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# Lithium stimulates glutamate "release" and inositol 1,4,5-trisphosphate accumulation via activation of the *N*-methyl-D-aspartate receptor in monkey and mouse cerebral cortex slices

(manic depression/bipolar disorder/primates)

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ABSTRACT **Beginning at therapeutic concentrations (1–1.5 mM), the anti-manic-depressive drug lithium stimulated the release of glutamate, a major excitatory neurotransmitter in the brain, in monkey cerebral cortex slices in a time- and concentration-dependent manner, and this was associated with increased inositol 1,4,5-trisphosphate [Ins(1,4,5)$P_3$] accumulation. (±)-3-(2-Carboxypiperazin-4-yl)propyl-1-phosphoric acid (CPP), dizocilpine (MK-801), ketamine, and $Mg^{2+}$-antagonists to the *N*-methyl-D-aspartate (NMDA) receptor/channel complex selectively inhibited lithium-stimulated Ins(1,4,5)$P_3$ accumulation. Antagonists to cholinergic–muscarinic, $\alpha_1$-adrenergic, 5-hydroxytryptamine$_2$ (serotoninergic), and $H_1$ histaminergic receptors had no effect. Antagonists to non-NMDA glutamate receptors had no effect on lithium-stimulated Ins(1,4,5)$P_3$ accumulation. Possible reasons for this are discussed. Similar results were obtained in mouse cerebral cortex slices. Carbetapentane, which inhibits glutamate release, inhibited lithium-induced Ins(1,4,5)$P_3$ accumulation in this model. It is concluded that the primary effect of lithium in the cerebral cortex slice model is stimulation of glutamate release, which, presumably via activation of the NMDA receptor, leads to $Ca^{2+}$ entry. Ins(1,4,5)$P_3$ accumulation increases due to the presumed increased influx of intracellular $Ca^{2+}$, which activates phospholipase C. These effects may have relevance to the therapeutic action of lithium in the treatment of manic depression as well as its toxic effects, especially at lithium blood levels above 1.5 mM.**

The molecular mechanism of action of lithium in the treatment of manic depression (or bipolar disorder) is not known. Several theories have been advanced, including brain inositol depletion; amplification of cholinergic neurotransmission; and modulation of guanine nucleotide binding protein function, gene expression, protein phosphorylation, and Na,K-ATPase levels [see review by Jope and Williams (1)]. We previously showed that lithium increased accumulation of inositol 1,4,5-trisphosphate [Ins(1,4,5)$P_3$] in cerebral cortex slices of guinea pig, rabbit, and rhesus monkey (2, 3). In the case of guinea pig and rabbit, inositol 1,3,4,5-tetrakisphosphate was also increased. These effects are in contrast to cholinergically stimulated cerebral cortex slices of rat and mouse, where lithium decreased accumulation of Ins(1,4,5)$P_3$ and inositol 1,3,4,5-tetrakisphosphate (2, 4, 5). The explanation for the species differences lies in the fact that rat and mouse cerebral cortex slices are uniquely deficient in inositol (2, 6, 7), and when the incubation medium was supplemented with inositol, cerebral cortex slices of these two species also showed lithium-stimulated increases in Ins(1,4,5)$P_3$ and inositol 1,3,4,5-tetrakisphosphate (2).

In the case of rhesus monkey, neither inositol nor an agonist was required to demonstrate lithium-stimulated accumulation of Ins(1,4,5)$P_3$ (3). This suggested that an endogenous neurotransmitter was involved in the lithium effect. We show here that, beginning at therapeutic concentrations, lithium stimulated the release of glutamate in rhesus monkey and mouse cerebral cortex slices, and this in turn increased accumulation of Ins(1,4,5)$P_3$ via activation of the *N*-methyl-D-aspartate (NMDA) receptor.

## EXPERIMENTAL PROCEDURES

**Preparation and Handling of Monkey Brain Slices.** Except as modified below, slice preparation, restoration, labeling, quench, extraction of inositol phosphates, and separation by HPLC were as described by Lee *et al.* (2) and Dixon *et al.* (3). Fresh cerebral cortex was obtained twice monthly from rhesus monkeys being sacrificed at the Wisconsin Regional Primate Center, essentially as described (3). In brief, an adult female monkey was deeply anesthetized with sodium pentobarbital, the skull cap was removed, the brain was removed and immediately chilled, and coronal sections of cerebral cortex were prepared and cross-chopped (0.5 × 0.45 × 0.45 mm). Where indicated in the figure legends, the preparative buffer [nominally $Ca^{2+}$-free Krebs–Henseleit bicarbonate saline (KHBS)] was supplemented with 10 mM $Mg^{2+}$ to prevent damage from excessive NMDA receptor activation, especially in the early stages of slice preparation and restoration (8). Slicing and incubation procedures, perchloric acid quench, extraction of inositol phosphates, and separation by HPLC were as described by Lee *et al.* (2).

**Ins(1,4,5)$P_3$ Receptor Binding Assay.** Ins(1,4,5)$P_3$ mass was determined by the receptor binding assay as described (3) but with the following modification. Binding protein was prepared from beef liver. The buffer in the 5% sucrose washing layer was identical to the buffer in the assay (pH 9). Preparation of the binding protein from bovine adrenal glands required the use of a buffer of lower pH (pH 5) to produce a tight pellet. However, the Ins(1,4,5)$P_3$ binding protein prepared from beef liver, which was used for mass determination in this series of experiments, forms tight but easily resuspended pellets when centrifuged at pH 9.

**Measurement of Medium Glutamate.** After removal of slices, the incubation medium was centrifuged at 200 × *g* for

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Abbreviations: Ins(1,4,5)$P_3$, inositol 1,4,5-trisphosphate; CPP, (±)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphoric acid; NMDA, *N*-methyl-D-aspartate; 7-CKA, 7-chlorokynurenic acid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; AMPA, DL-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

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5 min to remove any particulate material. The supernatants were boiled for 5 min in a water bath and then stored at −20°C. Before assay, the samples were centrifuged at 16,000 × *g* to remove denatured protein and a small amount of buffer salts, which precipitate on freezing. Any particles present in the assay solution can cause drastic instability in the spectrofluorometric reading (see below). Glutamate was determined enzymatically by incubation of samples with glutamate dehydrogenase and $NAD^+$, followed by spectrofluorometric measurement of the formation of NADH (9). Samples containing 0.2–1.0 nmol of L-glutamate were incubated at 25°C for 30 min in a total vol of 1 ml of buffer of the following composition: 20 mM Tris base/0.4 M hydrazine hydrate/10 mM $MgCl_2$/5 mM EDTA, pH 8.5/0.6 mM NAD/1.2 units of bovine liver glutamate dehydrogenase (Sigma; specific activity, 43 units/mg). Controls without enzyme were also analyzed for each sample. Fluorescence was measured in an SLM 8000 spectrofluorometer (SLM Aminco, Urbana, IL) at an excitation wavelength of 340 nm and an emission wavelength of 453 nm.

**Protein Measurement.** The acid-insoluble protein was resuspended in 2 ml of 0.2 M NaOH. After 12 hr at room temperature, the suspension was diluted with 2 ml of water, and 50-μl aliquots were assayed by the microtiter protocol of the Pierce BCA protein assay. The microtiter plates were incubated at 37.5°C for 2 hr and read at 590 nm. Total $^3H$ radioactivity in the acid-insoluble fraction was also determined from aliquots of the above protein suspension.

**Statistical Analysis.** The figures illustrate representative experiments. Each experiment was carried out two or more times. The values are expressed as the mean ± SEM of 3–6 identical tissue incubations in each experiment.

## RESULTS

**Effects of Receptor Antagonists on Lithium-Stimulated Accumulation of Ins(1,4,5)$P_3$ in Monkey Cerebral Cortex Slices.** Fig. 1 shows the effects of receptor antagonists on lithium-stimulated Ins(1,4,5)$P_3$ accumulation. Atropine, phentolamine, ketanserin, and chlorpheniramine, antagonists at the cholinergic–muscarinic, $\alpha_1$-adrenergic, 5-hydroxytryptamine$_2$ (serotoninergic), and $H_1$ histaminergic receptors, respectively, had no effect on Ins(1,4,5)$P_3$ accumulation in the presence of lithium. On the other hand, (±)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphoric acid (CPP), which selectively blocks the ionotropic NMDA receptor in a competitive manner, reduced lithium-stimulated Ins(1,4,5)$P_3$ accumulation. Dizocilpine [(±)MK-801], ketamine, and 10 mM $Mg^{2+}$, which block the NMDA receptor ion channel in a noncompetitive manner, also reduced lithium-stimulated Ins(1,4,5)$P_3$ accumulation. 7-Chlorokynurenic acid (7-CKA; 10 μM), which blocks the glycine site on the NMDA receptor, had no effect. This is probably due to the fact that considerable glycine is present in the incubation medium and is not effectively displaced by that concentration of 7-CKA. 6,7-Dinitroquinoxaline-2,3-dione (DNQX), which blocks the DL-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) ionotropic receptor, also had no effect on basal or lithium-stimulated Ins(1,4,5)$P_3$ accumulation. This may be due to the fact that the permeability of the AMPA channel to $Ca^{2+}$ is much lower than that of the NMDA channel (10). Note that under the conditions of this experiment, in which slices were incubated with lithium for 1 hr, antagonists at the NMDA receptor/channel complex also reduced Ins(1,4,5)$P_3$ accumulation in the absence of lithium. Under these conditions, there is considerable accumulation of glutamate in the absence of lithium, which probably accounts for most, if not all, of Ins(1,4,5)$P_3$ accumulation found in the absence of lithium (see below). Under different incubation conditions, where the slices were first preincubated in $Ca^{2+}$-free medium, followed by CPP and finally $Ca^{2+}$ for 20 min, CPP selectively abolished the lithium stimulation with no effect on basal Ins(1,4,5)$P_3$ accumulation (Fig. 2). Fig. 3 shows the effects of lower concentrations of (+)MK-801, which would be expected to inhibit Ins(1,4,5)$P_3$ accumulation if this agent

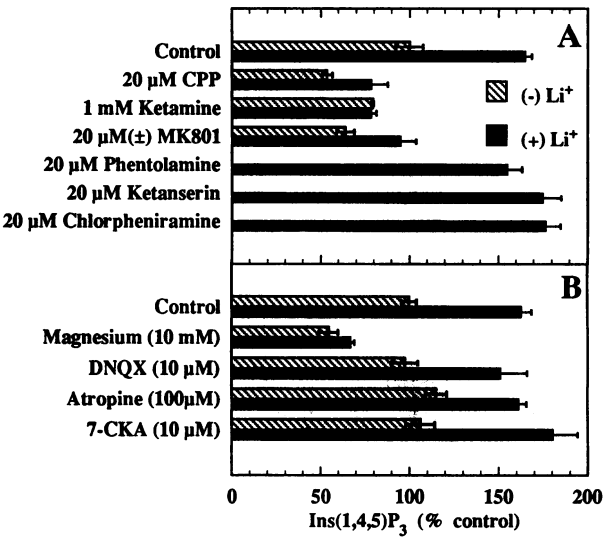


FIG. 1. Effect of neurotransmitter antagonists on accumulation of Ins(1,4,5)$P_3$ in monkey cerebral cortex slices with and without lithium, as determined by both the [$^3H$]inositol prelabeling technique and the receptor binding mass assay. (*A*) [$^3H$]Ins(1,4,5)$P_3$. Cerebral cortex slices, labeled with [$^3H$]inositol, were incubated for 1 hr with or without 25 mM LiCl, as indicated, and with and without various antagonists before perchloric acid quench. The *P* value of the increment due to $Li^+$ in the control was 0.002. There was no significant increase due to $Li^+$ in the presence of CPP or ketamine. (*B*) Ins(1,4,5)$P_3$ mass. Brain cortex slices were prepared and restored in nominally $Ca^{2+}$-free KHBS and then preincubated for 60 min in fresh buffer with or without 25 mM LiCl and with or without various antagonists. $CaCl_2$ (final concentration, 2.5 mM) was then added and the incubation mixture was quenched with perchloric acid after 20 min. Ins(1,4,5)$P_3$ mass was measured by the receptor binding assay as described. Error bars show SEM of triplicate tissue incubations. Results are expressed as percentage of control without lithium. Control = 9.51 ± 0.54 pmol/mg. The *P* values of the increment due to $Li^+$ are as follows: control, 0.001; $Mg^{2+}$, not significant; DNQX, 0.032; atropine, 0.004; 7-CKA, 0.01.

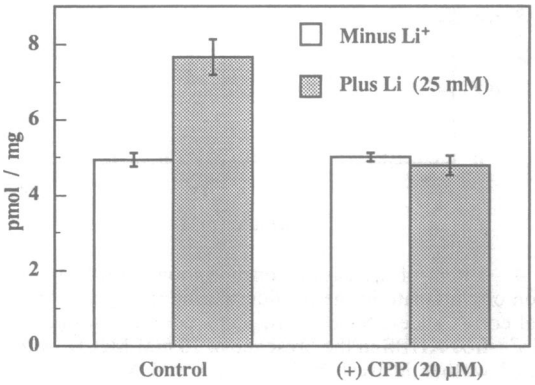


FIG. 2. Effect of CPP on the lithium-dependent increment of Ins(1,4,5)$P_3$ mass in monkey brain cerebral cortex slices. Slices were prepared and restored in nominally $Ca^{2+}$-free KHBS and then preincubated for 1 hr with or without 25 mM LiCl. After a 5-min incubation with or without CPP, $CaCl_2$ was added (final concentration, 2.5 mM) and the incubation was continued for 20 min before the quench. In the absence of CPP, the *P* value of the increment due to $Li^+$ was 0.008. In the presence of CPP, there was no significant increment due to $Li^+$.

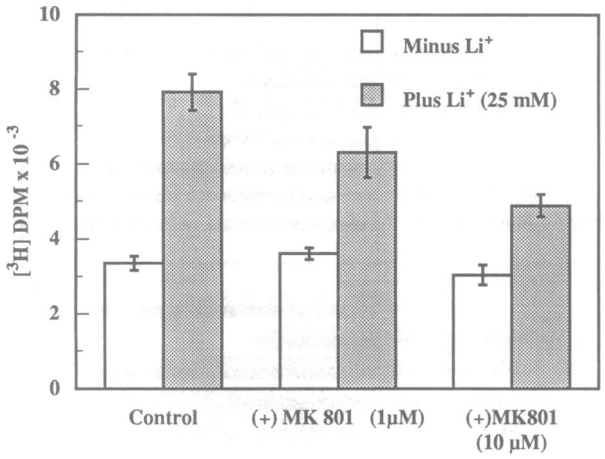


FIG. 3. Effect of (+)MK-801 on the lithium-dependent increment in [$^3$H]Ins(1,4,5)$P_3$ in monkey cerebral cortex slices. Slices were prepared, restored, and prelabeled in nominally $Ca^{2+}$-free KHBS and preincubated with or without 25 mM LiCl and with or without various concentrations of (+)MK-801 for 60 min. $CaCl_2$ was then added (final concentration, 2.5 mM) and the reaction mixture was quenched 20 min later with perchloric acid. MK-801 had no significant effect in the absence of $Li^+$. Both concentrations of MK-801 significantly reduced the $Li^+$ increment relative to the control with a $P$ value of 0.047 at 1 $\mu$M and 0.013 at 10 $\mu$M.

were acting specifically. There was inhibition of lithium-stimulated Ins(1,4,5)$P_3$ accumulation with 1 $\mu$M (+)MK-801, and this was greater at 10 $\mu$M (+)MK-801 without any effect on basal accumulation.

**Effects of Lithium on Glutamate "Release" in Monkey Cerebral Cortex Slices.** The effects of receptor/channel antagonists described above suggested that the lithium-stimulated accumulation of Ins(1,4,5)$P_3$ involved glutamatergic neurotransmission at the NMDA receptor. We therefore studied the effects of lithium on glutamate release. Release is operationally defined here as release from the slice into the incubation medium. It could be due to release from presynaptic vesicles, inhibition of reuptake into the presynaptic terminal, or inhibition of glutamate metabolism. In this paper, concentrations of lithium ranging from the therapeutic at 1–1.5 mM to the toxic (as high as 25 mM) were studied. Fig. 4 shows the effects of increasing concentrations of lithium on accumulation of glutamate in the incubation medium in monkey cerebral cortex slices. There was a significant increase in glutamate release at 1.5 mM lithium, which is the maximum therapeutic concentration. Glutamate release increased progressively with increasing lithium concentrations. Fig. 5 shows the effect of 10 mM lithium on the time-dependent accumulation of glutamate in the incubation medium. After a lag of 10 min, lithium progressively increased glutamate release over a 2-hr period. Fig. 6 shows the effects of 25 mM lithium on the release of glutamate and the accumulation of Ins(1,4,5)$P_3$. Ins(1,4,5)$P_3$ was present in the absence of lithium. This was presumably due at least in part to the presence of glutamate in the incubation medium in the absence of lithium. Further evidence for this is shown in the next section. Lithium caused a 3-fold increase in medium glutamate over control. Ins(1,4,5)$P_3$ was also increased. In general, the release of glutamate was more responsive to lithium than the level of Ins(1,4,5)$P_3$.

**Effects of Carbetapentane and CPP on the Release of Glutamate and Ins(1,4,5)$P_3$ Accumulation in the Presence and Absence of Lithium in Mouse Cerebral Cortex Slices.** Lithium also stimulated glutamate release and Ins(1,4,5)$P_3$ accumulation in mouse cerebral cortex slices (Fig. 7). CPP competitively binds at the glutamate site on the NMDA receptor. To

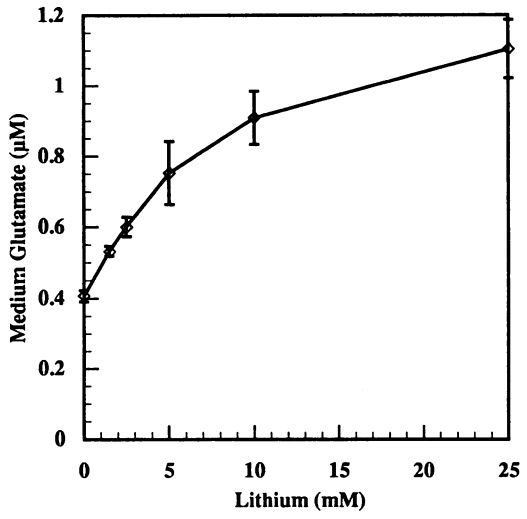


FIG. 4. Effect of increasing concentrations of lithium on accumulation of glutamate in the medium during incubation of monkey cerebral cortex slices. Slices were prepared and restored in nominally $Ca^{2+}$-free KHBS in the presence of 10 mM $MgCl_2$. After eight sequential washes in fresh buffer with 1 mM $MgCl_2$, slices were divided into 28 aliquots of ≈100 $\mu$l of gravity packed tissue and incubated twice for 30 min with complete buffer replacement at the end of each incubation. Medium volume was 3.2 ml throughout. Slices were then incubated with and without various concentrations of $Li^+$ and with 1.3 mM $CaCl_2$ for 60 min before samples of medium were separated from the slices for glutamate analysis. The $P$ values of the increment due to $Li^+$ are as follows: 0.001 at 1.5, 2.5, and 5 mM LiCl; 0.006 at 10 mM LiCl; <0.001 at 25 mM LiCl. Error bars show SEM of four tissue incubations. Tissue averaged 1.16 mg of protein per sample. Medium volume was 3.2 ml.

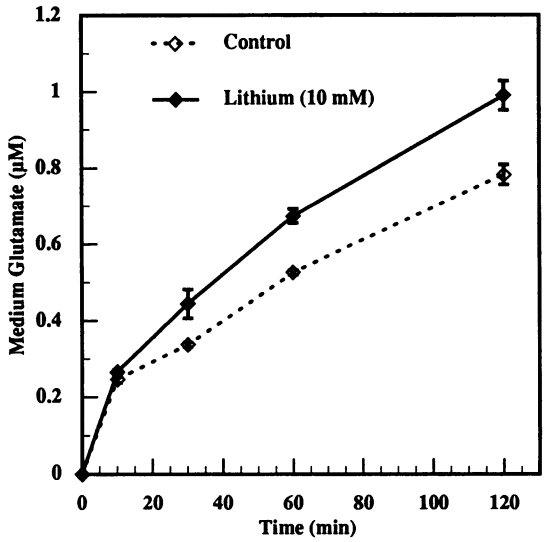


FIG. 5. Time-dependent accumulation of glutamate in the medium during incubation of monkey cerebral cortex slices with or without lithium. Slices were prepared and restored in nominally $Ca^{2+}$-free KHBS with 10 mM $MgCl_2$. Slices were then divided into aliquots and preincubated in 3.2-ml portions of nominally $Ca^{2+}$-free KHBS with 1 mM $MgCl_2$ for 30 and 60 min, with complete buffer replacement after each incubation. The final incubations were initiated by addition of fresh buffer with 1.3 mM $CaCl_2$ and with or without 10 mM LiCl. The incubations were stopped at the times indicated, the medium was removed for glutamate analysis, the tissue was homogenized with perchloric acid (3%), and the acid-insoluble protein was determined for normalization. Tissue slices averaged 1.14 mg of protein per sample. Error bars show SEM of triplicate slice incubations. The $P$ values of the increment due to $Li^+$ are as follows: 10 min, not significant; 30 min, 0.045; 60 min, 0.002; 120 min, 0.012.

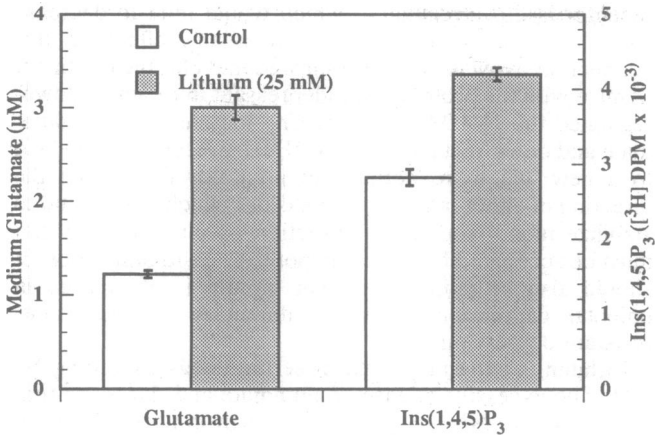


FIG. 6. Effect of lithium on medium glutamate and [$^3$H]Ins(1,4,5)$P_3$ in monkey cerebral cortex slices. Slices were prepared and prelabeled in nominally $Ca^{2+}$-free KHBS with 10 mM $MgCl_2$, and, after eight sequential washes with 1 mM $MgCl_2$, they were separated into aliquots and preincubated for 30 min with and without 25 mM LiCl and finally incubated for 20 min after addition of $CaCl_2$ (final concentration, 1.3 mM). Samples of medium were taken just before quench for glutamate analysis. Error bars show SEM of four separate tissue incubations. Tissue averaged 1.58 mg of protein per sample. Medium volume was 3.2 ml. The *P* value of the increment due to $Li^+$ was 0.001 for both glutamate and Ins(1,4,5)$P_3$.

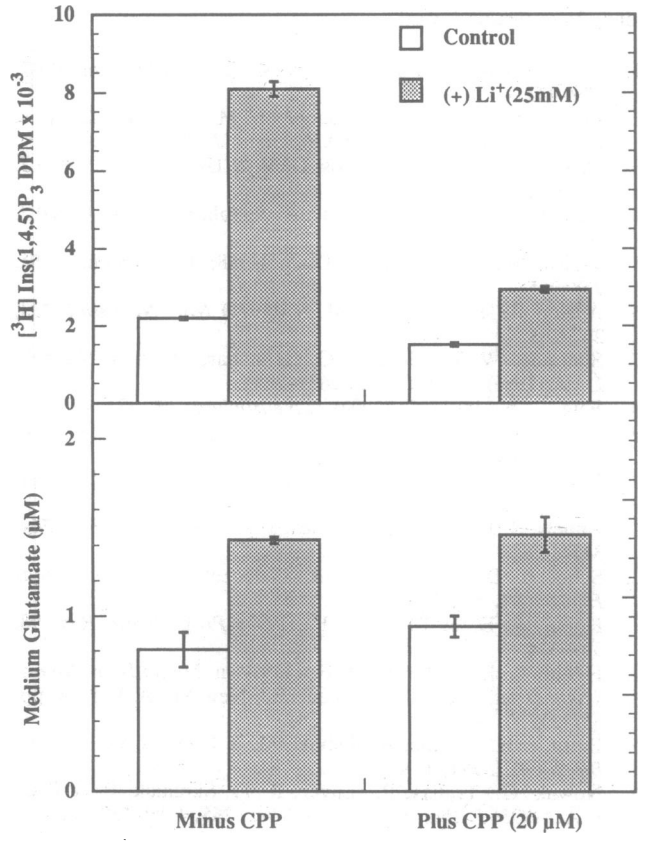


FIG. 7. Effect of CPP on lithium-dependent increments in [$^3$H]Ins(1,4,5)$P_3$ and medium glutamate of mouse cerebral cortex slices. Mouse cerebral cortex slices were prepared and prelabeled in nominally $Ca^{2+}$-free KHBS, separated into aliquots, preincubated with and without LiCl and CPP for 60 min, and then incubated with 1.3 mM $CaCl_2$ for 20 min before quench. Samples of incubation medium were taken just before quench for measurement of glutamate. Error bars show SEM of four separate tissue incubations. Protein averaged 1.24 mg of protein per sample. CPP significantly reduced Ins(1,4,5)$P_3$ both with and without $Li^+$ (all *P* values < 0.001). CPP had no significant effect on glutamate in the medium.

establish that the CPP effects on Ins(1,4,5)$P_3$ accumulation were specific for the NMDA receptor and not due to any effect on glutamate release, the effect of CPP on glutamate release was studied. CPP markedly inhibited lithium-stimulated Ins(1,4,5)$P_3$ accumulation, while it had no effect on glutamate in the incubation medium. It is also clear from these results that accumulation of Ins(1,4,5)$P_3$ is not necessary for lithium stimulation of glutamate release. Carbetapentane, which inhibits depolarization-induced glutamate release (11) and which inhibited lithium-stimulated glutamate release in monkey cerebral cortex slices (J.F.D. and L.E.H., unpublished observations), essentially abolished lithium-stimulated Ins(1,4,5)$P_3$ accumulation in mouse cerebral cortex slices (Fig. 8). This further supports the conclusion that lithium-stimulated Ins(1,4,5)$P_3$ accumulation is due to glutamate release. Carbetapentane also inhibited to some extent basal Ins(1,4,5)$P_3$ accumulation, suggesting that at least part of the Ins(1,4,5)$P_3$ seen in the absence of lithium was due to lithium-independent glutamate release.

## DISCUSSION

We show here that our previous observation of lithium-induced Ins(1,4,5)$P_3$ accumulation in rhesus monkey cerebral cortex slices in the absence of added agonists is due to lithium-stimulated glutamate release, which elevates Ins(1,4,5)$P_3$ via activation of the NMDA receptor. Several lines of evidence support this hypothesis: (*i*) Most importantly, lithium stimulates glutamate release in monkey and mouse cerebral cortex slices. (*ii*) Antagonists to the NMDA receptor/channel block the elevating effect of lithium on Ins(1,4,5)$P_3$ accumulation. (*iii*) Carbetapentane, which blocks glutamate release, inhibits both basal and lithium-stimulated Ins(1,4,5)$P_3$ accumulation. These are criteria that support involvement of a neurotransmitter—i.e., glutamate in this instance.

Although not a metabotropic receptor, activation of the NMDA receptor increases Ins(1,4,5)$P_3$ formation (10). This is presumably due to increased influx of $Ca^{2+}$, which activates phospholipase C.

It is of interest that the increase in Ins(1,4,5)$P_3$ is essentially abolished by the highly specific NMDA receptor an-

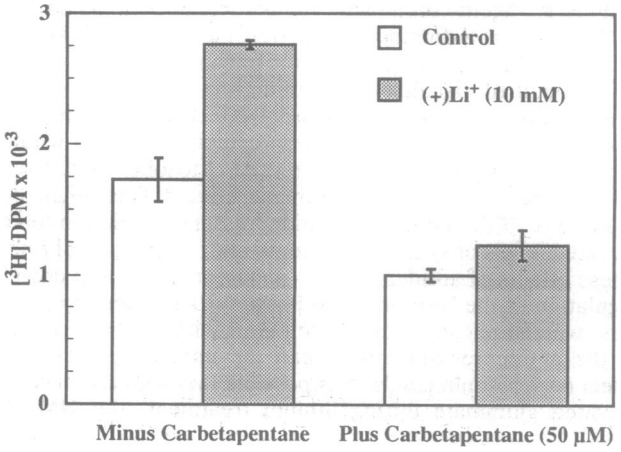


FIG. 8. Effect of carbetapentane on lithium-dependent increment in [$^3$H]Ins(1,4,5)$P_3$ in mouse cerebral cortex slices. Mouse cerebral cortex slices were prepared and prelabeled in nominally $Ca^{2+}$-free KHBS and preincubated for 30 min with or without 10 mM LiCl with or without 50 μM carbetapentane. $CaCl_2$ (final concentration, 1.3 mM) was then added and the incubation was continued for 20 min before quench. Error bars show SEM of four separate tissue incubations. Tissue averaged 1.29 mg of protein per sample. The *P* value of the decrement due to carbetapentane without $Li^+$ was 0.006 and with $Li^+$ was <0.001.

tagonist CPP as well as other NMDA receptor/channel complex antagonists, while antagonists to other glutamate receptors (Fig. 1; J.F.D. and L.E.H., unpublished observations) had no effect. This is presumably due to the fact that the $EC_{50}$ for activation of metabotropic receptors is 1–2 orders of magnitude higher than that for the NMDA receptor (12) and that the concentrations of glutamate achieved at the synapses in cerebral cortex slices in the presence of lithium are insufficient to activate the metabotropic receptors. In addition, the permeability of the AMPA and kainate channels to $Ca^{2+}$ may be insufficient to cause formation of Ins(1,4,5)$P_3$ (10).

Earlier studies reported inhibitory effects of lithium on Ins(1,4,5)$P_3$ accumulation in mouse and rat cerebral cortex slices in the absence of supplementary inositol and in the presence of high concentrations of cholinergic agents (2, 4). This is due to inositol depletion in brain cortex slices under these conditions. We have previously discussed the possible reasons for this excessive inositol depletion in rodents (2, 3, 13, 14): (*i*) Brain inositol is already limited in mouse and rat as compared to higher species. (*ii*) Lengthy incubation of rat cerebral cortex slices reduces cellular inositol by 80%. (*iii*) High concentrations of cholinergic agents (1 mM carbachol has generally been used) cause a particularly large breakdown of phosphatidylinositol and when lithium is present, so as to inhibit inositol monophosphatases and inositol 1-polyphosphatases, large quantities of inositol are trapped in the form of inositol phosphates. (*iv*) It has recently been shown that cholinergic agents markedly inhibit inositol uptake into astrocytoma cells (15). It is possible that a similar effect occurs in neuronal cells or that the effect on glial cells has secondary effects on neuronal cells to depress cellular inositol concentrations.

In contrast, as reported here, when mouse cerebral cortex slices were incubated in the absence of added cholinergic agent, lithium increased Ins(1,4,5)$P_3$ accumulation, even without addition of inositol (Fig. 8). Low endogenous levels of glutamate are likely to result in significantly less breakdown of phosphatidylinositol than high levels of cholinergic agents. Thus, minimal amounts of inositol would be trapped in the presence of lithium, and inhibition of Ins(1,4,5)$P_3$ accumulation would not occur, despite the factors that favor inositol depletion in this species. Also, inhibitory effects of cholinergic agents on inositol uptake would be avoided.

Glutamate is a dominant neurotransmitter in cerebral cortex and mediates excitatory neurotransmission. Thus, it is not difficult to visualize how lithium-induced glutamate release might exert an antidepressant effect in manic depression via activation of the NMDA receptor. This would presumably be mediated by increased cytosolic $Ca^{2+}$. How might glutamate exert an antimanic effect? This might be analogous to the actions of antidepressants, which involve elevated levels of synaptic serotonin and/or norepinephrine. These effects of antidepressants apparently lead to a down-regulation of the serotonin and possibly $\beta$-adrenergic receptors, which appears to correlate better with the time course of the antidepressant effect than the immediate elevating effect on serotonin levels. It is possible that with chronically elevated glutamate during lithium treatment, the NMDA receptor is down-regulated, which would tend to decrease $Ca^{2+}$, and this could lead to an antimanic effect. In this connection, Nowak *et al.* (16) showed that chronic treatment of mice with the prototype antidepressant imipramine down-regulated the NMDA receptor. Chronic elevation of glutamate and down-regulation of the NMDA receptor could lead to a new and more normal set point for $Ca^{2+}$ from the direction of either mania or depression, which would tend to stabilize mood from either direction—a circumstance that must be accounted for by any hypothesis of lithium action. It should also be pointed out that lithium is often used in unipolar depression or as an adjunct in antidepressant-resistant depression.

Lithium is extremely toxic at serum levels exceeding 1.5 mM. The excessive release of glutamate and thus overstimulation of the NMDA receptor at high concentrations of lithium may be a mechanism that contributes to lithium toxicity.

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