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COMMENTARY

LITHIUM AND BRAIN SIGNAL TRANSDUCTION SYSTEMS

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Recent years have seen the achievement of much exciting progress in identifying the biochemical responses of the brain to lithium, one of the most interesting drugs used in psychiatry. Lithium is a primary treatment for mania and bipolar affective disorders, so a major research focus has been directed towards solving the intriguing puzzle of its therapeutic mechanism of action. Increasing evidence points to signal transduction systems, especially the phosphoinositide second messenger system, as important targets for lithium. Many different and complex effects of lithium have been reported, providing a challenge to determine which are important for *in vivo* therapeutic or toxic responses. This necessitates a critical analysis of experimental protocols and results to place the findings in a therapeutically relevant framework. Therefore, we consider the doses or concentrations of lithium used, potential species differences, the relevance of *in vitro* manipulations to the *in vivo* situation, and effects of acute compared with chronic lithium treatment, since it is widely acknowledged that chronic treatment is required for a therapeutic response. This review focuses on identifying important questions remaining to be addressed while discussing disparate findings in studies of responses to lithium with an emphasis on the phosphoinositide system, reflective of the current investigational emphasis on this topic. Many authors have concluded that the effects of lithium on the brain are unique, so that extrapolations from other tissues are unwarranted or tenuous at best. Therefore, the present discussion is limited to investigations on brain tissue or relevant cultured cells. Other reviews have covered in detail the effects of lithium on tissues other than the brain and the early findings with neural tissue [1–4].

Phosphoinositide second messenger system

There is no question that lithium affects phosphoinositide metabolism, apparently in multiple ways. The important questions remaining to be resolved are how does lithium do so and which of its effects on phosphoinositide metabolism are therapeutically important.

The components of the phosphoinositide second messenger system have been thoroughly reviewed previously [1–4]. The major inositol-containing lipid, phosphatidylinositol (PI†), is sequentially phosphorylated to form phosphatidylinositol 4-phosphate (PIP) and phosphatidylinositol 4,5-bisphosphate (PIP_2). There is evidence that each of these lipids can be hydrolyzed, although the hydrolysis of PIP_2 to inositol 1,4,5-trisphosphate (IP_3) is usually emphasized. IP_3 acts as a second messenger to release intracellular sequestered calcium. Multiple enzymes contribute to the metabolism of IP_3, leading to its phosphorylation to inositol 1,3,4,5-tetrakisphosphate (IP_4) or dephosphorylation to any of several inositol bisphosphates (IP_2), and penultimately to inositol monophosphate (IP_1) which is dephosphorylated by inositol monophosphatase to free inositol. Hydrolysis of phosphoinositides also produces diacylglycerol (DAG) which activates protein kinase C. DAG is converted sequentially to phosphatidic acid and to CDP-DAG (also called CMP-phosphatidate or phosphatidyl-CMP) which is combined with free inositol by PI synthase to re-form PI. Activation of this system is induced by agonists stimulating receptors that are coupled to appropriate G-proteins. The stimulated G-protein, in turn activates phospholipase C to cleave phosphoinositides.

Lithium was first shown to affect the phosphoinositide system in 1971 when it was found that administration of lithium to rats decreased brain inositol concentrations [5]. In 1974 lithium was reported to inhibit inositol monophosphatase [6]. The significance of these findings became apparent when Allison and Sherman and their colleagues made several key discoveries [7–12]. Rats treated with lithium were found to have increased brain IP_1 concentrations concurrent with the reduced inositol levels [8–10], and the reduction of inositol and accumulation of IP_1 were blocked by cholinergic antagonists [7, 8]. However, relatively high doses of lithium were required to obtain depletion of inositol, as discussed below. Reduced inositol and increased IP_1 concentrations caused by lithium have been attributed to inhibition by lithium of inositol monophosphatase [10]. Lithium inhibited inositol monophosphatase uncompetitively with a K_i of

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† Abbreviations: PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_1, inositol monophosphate; IP_2 inositol bisphosphate; IP_3, inositol 1,4,5-trisphosphate; IP_4, inositol 1,3,4,5-tetrakisphosphate; and DAG, diacylglycerol.

0.8 mM [10], an exciting observation since this is within its therapeutically effective serum concentration range (about 0.5 to 1.4 mM). The finding that lithium inhibited inositol monophosphatase was confirmed and extended by other investigators [13–16], including the observation that inositol polyphosphate 1-phosphatase was also inhibited by lithium [17, 18]. These results led to the critical discovery by Berridge *et al.* [19] that lithium could be used in *in vitro* assays of phosphoinositide metabolism to cause accumulation of IP_1 to a degree that was proportional with receptor activation, an innovation that greatly simplified the measurement of phosphoinositide hydrolysis and which has been employed in numerous investigations.

Lithium and phosphoinositide metabolism

Berridge *et al.* [19] proposed that the therapeutic effect of lithium may derive from reduced agonist-induced phosphoinositide hydrolysis resulting from the inhibition by lithium of inositol monophosphatase causing depletion of inositol, which in turn depletes phosphoinositides. This has become known as the inositol depletion hypothesis and has been widely accepted in part because of its attractive simplicity and wide dissemination, although as discussed below many questions remain regarding the experimental basis of this hypothesis and its applicability to the *in vivo* responses to lithium administration. It is important to emphasize that there exist two different issues: lithium may influence individually either agonist-induced phosphoinositide hydrolysis or inositol availability without a concomitant alteration of the other, or both may be altered by lithium individually or in a connected manner. A direct test of the effects of lithium in intact cells on receptor-coupled phosphoinositide hydrolysis and on inositol availability for phosphoinositide synthesis is difficult to achieve because chronic lithium treatment is necessary to model its therapeutic use, which limits the *in vitro* approaches that can be used, and because, by inhibiting inositol phosphate phosphatases, lithium causes accumulation of inositol phosphates regardless of whether lithium increases, decreases, or does not affect phosphoinositide hydrolysis (i.e. even if lithium directly inhibited this process, IP_1 would accumulate above control values due to inhibition by lithium of inositol monophosphatase, masking any inhibition lithium may cause). Therefore, several less direct methods have been used to identify the effects of lithium on phosphoinositide metabolism and to test the inositol depletion hypothesis.

The primary observation used in the development of the inositol depletion hypothesis is the reduction of inositol that has been reported to occur in rat brain after administration of lithium [19]. This response to lithium is well-established, but the doses of lithium used, the effects of chronic compared with acute lithium administration, and the extent of the reduction of inositol concentrations must be considered. It is sometimes overlooked that only partial inhibition of inositol monophosphatase will be obtained at therapeutic concentrations of lithium so some inositol will continue to be generated under these conditions [20]; it is only with toxic concentrations of lithium that more complete inhibition is approached. Acute administration of LiCl using a dose of about 3 mmol/kg is often considered to produce relevant lithium concentrations [e.g. 21, 22], whereas higher doses are toxic. For example, 60% of rats given LiCl at 5.4 mmol/kg in five daily doses died [9], clearly establishing this as too high a dose to use to study therapeutically relevant effects of lithium. The LD_{50} of LiCl administered intraperitoneally to adult male rats was reported to be 12 mmol/kg [23], so data obtained with high doses of lithium often are near this value. Only at toxic doses did lithium reduce inositol concentrations in rat brain [24], and even then the reduction was limited. LiCl administered at 3.6 [9] or 4 [11] mmol/kg did not lower rat brain inositol levels but LiCl at 6 to 14 mmol/kg reduced rat cortical inositol concentrations by 35% [11]. Additionally, in rats of another strain LiCl (5 mmol/kg) did not reduce inositol concentrations in six brain regions [25]. Thus, moderate doses of lithium either do not or only slightly influence rat brain inositol concentrations. Even with toxic doses of lithium it is unknown if such a modest decrease (35%) of inositol is sufficient to limit phosphoinositide synthesis. Thus, more investigations are required to determine if acute administration of lithium lowers brain inositol enough to have a functional influence on phosphoinositide metabolism and, if so, what doses of lithium are required and which species are susceptible. Because of the limited effects of moderate doses of lithium on inositol, it has been suggested that inositol depletion may be limited to selected cells [4], resulting in greater depletion at these sites, although the mechanism for such a selectivity is not known. There is some evidence for selectivity, as the original work by Allison and Blisner [7] showed that cholinergic muscarinic antagonists completely blocked lithium-induced depletion of inositol in rat cortex. This was the first of several findings that have indicated that lithium has selective effects on the phosphoinositide system associated with cholinergic cells, as discussed further below. Therefore it is now important to establish (i) whether or not therapeutically relevant doses of lithium deplete inositol sufficiently to reduce phosphoinositide concentrations and if this subsequently results in lowered agonist-stimulated second messenger formation, and (ii) if there are localized sites at which these effects are pronounced.

Most relevant to potential therapeutic mechanisms of action of lithium are measurements of brain inositol after chronic, rather than acute, lithium administration. Nine daily injections of lithium (3.6 mmol/kg) did not alter rat brain inositol levels, although IP_1 increased several-fold [9]; after 18 and 22 days of dietary lithium rat brain inositol was reduced only 19% and 8%, respectively [11]; 13 days of dietary lithium treatment did not alter the inositol concentration in rat striatum and increased cerebral cortical inositol by 30% [26]; and normal levels of inositol were found in six regions of rat brain after 2 weeks of lithium (2.5 mmol/kg) [24]. These results suggest that the more therapeutically relevant chronic administration of lithium has an even smaller inositol-depleting effect in rat brain than does acute lithium

treatment. Thus, it remains to be demonstrated that chronic lithium treatment does reduce brain inositol concentrations and, if so, that this small depletion of inositol can result in a significant depletion of phosphoinositides and account for the therapeutic response to lithium or if there is a greater localized depletion of inositol and phosphoinositides which has not yet been identified.

Another approach to test if phosphoinositide depletion resulting from lithium-induced inositol depletion occurs and causes less stimulation-induced phosphoinositide metabolism is to measure the production of the inositol phosphate products. The *in vivo* concentration of IP_3, the primary second messenger produced by PIP_2 hydrolysis, was measured after chronic lithium administration and was found to increase [27] or not change [28] in mice and rats, respectively, indicating further that, if it occurred, *in vivo* depletion of inositol did not impair significantly the normal production of IP_3. Further, after chronic lithium treatment, stimulation with *in vivo* administration of pilocarpine increased the IP_3 concentration in mouse and rat brain [27, 28], showing that there was a substantial pool of PIP_2 available in a stimulus-responsive compartment even after chronic lithium administration. Possible evidence that these pools can be depleted under extraordinary circumstances was provided by the finding of reduced IP_3 levels after about 40 min of severe seizure activity in lithium-treated rats [28], but so many other metabolic changes are likely to have occurred that phosphoinositide depletion is only one of several possible causes for this result. These findings follow observations from earlier studies in which massive levels of IP_1 were found *in vivo* in rat brain after seizures induced by co-administration of lithium and cholinergic agonists [12, 29–33]. For example, LiCl (3 mmol/kg) and pilocarpine (30 mg/kg) individually increased the rat cortical IP_1 concentration 4-fold and 2-fold, respectively, but given together IP_1 was increased 40-fold [29], indicating that there was a substantial amount of phosphoinositides available for stimulation-induced hydrolysis after lithium treatment. Similar accumulations of IP_1 were obtained after acute and chronic lithium administration [33]. In summary, *in vivo* measurements of IP_3 and IP_1 have not provided evidence to support the hypothesis that lithium depletes inositol or impairs phosphoinositide hydrolysis *in vivo*. However, these assays are limited to measurements in entire brain regions, so effects localized to specific neurotransmitter systems or within selective subcellular locations would not be detected. Even with administration of pilocarpine to lithium -treated rats the increases in IP_3 and IP_1 cannot be attributed to cholinergic responses since seizures occur and these are likely to activate many systems [34].

The simplest evidence to support the inositol depletion hypothesis would be if lithium treatment were shown to deplete selectively phosphoinositides in brain. Joseph *et al.* [35] found that chronic dietary lithium reduced rat cortical PI by 24%. However, this reaction to lithium was not specific for PI since the concentrations of phosphatidylethanolamine and phosphatidylcholine were also altered, and even acute lithium treatment decreased phosphatidylethanolamine. Other investigators found that chronic lithium treatment did not decrease the rat brain concentrations of PI [36, 37], PIP, or PIP_2 [37]. Acute administration of a high dose of lithium which lowers inositol concentrations did not reduce rat [11] or mouse [12] brain phosphoinositide concentrations. Experiments to detect subtle changes in lipid synthesis have also been carried out by measuring the incorporation into phospholipids of $^{32}P_i$ administered *in vivo*. Acute lithium treatment did not affect $^{32}P_i$ incorporation into any of the inositol lipids in rat cortex [11]. Two, four and six days of lithium treatment all reduced the incorporation of $^{32}P_i$ into phosphatidylethanolamine, the two shorter treatments reduced labeling of PI, and the longest treatment increased PIP_2 labeling in rat brain, but all changes were less than 20% [38]. Taken together, these studies indicate that a significant selective effect of lithium treatment on brain phosphoinositide concentrations has not been observed. This lack of a decrease in phosphoinositide concentrations may be due to a very localized effect occurring only in highly active sites, an hypothesis that cannot be tested with available techniques. Nonetheless, since phosphoinositide concentrations have not been found to be lowered by lithium treatment and the corresponding amount of inositol depletion, it remains to be established to what degree inositol must be reduced to affect phosphoinositide levels and if lithium treatment causes such a response.

It was not until several years after Berridge *et al.* [19] first proposed that lithium may attenuate receptor-coupled phosphoinositide hydrolysis that three groups published evidence that chronic lithium treatment of rats attenuated agonist-stimulated phosphoinositide metabolism measured in brain slices, but rather than supporting the inositol depletion hypothesis these reports suggested that lithium inhibited phosphoinositide metabolism by other mechanisms [39–43]. Casebolt and Jope [40] concluded "although *in vivo* depletion of specific pools of inositol phospholipids by chronic lithium treatment cannot be ruled out entirely, several results suggest that this may not be a likely consequence in brain of long-term lithium treatment." Kendall and Nahorski [41] concluded "we do not feel that these effects seen *ex vivo* simply reflect depletion of appropriate pools of phosphoinositides required for receptor activity." Godfrey *et al.* [43] concluded that "chronic *in vivo* lithium treatment reduces agonist-stimulated inositol phospholipid metabolism in cerebral cortex; this persistent inhibition appears to be at the level of G-protein–phospholipase C coupling." Thus, although the general hypothesis that lithium inhibits agonist-induced phosphoinositide hydrolysis was confirmed in all of these studies, each of the groups suggested that mechanisms other than inositol depletion, most notably G-protein coupling to receptors or phospholipase C, accounted for the effects of lithium. Nonetheless, the inositol depletion hypothesis continues to be considered since the precise site of the actions of lithium has not been identified.

In vitro *inositol depletion and lithium*

In contrast to the *in vivo* situation, it is clear that

in vitro, for example with rat brain slices, lithium can contribute to depletion of inositol. This difference may have led to some misconceptions resulting from applying *in vitro* results to explain the *in vivo* actions of lithium. Measurements of inositol in washed rat brain slices have revealed that inositol is depleted by about 80% in the absence of lithium [12]. Thus, the highly inositol-depleted slices can be artificially sensitive to the additional inositol-depleting effects of lithium but the inositol depletion occurring prior to the addition of lithium clearly limits the extrapolation of the results to the *in vivo* situation. Several investigations using brain slices have demonstrated that *in vitro* effects of lithium are reversed or blocked by the addition of supplemental inositol (discussed below), convincing evidence that in inositol-depleted brain slices, lithium blockade of inositol monophosphatase results in inositol depletion below a critical level necessary to maintain phosphoinositides. Of the studies indicating that in inositol-depleted brain slices lithium can cause inositol depletion-dependent effects, two groups of studies are discussed below, those in which the concentration of CDP-DAG or of inositol polyphosphates was measured.

When inositol is present at insufficient concentrations for maximal PI synthase activity, the other substrate, CDP-DAG, may accumulate. This was demonstrated in rat cortical slices in which incubation with carbachol plus lithium caused a large accumulation of CDP-DAG [44]. As mentioned above, washed rat brain slices are highly depleted of inositol, so stimulation of phosphoinositide hydrolysis with carbachol and inhibition of inositol recycling by lithium resulted in the accumulation of CDP-DAG. Even under these conditions the production of CDP-DAG was linear for at least 60 min, demonstrating that the endogenous phosphoinositide pool can support substantial stimulation-induced second messenger formation even with minimal resynthesis of phosphoinositides. Inclusion of inositol in the medium prevented the accumulation of CDP-DAG [44]. Other agents that stimulated phosphoinositide hydrolysis, including norepinephrine, serotonin, and K^+, also caused accumulation of CDP-DAG in the presence of lithium [44]. Notably, even without lithium, carbachol stimulated accumulation of CDP-DAG, indicating the inositol-depleted condition of the slices [44]. Many of these findings were confirmed by others [45] and similar results were obtained with NG 108-15 cells stimulated with bradykinin [46]. These results are consistent with the conclusion that rat cortical slices and some cultured neuroblastoma cells retain insufficient inositol to fully utilize the CDP-DAG produced by agonist-stimulated phosphoinositide hydrolysis in the presence of lithium.

Recent studies have provided further insights into CDP-DAG accumulation. Heacock *et al.* [47] measured in the presence of lithium the responses to agonists for six different phosphoinositide-linked receptors in slices from three regions of rat brain. Only with carbachol in cortical and hippocampal slices was there a close correspondence between the production of inositol phosphates and CDP-DAG accumulation, whereas with carbachol in cerebellar slices and the five other agonists in each region the two measures differed significantly [47]. These results demonstrated that the processes supplying and using inositol and CDP-DAG and their responses to lithium vary among brain regions and among different phosphoinositide-linked systems and that inositol depletion as detected by CDP-DAG accumulation is a limited localized response to lithium even in inositol-depleted brain slices. This conclusion was supported further by the finding that carbachol-stimulated CDP-DAG formation in SK-N-SH neuroblastoma cells was not affected by lithium or supplemental inositol [48]. After growing cells in inositol-deficient medium to artificially deplete inositol to a level comparable with brain slices, lithium enhanced CDP-DAG accumulation, indicating that inositol must be pre-depleted for the inositol-reducing effect of lithium to be influential. Carbachol in the absence of lithium caused CDP-DAG accumulation in SK-N-SH cells that had not been depleted of inositol which was interpreted to indicate that inositol levels are normally saturating for PI synthase operating near its V_{max}, hence, CDP-DAG could not be used as rapidly as it was produced [48]. Taken together, the measurements of CDP-DAG indicate that inositol must be pre-depleted to low levels before the inhibition by lithium of inositol recycling causes significant accumulation of CDP-DAG and, furthermore, that cholinoceptive cells may be most sensitive to inositol depletion.

A series of findings similar to those with CDP-DAG has been obtained with measurements of the accumulation of inositol polyphosphates. In [^{3}H]-inositol-prelabeled rat cerebral cortical slices stimulated with carbachol, *in vitro* addition of lithium enhanced the accumulation of labeled IP_1 and IP_2 but inhibited IP_3 accumulation [49]. After development of methods to separate inositol polyphosphates, the same investigators [50] identified the accumulation of IP_4 rather than of IP_3 as the product inhibited by lithium. In mouse cortical slices the inhibition by lithium of IP_4 accumulation was selective for carbachol stimulation, as no inhibition by lithium was detected after other stimulants [51]. Later studies found inhibition by lithium of both IP_3 and IP_4 [45, 52] or only IP_4 [53] accumulation after carbachol stimulation, and inositol supplementation partially blocked the inhibition. The mechanism causing this inhibitory effect of lithium was attributed, at least in part, to inositol depletion. This was confirmed by Lee *et al.* [54] who found in rat and mouse cortical slices that inositol supplementation completely blocked inhibition by lithium of carbachol-induced accumulation of IP_3 and IP_4. Not only did supplemental inositol block depletion, but it also enabled the investigators to observe that lithium enhanced the production of IP_3 and IP_4 in the presence of sufficient inositol [54]. Further, lithium did not depress carbachol-induced IP_3 and IP_4 accumulation in guinea pig cortical slices, a finding attributed to the guinea pig slices retaining higher inositol concentrations than rat or mouse slices prior to exposure to lithium [54]. This was confirmed by measuring CDP-DAG accumulation and showing that about an order of magnitude lower inositol concentration was sufficient for blocking CDP-DAG

accumulation in guinea pig slices compared with rat and mouse brain slices [54]. Most importantly, lithium enhanced carbachol-induced IP_3 and IP_4 accumulation in monkey cortical slices without the addition of supplemental inositol, indicating that primate brain retains greater inositol levels than rodent brain and therefore lithium is less likely to cause inositol depletion in primate brain [55]. These findings further emphasize the artificial depletion of inositol that occurs in mouse and rat brain slices and the sensitivity to lithium that this imposes on the phosphoinositide system with conventional experimental incubation procedures. The findings that lithium enhanced IP_3 and IP_4 production in rat brain slices with supplemental inositol added and in guinea pig and monkey brain slices without added inositol most closely model the *in vivo* situation after chronic lithium treatment in which IP_3 production was not impaired after lithium treatment [27, 28]. Therefore, it is apparent that the inositol-depleting effect of lithium observed in some cases in brain slices is dependent on the inositol-depleted state of the slices, is species-selective, and cannot be extrapolated to brain *in vivo*.

Lithium and the cholinergic system

A number of diverse observations suggest that there is a selective, but as yet ill-defined, interaction of lithium with cholinergic cells. As mentioned above, in rat cortex the *in vivo* lithium-induced depletion of inositol and accumulation of IP_1 was blocked by muscarinic antagonists [7], indicating that cholinergic receptor-linked phosphoinositide hydrolysis contributes disproportionally to these *in vivo* responses to lithium. This may be related to the finding that lithium increases acetylcholine synthesis and release in rat brain [56] which would cause lithium to increase endogenous cholinergic activity while simultaneously blocking inositol monophosphatase, leading to accumulation of IP_1 and hypothetically reduced inositol in cholinoceptive cells. In accordance with this proposal, based on measurements of inositol isomers that accumulated, Sherman *et al.* [9] proposed that lithium simultaneously stimulated muscarinic receptor-coupled phosphoinositide hydrolysis and inhibited inositol monophosphatase.

Increased cholinergic activity after lithium treatment of rats has been substantiated further by studies showing that responses to cholinergic agonists given to rats are potentiated by preadministration of lithium, as shown by lowered seizure thresholds. Acute or chronic lithium administration to rats lowered the seizure threshold to all cholinergic agonists that have been tested, including pilocarpine, arecoline, physostigmine and diisopropylfluorophosphate [29–34, 57–59]. This is a cholinergic-specific effect of lithium because seizures are blocked by administration of muscarinic antagonists, and lithium does not affect responses to a number of other noncholinergic convulsant treatments [60]. Although seizures have been used as an experimental end-point in many of these studies, it is assumed that lithium has the same potentiating effect on responses to endogenous acetylcholine as evidenced, for example, by the potentiated responses to acetylcholinesterase inhibitors.

Associated with the seizures induced in rats by coadministration of lithium and pilocarpine is a massive increase in the synthesis and release of acetylcholine [61, 62]. The increased synthesis of acetylcholine has been attributed to activation of high affinity choline uptake [63], the rate limiting step in acetylcholine synthesis [64]. Contributing to increased acetylcholine release is the blockade by lithium of presynaptic inhibitory autoreceptors that normally reduce acetylcholine release [65]. These many different experimental approaches demonstrate that there is substantial evidence indicating that moderate doses of lithium administered acutely or chronically enhance cholinergic activity *in vivo* [66].

The cause of the seizures induced by administration of cholinergic agonists to lithium-treated rats involves cholinergic activation, as briefly reviewed above, but the precise mechanisms resulting in seizures are not known and it is not clear how the phosphoinositide system is involved. Evidence cited previously and other experiments [57] indicate that lithium enhances both presynaptic and postsynaptic cholinergic activity. How this is transduced to the genesis of seizures and whether or not studies of these seizures will reveal insights into the mechanism of action of lithium remain to be seen. However, several agents have been applied to the lithium/pilocarpine seizure model to attempt to identify mechanisms that may contribute to the evolution of the seizures and the role of lithium. For example, administration of pertussis toxin (in the absence of lithium), which inactivates the G_i/G_o family of G-proteins, potentiated pilocarpine-induced seizures to the same extent as did lithium [59]. This raised the possibility that impaired G-protein function caused by lithium (which is discussed in more detail below) may contribute to seizures. Also, depletion of norepinephrine by administration of DSP-4 potentiated pilocarpine-induced seizures, raising the possibility that impaired noradrenergic activity in response to lithium could contribute to the proconvulsant interaction of lithium with pilocarpine [60]. Further evidence indicated that enhanced responses of *N*-methyl-D-aspartate receptors contribute to the seizures [34].

The relationship of the phosphoinositide system to the potentiated cholinergic responses after lithium treatment has been difficult to identify. One possibility that has been raised is that the phosphoinositide pool or the inositol source for phosphoinositide synthesis may be especially small or rapidly used in cholinergic neurons. As discussed above, this was indicated *in vitro* because even in rat and mouse brain slices, which have low inositol levels, lithium selectively reduced carbachol-induced IP_3 and IP_4 production but not that induced by other agonists [49–54]. Even more clear was the close correlation between carbachol-stimulated CDP-DAG accumulation and inositol phosphate accumulation found in rat brain slices exposed to lithium [47]. However, it is difficult to relate effects in slices that are greatly depleted of inositol to effects in the intact brain *in vivo*. Intriguing results were obtained

in a novel experiment which demonstrated that intracerebroventricular (i.c.v.) administration of a very large dose of inositol to mice or rats reduced the seizure responses (i.e. increased latency and/or reduced severity) to administration of lithium plus pilocarpine, suggesting that inositol depletion contributes to the genesis of seizures [67, 68]. At present, this finding is difficult to reconcile with the measurements of the *in vivo* concentration of IP_3 after pilocarpine administration with or without lithium treatment that revealed no evidence for impaired formation of IP_3 in mouse [27] or rat brain [28] except after long-term (40 min) seizure activity, indicating that there was an adequate supply of phosphoinositides for IP_3 formation upon stimulation of cholinergic receptors after acute or chronic lithium treatment. Also, following seizures there is a massive accumulation of IP_1 in rat brain, indicating that a large store of phosphoinositides is available for hydrolysis [29]. The basis for these apparently contradictory findings is unknown. Potential explanations include the possibilities that the inositol administered had an effect other than as a source of phosphoinositides in cholinergic cells (e.g. it may provide inositol to other cells, such as adrenergic cells which have anticonvulsant activity against seizures induced by lithium plus pilocarpine) [60], that brain region measurements of IP_1 and IP_3 are not specific enough to detect cell-selective depletions, or that the administered inositol serves to remove accumulated CDP-DAG. In SK-N-SH neuroblastoma cells CDP-DAG accumulated after carbachol stimulation even in the absence of lithium [48]. Accumulation of CDP-DAG may lead to increased levels of DAG and subsequently increased activation of protein kinase C. Lithium increased DAG levels in NG108 cells both in the absence and presence of agonists [69]. Activation of protein kinase C by phorbol esters administered i.c.v. to mice has been shown to cause seizures [70]. Thus, protein kinase C activated by increased DAG levels may contribute to seizures induced by administration of lithium plus cholinergic agonists and inositol may counteract protein kinase C activation by reducing DAG formation. Clearly, further investigations are required to resolve the mechanism by which inositol administration reduces seizure activity induced by administration of lithium and cholinergic agonists and to determine how these interactions are related to the therapeutic effect of lithium.

Although all of the mechanisms are not yet clear, lithium certainly appears to have selective modulatory effects on the activity of the cholinergic system but, as demonstrated by the inositol administration experiments, it is not clear whether lithium enhances postsynaptic cholinergic responses or if depletion of inositol occurs and impairs second messenger production after cholinomimetics and this results in the genesis of seizure activity. This fundamental question should be addressed in the near future.

Other actions of lithium on the phosphoinositide system and on other signal transduction systems

It is now apparent that lithium affects the phosphoinositide system at sites in addition to the inositol phosphatases and that lithium influences other signal transduction systems (discussed below). However, because much of the emphasis in studies of the influence of lithium on phosphoinositide metabolism has been related to inhibition of inositol phosphatases, significant gaps remain in the understanding of other actions of lithium. Three general actions of lithium have been considered to be potentially important, including modulation of G-protein function, gene expression, and protein phosphorylation, although specific mechanisms by which lithium acts remain unclear and definitive evidence relating these to therapeutic responses remains to be forthcoming.

Lithium impairs G-protein function

It was rather remarkable that when three groups first reported [39–43] that *in vivo* lithium administration to rats reduced agonist-stimulated phosphoinositide hydrolysis in brain slices, each of the groups suggested that mechanisms other than inositol depletion and perhaps involving G-protein function were likely to contribute to the mechanism of action of lithium, as reviewed earlier in this article. Evidence indicative that lithium modifies G-protein function using experimental methods other than measurements of phosphoinositide hydrolysis was provided by Avissar *et al.* [71]. They found that lithium (*in vitro* or chronic *in vivo* treatment) completely abolished agonist-induced [^{3}H]GTP binding in cortical membranes, indicating that G-proteins were not activated in the presence of lithium. Those investigators studied this effect of lithium in further detail [72], most notably showing that it was reversed by Mg^{2+} [73], but other groups have yet to publish similar results and contradictory results were reported recently [74]. Also, in contrast to the results of Avissar *et al.* [71] with rat brain membranes, lithium enhanced [^{3}H]GTP binding to PC12 membranes [75]. Thus, the results from GTP-binding experiments are intriguing, but it is not yet clear how lithium affects the activation of G-proteins and further studies directed towards clarifying this important issue are needed.

A direct confirmation of the hypothesis that mechanisms other than inositol depletion contribute to lithium-induced impairment of agonist-stimulated phosphoinositide hydrolysis was achieved by measuring GTP- and agonist-induced phosphoinositide hydrolysis in rat brain membranes with exogenously supplied [^{3}H]PI [76]. Utilization of exogenous substrate bypassed any potential influence of depletion of endogenous phosphoinositides, and the reduction of agonist- and GTPγS-stimulated PI hydrolysis observed in membranes from chronic lithium-treated rats confirmed that other sites, most likely G-protein function, were modulated by lithium [76]. A mechanism by which lithium might impair G-protein function was identified recently in a study showing that chronic lithium treatment interfered with G-protein dissociation into activated subunits in rat brain (although G_i rather than a phosphoinositide-linked G-protein was investigated) [77]. A variety of other studies of lithium in neuronal tissue [59, 65, 78] and numerous studies with nonneuronal tissues also provide evidence that lithium modulates G-protein function and that these effects may be

related to its therapeutic effects in human patients [reviewed in Ref. 79].

The influence of lithium on G-proteins appears to contribute to the modulation of cyclic AMP production by lithium. Several *in vitro* and *in vivo* effects of lithium on cyclic AMP synthesis have been reported, as discussed in recent comprehensive reviews [79–82]. Those effects of lithium that appear likely to involve modulation of G-protein function include increased basal and reduced stimulus-induced cyclic AMP production. For example, both of these responses to lithium were demonstrated to occur in rat brain *in vivo* by microdialysis measurements of cyclic AMP, as chronic lithium administration increased basal cyclic AMP release and decreased norepinephrine-stimulated cyclic AMP production [77, 83]. The increased basal cyclic AMP production caused by lithium was proposed to be caused by stabilization of the inactive heterotrimeric form of G_i, thus reducing the inhibitory control of cyclic AMP production [77]. Impaired G_i function by lithium has also been implicated in studies of the function of receptors coupled through G_i to inhibition of cyclic AMP synthesis. For example, lithium reduced serotonin-induced inhibition of cyclic AMP synthesis in rat brain [84–86]. Lithium also reduced inhibition by carbachol of forskolin-stimulated adenylyl cyclase activity in rat hippocampal membranes [87] but, in contrast, lithium enhanced inhibition by carbachol of dopamine-stimulated adenylyl cyclase in striatal slices [26]. Involvement of G-proteins in the inhibitory effects of lithium on responses to agonists activating receptors coupled to G_s and stimulation of cyclic AMP production has also been reported [79–82]. For example, elevated GTP concentrations eliminated the inhibitory effect of lithium on isoprenaline-stimulated cyclic AMP production in rat brain membranes [88]. Although differences among strains of mice in the inhibitory effect of lithium on stimulated cyclic AMP production have been reported [89], it is notable that inhibition by lithium (1 mM) of norepinephrine-induced cyclic AMP synthesis was demonstrated in human brain [90]. Recent evidence has demonstrated that *in vitro* concentrations of lithium of less than 1 mM can inhibit rat cortical cyclic AMP production [91]. Thus, evidence has accumulated indicating that therapeutic levels of lithium modulate both inhibition and stimulation of cyclic AMP production and that at least some of these effects appear to be due to altered G-protein function. Circumstantially, since lithium modulates both phosphoinositide hydrolysis and cyclic AMP production, G-proteins as targets of the lithium's actions of lithium provide a common site at which lithium could affect both second messenger systems.

It is now evident that G-protein function is an important site of modulation of signal transduction processes, for example activity-dependent modulation and hormonal regulation of G-protein function have been described in a number of systems [92, 93]. As more is learned about the mechanisms modulating G-protein function, potential influences of lithium should be a major topic for further investigation.

Lithium alteration of gene expression, including genes coding for proteins involved in signal transduction

Although molecular biological approaches have been applied only recently to studying the effects of lithium on gene expression, a relatively large number of transcripts have been reported to be influenced by lithium (Table 1). Among these studies, lithium has been reported to alter the protein and mRNA levels of a number of components of signal transduction systems. The mechanisms for these effects are unknown; they may include direct effects on gene expression, mRNA processing, and protein synthesis or degradation, or they may be secondary to the modulation by lithium of the activity of signal transduction processes. Regardless of the mechanism, since lithium must be administered chronically for a therapeutic effect and since many patients are maintained on lithium for long periods of time, the effects of lithium on gene expression and protein concentrations must be considered as important components of its effects on neuronal function.

Besides affecting G-protein function, lithium also influences G-protein mRNA and protein concentrations. Li *et al.* [94] found that chronic lithium treatment reduced by about 30% rat cerebral cortical $G_s\alpha$, $G_{i1}\alpha$, and $G_{i2}\alpha$ mRNA levels. Colin *et al.* [95] also reported reduced $G_{i1}\alpha$ and $G_{i2}\alpha$ mRNA levels in rat cerebral cortex after chronic lithium treatment, whereas $G_s\alpha$, $Go\alpha$, and $G\beta$ mRNA levels were not different from controls. In the latter report, G_i protein levels were also found to be reduced [95]. Lesch *et al.* [96] reported increased $G_i\alpha$ protein concentration in rat hippocampus and hypothalamus and unaltered $G_s\alpha$ and $G_o\alpha$ protein concentrations in several brain regions after chronic lithium treatment. In an impressively detailed study, Lesch and Manji [97] also reported selective changes in G-protein concentrations and mRNA levels in several regions of rat brain after treatments with several antidepressants. These studies do not identify mechanisms, but they indicate that G-protein levels and gene expression are influenced by chronic treatment at therapeutic doses of drugs used to treat affective disorders. Chronic lithium treatment appears to have differential effects on protein and mRNA levels among the various G-proteins and among different brain regions, responses that should be confirmed, related to second messenger system activities, and mechanisms of modulation identified.

The expression of other genes coding for proteins involved in signal transduction is also modulated by lithium. Chronic lithium treatment (4 weeks; 1 mM serum lithium) increased rat cortical mRNA levels of types 1 and 2 adenylyl cyclase by 50–60% [95]. This well-controlled study also showed that the mRNA levels were not changed after a lower dose of lithium or after a shorter (6 day) treatment period [95]. Immunoblotting demonstrated elevated protein concentrations concomitant with the increased mRNA levels after chronic lithium treatment [95]. These findings are consistent with the increased basal activity of adenylyl cyclase observed in rat brain after chronic lithium treatment that was discussed previously in this review.

Table 1. Lithium-induced alterations in mRNA levels

Target	Effect of Lithium	Tissue	Lithium treatment	Reference
$G_{i1}\alpha$, $G_{i2}\alpha$	↓ mRNA	Rat cerebral cortex	↓ 21 days dietary 0.2% LiCl	94
$G_{i1}\alpha$, $G_{i2}\alpha$	↓ mRNA	Rat cerebral cortex	4 weeks dietary 0.24% Li_2CO_3	95
$G_s\alpha$	↓ mRNA	Rat cerebral cortex	21 days dietary 0.2% LiCl	94
$G_s\alpha$, $G_o\alpha$, $G\beta$	No change in mRNA	Rat cerebral cortex	4 weeks dietary 0.24% Li_2CO_3	95
Adenylyl cyclase, types 1 and 2	↑ mRNA	Rat cerebral cortex	4 weeks dietary 0.24% Li_2CO_3	95
c-fos TIS1, TIS7, TIS8, TIS11, TIS21*	↑ mRNA induced by carbachol	Rat neocortical astrocytes	5 mM LiCl	98
c-fos	↑ mRNA induced by carbachol, bradykinin, or PMA	PC12 cells	10 mM LiCl†	99, 100
c-fos	↑ mRNA induced by pilocarbine	Rat cerebral cortex	Acute LiCl‡ (3 mmol/kg)	101
Neuropeptide Y	↑ mRNA	Rat hippocampus	Acute LiCl (1.5 mmol/kg), 2x, 12 hr apart	103
PEPCK	↓ mRNA	Rat FTO-2B hepatoma cells	20 mM LiCl	104
PEPCK	↓ mRNA	Rat liver	Acute LiCl (2 mmol/kg), 2x, 90 min apart	104
Neurotensin/ Neuromedin	↑ mRNA induced in combination with NGF, dexamethasone and forskolin	PC12 cells	LiCl (5–40 mM)	105
Prodynorphin	↑ mRNA	Rat striatum	4 days LiCl (4 mmol/kg)	106
Preprotachykinin	↑ mRNA	Rat striatum	4 days LiCl (4 mmol/kg)	107
Tyrosine hydroxylase	↑ mRNA	PC12 cells differentiated with NGF	0.1 mM LiCl	108
Glucocorticoid receptor (type II)	↑ mRNA (female>male)	Rat hypothalamus, hippocampus, and amygdala	10 days LiCl (6 mmol/kg)	109

Abbreviations: NGF, nerve growth factor; PEPCK, phosphoenolpyruvate carboxykinase; and PMA, phorbol 12-myristate 13-acetate. ↓, decreased; ↑, increased.

* Multiple names have been given to the TIS genes, including TIS28 (c-fos), TIS1 (nur77; NGF1B), TIS7 (PC4), TIS8 (egr-1, krox-24, zif-268, NGF1A).

† A 1 mM concentration of LiCl also increased c-fos mRNA induced by carbachol but not by bradykinin or PMA.

‡ Five to six days of 3 mmol/kg/day LiCl administration also potentiated c-fos mRNA induced by pilocarpine.

c-Fos is the product of an immediate early gene that is induced by stimuli that activate protein kinase C as well as by other mechanisms. Administration of lithium potentiated the increased c-fos mRNA levels that followed treatment of rats with pilocarpine or of cultured cells with carbachol or a phorbol ester that activates protein kinase C [98–101]. The site of action of lithium was concluded to be at or distal to protein kinase C. These findings are important because c-fos is a transcription factor regulating the expression of other genes, so by modulating c-fos mRNA expression lithium can affect the expression of secondary genes. The carbachol-induced expression of other genes coding for proteins that are transcription factors was also reported to be enhanced by lithium in cultured astrocytes [98]. It would be of interest to determine if the potentiating effects of lithium on the expression of these immediate early genes are responsible for the enhanced expression of other genes that has been observed after lithium treatment or if there is a common mediator, such as protein kinase C, that is modulated by lithium, thereby altering the expression of a number of genes. Similarly with the transcription factor proteins encoded by the immediate early genes, ornithine decarboxylase, the polyamine synthetic enzyme, is increased rapidly in brain following many stimuli. Dexamethasone administration to rats increased brain ornithine decarboxylase activity 4-fold 6 hr after treatment, and chronic, but not acute, lithium administration abolished this response [102]. Although enzyme activity rather than mRNA levels were measured, this finding may be the result of an effect of lithium on gene expression.

Lithium has also been reported to alter the mRNA levels of a number of neuropeptides, tyrosine hydroxylase, and a glucocorticoid receptor (Table

1). Each of these effects of lithium is intriguing in light of the known roles of each protein product in neuronal function and may contribute to critical aspects of the therapeutic effects of lithium, although further studies will be required before each of these findings can be integrated into a composite mechanism of action. In the near future, further clarification of how lithium alters mRNA levels and how this is related to its therapeutic responses should be obtained.

Lithium alteration of protein phosphorylation

Since lithium influences the activity of second messenger systems, it is reasonable to hypothesize that protein phosphorylation mediated by the associated kinases may be altered after lithium administration. Equally important is the possibility that lithium may directly affect phosphorylation or dephosphorylation of key proteins. It is unfortunate that only a few studies have addressed this issue, especially considering the diverse evidence that altered protein kinase C activity may be a consequence of lithium treatment. The first studies of this topic found that chronic lithium administration increased the incorporation of $^{32}P_i$ administered to rats *in vivo* into several cortical synaptosomal proteins [110] and increased the *in vitro* phosphorylation with [^{32}P]ATP of a 64-kDa protein in rat cortical and hippocampal membranes [111]. A few studies have raised the possibility that the phosphorylation of cytoskeletal proteins is altered by lithium. In PC12 cells lithium reduced the phosphorylation of a 64-kDa protein that was tentatively identified as a microtubule-associated protein [112], a class of proteins that are now recognized as important components in neuronal plasticity processes [113]. Lithium *in vitro* inhibited the phosphorylation of microtubule-associated protein 2 in rat cortical microtubule preparations [114] and of neurofilament proteins in cultured chick sensory neurons [115]. It would be interesting if changes in cytoskeletal protein phosphorylation induced by lithium were related to similar effects induced by antidepressants [116].

Relatively few studies have been done to determine if lithium influences proteins phosphorylated by cyclic AMP-dependent protein kinase, a surprising situation since lithium has been known for many years to modulate cyclic AMP production. Chronic lithium treatment was found to alter the *in vitro* phosphorylation by cyclic AMP-dependent protein kinase of a few proteins in rat hippocampus [117]. Chronic, but not acute, lithium treatment increased by 30% the rat frontal cortex (but not other regions) concentration of DARPP-32 (32-kDa dopamine- and cyclic AMP-regulated phosphoprotein), a major substrate for cyclic AMP-dependent protein kinase, as did two antidepressants but not several other types of psychotropic drugs [118]. Active DARPP-32 is a protein phosphatase inhibitor [119], so increased levels after lithium may alter the phosphorylation state of other proteins. Chronic treatment with lithium, as well as with antidepressants, induced the translocation of cyclic AMP-dependent protein kinase from the cytosol to the nucleus [120]. Thus, there is evidence that lithium modulates cyclic AMP production, the intracellular location of cyclic AMP-dependent protein kinase, the phosphorylation of proteins catalyzed by this kinase, and the amount of the protein phosphatase inhibitor DARPP-32.

Measurements of protein kinase C activity and its distribution between soluble and particulate fractions from rat hippocampus revealed that neither was modified by chronic lithium treatment [117]. However, lithium treatment altered the *in vitro* phosphorylation mediated by protein kinase C of several proteins in particulate and soluble hippocampal fractions [117]. It was suggested that the selective effects on protein substrate phosphorylation could be due to cell-selective responses or to differential effects on protein kinase C isozymes [117]. Measurements of neurotransmitter metabolism have also indicated that lithium influences protein kinase C-mediated phosphorylation [65, 121]. Recently, Lenox *et al.* [122] confirmed that *in vivo* lithium administration did not alter the activity or cytosol/membrane distribution of protein kinase C in rat hippocampus and found that lithium *in vitro* did not alter the phorbol ester-induced membrane translocation of protein kinase C. Most interestingly, in chronic lithium-treated rats there was a 45% reduction in the *in vitro* phosphorylation of the prominent protein kinase C substrate, MARCKS (myristoylated alanine-rich C kinase substrate), and of a 45-kDa protein in the soluble fraction from the hippocampus [122]. Immunoblotting revealed that the concentration of MARCKS protein was reduced after chronic lithium treatment [121]. Although direct investigations of the effects of lithium on protein kinase C-mediated phosphorylation are few, that this interaction may be central to the issue of the therapeutically important sites of action of lithium is indicated by a growing and diverse, but indirect, body of evidence that includes the following findings. Protein kinase C modulates the activities of receptor-coupled phosphoinositide hydrolysis and cyclic AMP production, two systems influenced by lithium. As noted earlier, lithium may alter the concentration of diacylglycerol, an activator of protein kinase C. Activation of protein kinase C is one mechanism for inducing immediate early gene transcription, a response potentiated by lithium. Protein kinase C phosphorylates, and presumably regulates the function of, some G-proteins [123–125], and G-protein function and expression are modulated by lithium. Protein kinase C is involved in the modulation of neurotransmitter synthesis and release that is influenced by lithium and in the seizures induced by coadministration of lithium and cholinergic agonists, as was reviewed above. Altogether, we suggest that this evidence indicates that modulation by lithium of protein kinase C-dependent phosphorylation of specific proteins may play a key role in the therapeutic response to lithium administration.

Conclusion

Much has been learned about the neurochemical effects of lithium in the last few years and exciting progress has been achieved in focusing on specific sites that appear to be important targets of lithium.

The phosphoinositide second messenger system stands out as a primary candidate for a therapeutically relevant site of action of lithium, but several questions remain to be resolved. Most notable is the problem of applying results from *in vitro* preparations (where inositol is artificially depleted, making the system sensitive to the inositol-depleting effect of lithium) to *in vivo* processes where toxic, not therapeutic, concentrations of lithium appear to be required to reduce the inositol concentration, and even then lithium causes only relatively slight decreases of inositol. Nevertheless, although reductions of inositol in brain *in vivo* have not been reported after relevant lithium treatments, evidence has accumulated that not all phosphoinositide-linked systems are alike, so selective depletion of inositol may occur in specific cells or intracellular locations and this may be a key to the selective effects of lithium on cholinergic cells as well as to the therapeutic response. However, experimental techniques must be developed that can detect isolated depletions of inositol to test this modified inositol depletion hypothesis. But beyond the inositol depletion hypothesis, lithium has been shown to perturb other aspects of phosphoinositide metabolism, such as agonist-induced phosphoinositide hydrolysis and the *in vivo* concentrations of inositol phosphates. Especially interesting is the finding that lithium apparently has unique influences on cholinergic activity, including increasing cholinergic agonist-induced gene expression, which indicates that lithium increases a signal transduction process between the muscarinic receptor and gene expression, a process thought to involve phosphoinositide hydrolysis and activation of protein kinase C. Recent observations demonstrate that lithium affects G-protein function, gene expression, protein phosphorylation and other processes, each of which may have a far-reaching effect on overall cellular function. We must ask if and how each of these interactions contributes to the therapeutic, or toxic, responses to lithium and if each represents one of many sites of action of lithium, or if there is a single or a very few major sites of action of lithium that themselves influence many secondary and tertiary processes. Studies of the molecular mechanisms responsible for the novel actions of lithium should continue to provide stimulating research and valuable insights about the complex regulation of signal transduction processes in the brain and their influence in psychiatric disorders.

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