Laboratories for Therapeutic Research.

Adults and Children (Williams & Wilkins, Baltimore), pp. 331–350.