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MOLECULAR TARGETS OF LITHIUM ACTION

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■ **Abstract** Lithium is highly effective in the treatment of bipolar disorder and also has multiple effects on embryonic development, glycogen synthesis, hematopoiesis, and other processes. However, the mechanism of lithium action is still unclear. A number of enzymes have been proposed as potential targets of lithium action, including inositol monophosphatase, a family of structurally related phosphomonoesterases, and the protein kinase glycogen synthase kinase-3. These potential targets are widely expressed, require metal ions for catalysis, and are generally inhibited by lithium in an uncompetitive manner, most likely by displacing a divalent cation. Thus, the challenge is to determine which target, if any, is responsible for a given response to lithium in cells. Comparison of lithium effects with genetic disruption of putative target molecules has helped to validate these targets, and the use of alternative inhibitors of a given target can also lend strong support for or against a proposed mechanism of lithium action. In this review, lithium sensitive enzymes are discussed, and a number of criteria are proposed to evaluate which of these enzymes are involved in the response to lithium in a given setting.

INTRODUCTION

Lithium has been used for more than fifty years as the primary therapy for bipolar disorder (BPD) (1, 2), but its mechanism(s) of action is still unknown (3–10). Other putatively therapeutic uses prior to the work of Cade had been described for lithium salts, and an effect of lithium on embryonic development has been recognized for at least 100 years (11). Lithium also affects metabolism, neuronal communication, and cell proliferation in a diverse array of organisms, from cellular slime molds to humans. Some attempts have been made to explain the mechanism of lithium action in these diverse settings through a single unifying hypothesis. These efforts have guided valuable research on the pharmacology of lithium action, but it remains unclear whether these or other possible mechanisms are sufficient to explain any or all of the effects of lithium. In this review, we focus on potential targets that are known to be directly inhibited by lithium in vitro. The vast and expanding

literature on downstream consequences of lithium action cannot be adequately reviewed here; for this and related topics, readers are referred to several excellent reviews (3–7, 12; see also 9, 10).

The clinical importance of defining the direct target(s) of lithium action is twofold. First, while lithium is highly effective in treating BPD in many patients, the therapeutic window between effective dosing and toxicity is narrow, side effects are common even within the therapeutic dose range, and a significant number of patients do not respond (3, 8). While anticonvulsants such as valproic acid offer an alternative mode of therapy, an understanding of the targets of lithium (and of valproic acid) will make it possible to identify additional therapies for this common disorder. Second, little is known about the pathogenesis of BPD or other mood disorders, and therefore identification of the molecular target of lithium should shed light on the etiology of this disorder. Finally, it is of great scientific interest to understand how a simple, small cation like lithium can demonstrate such relative specificity in its range of actions when other monovalent cations have no apparent effect. It is indeed remarkable that millions of people can actually tolerate this simple drug with a minimum of prohibitive side effects.

Lithium is clearly able to inhibit multiple enzymes, and it is improbable that all of the actions of lithium can be explained by interaction with a single target. Since these diverse potential targets are often present in the same cell, it remains a challenge to distinguish which, if any, of the known targets of lithium is involved in the in vivo response to lithium. Thus, after a brief discussion of some of the better characterized effects of lithium, we also discuss criteria that can be applied to validate a putative target in a given setting.

EFFECTS OF LITHIUM

Lithium has numerous effects in humans and in model organisms; it would be difficult to describe all of them in detail here. A few of the more common effects of lithium are described to provide a physiological context for the discussion of the potential molecular targets of lithium action that follows.

Neuropsychiatric

Bipolar disorder is a common psychiatric ailment characterized by cycling periods of extreme elation (mania) and severe depression (9); features of psychosis, including delusions and hallucinations, can be associated with either extreme. Although lithium salts were used in the nineteenth century for various and sometimes dubious purposes, for example as a soporific and a gout treatment (2, 13–15), the use of lithium to treat the manic phase of BPD was first described by Cade in 1949, after noticing the sedative effect of lithium on guinea pigs (1). Cade boldly extended his observations to humans with BPD, providing a potent

therapy for BPD. The occurrence of BPD has been estimated to be in 0.3% to 1.5% of the population, based on epidemiological studies sampling diverse regions of the world (16). Although a genetic link is suspected given the clustering of BPD in genetically homogenous populations (17–21), specific loci have not been identified (22, 23). In addition to controlling mania, lithium has been used as a mood stabilizer in the control of bipolar depression and may also have a therapeutic value as adjunctive therapy in the treatment of unipolar depression (8, 24) and in cluster headache. Lithium in cell culture models confers protection from excitotoxic neurotransmitters in cortical and cerebellar cells and leads to synaptic remodeling in cerebellar cells (25–27). As described below, lithium can cause an increase in neurotransmitter release in *Drosophila* neuromuscular junctions.

Developmental Effects

Lithium alters the development of phylogenetically diverse organisms (Figure 1) (11, 28–32). For example, in *Dictyostelium*, a simple eukaryotic organism, exposure to lithium during early development blocks spore cell fate and promotes the formation of stalk cells (28, 29). In sea urchins, lithium causes vegetalization of animal blastomeres (32–34). In vertebrates such as *Xenopus* and zebrafish, lithium causes expansion of dorsal mesoderm, leading to duplication of the dorsal axis in *Xenopus* or, in extreme cases, entirely dorsalized embryos lacking ventral tissues (30, 31). In mammals, isolated nephrogenic mesenchyme undergoes mesenchymal to epithelial differentiation when exposed to lithium (35), and mouse mammary tumor cells have an increased proliferative index in the presence of lithium (36). In humans teratogenic effects of lithium have been reported at surprisingly low frequency when one considers the dramatic effects on the development of lower vertebrates. Some studies have indicated an increased frequency of congenital heart defects, particularly Ebstein's anomaly, characterized by downward displacement of the tricuspid valve in the right ventricle of the heart, but the reported frequency of this defect has varied widely (9).

Metabolic Effects

Lithium can stimulate glycogen synthesis in mammals through activation of glycogen synthase, mimicking insulin action (37–41). In addition, lithium therapy in humans is associated with subclinical hypothyroidism and nontoxic goiter, nephrogenic diabetes insipidus (decreased renal concentrating ability), weight gain, hyperparathyroidism, and a large number of other less common side effects (8, 9).

Hematopoiesis

One of the most prevalent benign side effects of lithium therapy in humans is an increase in the number of circulating granulocytes (up to 1.5-fold), predominantly

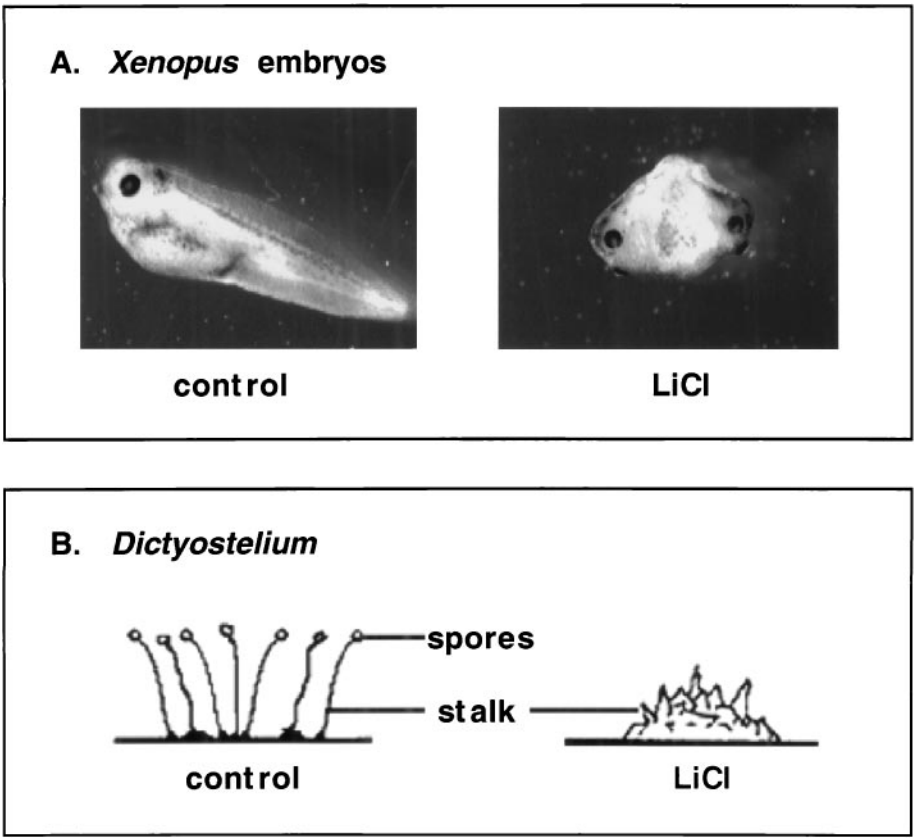

Figure 1 The effects of lithium on developing systems. (*a*) Exposure of *Xenopus* embryos to lithium during an early stage of development causes expansion and duplication of dorsal and anterior structures (30). On the left is a control tadpole. On the right, a lithium-treated embryo with duplicated dorsal and anterior structures. (*b*) *Dictyostelium discoideum* is a simple eukaryote that, upon starvation, develops into two general cell types, spores (within a fruiting body) and stalk cells (supporting the fruiting body). Exposure to lithium during development diverts cells away from the spore cell fate with expansion of the stalk cell population (adapted from Reference 28).

neutrophils, although effects on other lineages have also been reported (42–44). Lithium appears to increase the level of pluripotent hematopoietic stem cells, either indirectly by stimulating the release of cytokines or directly by acting on stem cells (or both) (44–48). Thus, lithium treatment reduces chemotherapy-induced neutropenia and febrile complications of marrow suppressive therapies in a number of clinical trials (49–52) (reviewed in 44). In spite of these early encouraging studies, lithium has not been used extensively in neutropenic patients, perhaps because of its narrow therapeutic window.

MOLECULAR TARGETS OF LITHIUM

Considerable information has been gained in the past 50 years concerning some of the indirect physiological consequences of lithium therapy (3, 4, 6, 53–56). Lithium can affect neurotransmitter release, metabolism of biogenic monoamines, and neuronal signal transmission through perturbation of the distribution of sodium, magnesium, and calcium (9, 10). Lithium can inhibit depolarization-induced and calcium-dependent release of norepinephrine and dopamine, (57), and conversely, may stimulate the release of serotonin (58). A number of phosphoproteins have been identified whose phosphorylation or expression level is sensitive to lithium treatment, including neurofilament proteins (59), microtubule-associated proteins (60), and protein kinase C (PKC) substrates such as the MARCKS protein, which is downregulated in response to lithium therapy (4, 6, 53). Lithium also inhibits ADP-ribosyl transferase in extracts derived from rat frontal cortex (61). Because of the characteristic delay in clinical response to lithium therapy, an effect of lithium on neuronal gene expression has also been proposed (56): In support of this, lithium has been shown to activate AP-1-dependent transcription (62–67) and Tcf/Lef-dependent transcription (see below). A number of studies have indicated that lithium interferes with signal transduction through G protein–coupled pathways, inhibiting the G proteins themselves or downstream effectors, including adenylyl cyclase, phospholipase C, and protein kinase C (4, 68). However, in each case, these effects of lithium have not been shown to be direct, largely because of the difficulty in reconstituting these signaling systems from purified components.

These observations are not necessarily in conflict with each other, and each could lie in a pathway regulated by a common signaling molecule that is the direct target of lithium action. Our focus ison targets known to be inhibited directly by lithium (Table 1). These include inositol monophosphatase (69) and the large family of related phosphomonoesterases (70), as well as the recently identified target glycogen synthase kinase-3β (GSK-3β) (71). Since each of these potential targets is expressed widely, and generally in the same cell types, it remains a

TABLE 1 Lithium-sensitive enzymes

Target	Ki (mM)	Type of inhibition	Reference(s)
IMPase	0.8	Uncompetitive	72
IPPase	0.3	Uncompetitive	91
FBPase	0.3–0.8		193
BPntase	0.16–0.3	Uncompetitive	85, 90
Hal2p	0.1	Un-/noncompetitive	86
GSK-3β	1–2	Uncompetitive	71, 101

challenge to distinguish which, if any, are involved in a given response to lithium. To validate a molecule as a relevant direct target of lithium action, one would expect the following:

1. The target should show direct sensitivity to lithium (and not to other monovalent cations) in vitro.
2. The target should be sensitive to lithium in vivo at physiological concentrations of lithium.
3. Loss-of-function mutations in the putative target should be phenocopied by exposure to lithium.
4. Alternative inhibitors of the target molecule should mimic the effect of lithium.
5. The effect of lithium should be reversed by appropriate modulation of components downstream of the putative target.
6. A lithium-resistant form of the molecule should block the effect of lithium in vivo.

Inositol Depletion Hypothesis

One of the most compelling hypotheses to explain lithium action is the inositol depletion hypothesis (Figure 2), which is based on the observation that lithium inhibits inositol monophosphatase (IMPase) in vitro at a Ki (0.8 mM) within the therapeutic range (0.5 to 1.5 mM) for lithium treatment of patients with bipolar disease (69, 72). Since IMPase regenerates inositol from inositol monophosphate (IMP), inhibition of this step could deplete inositol if the cells do not have an alternative source. In principle, this should lead to depletion of phosphatidylinositol bisphosphate (PIP_2), a necessary precursor for the generation of the second messenger inositol-1,4,5 trisphosphate (IP_3) in response to extracellular signals. For example, numerous neurotransmitters bind to G protein–coupled receptors that activate phospholipase C, which hydrolyzes PIP_2 to diacylglycerol (DAG) and IP_3; DAG activates PKC, while IP_3 causes release of calcium from intracellular stores into the cytoplasm. The net effect of lithium, then, would be to block ligand-dependent signaling through PKC and IP_3/calcium.

Lithium is an uncompetitive inhibitor of IMPase, and it has been argued that this may explain why lithium therapy is effective in BPD but has no apparent effect on the psychiatric state of normal subjects (73). For an uncompetitive inhibitor, the fractional inhibition is proportional to the level of enzyme-substrate complex, which means that at higher concentrations of substrate (e.g. IMP), lithium would have a greater effect. Thus, if mania or other disturbances of mood arise because of excess signaling that leads to increased IMP levels, lithium would inhibit IMPase to a greater extent than if given to "normal" patients with lower levels of IMP. It follows, then, that an uncompetitive inhibitor cannot be overcome by increased concentration of substrate, in contrast to a competitive inhibitor.

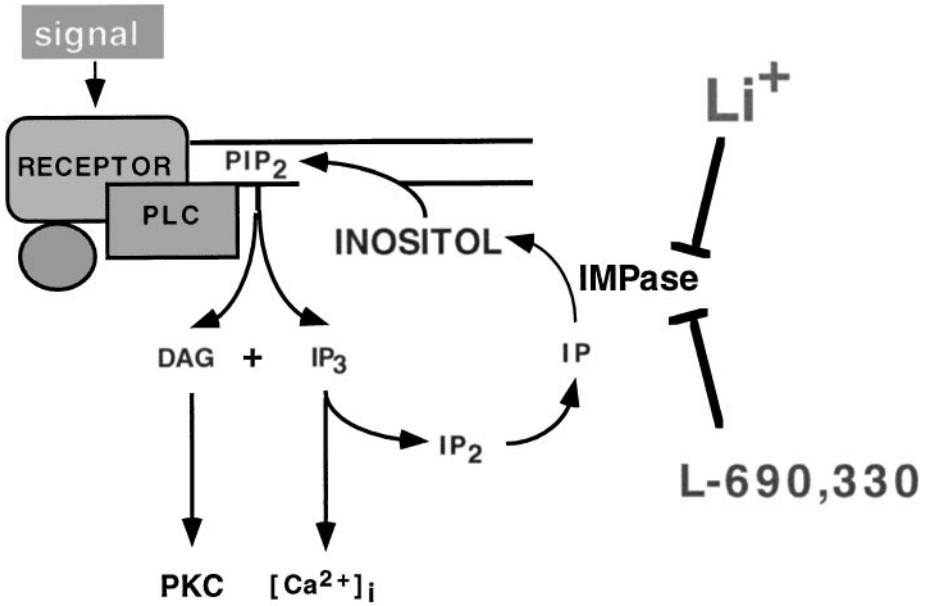

Figure 2 Inositol depletion hypothesis. Ligand binding to a surface receptor activates phospholipase C (PLC), which hydrolyzes the phospholipid PIP_2 to yield two second messengers: diacylglycerol (DAG) and inositol-1,4,5 trisphosphate (IP_3). As shown in this simplified model, lithium inhibits IMPase, which regenerates inositol from inositol monophosphate (IP). If this inhibition is sufficient to deplete inositol, then it should also deplete PIP_2 and prevent the formation of IP_3 and DAG, thus indirectly inhibiting transmembrane signaling. The bisphosphonate compound L-690,330 is an IMPase inhibitor that is 1000-fold more potent than lithium.

The inositol depletion hypothesis is supported by observations that lithium can inhibit IMPase in vivo (74), leading to accumulation of IMP. However, reduction of inositol in mammals is seen only at toxic doses of lithium; therapeutic doses in mammals do not deplete inositol, PIP_2, or IP_3 in vivo, either acutely or after chronic administration (3, 4). Numerous studies have shown inositol depletion in brain slices treated with lithium, but in these in vitro assays, a marked drop in inositol reserves is incurred even before addition of lithium (3, 4). Still, therapeutic concentrations of lithium could lower inositol levels within small, restricted regions of the brain or within specific subcellular pools that are particularly sensitive to inhibition of IMPase, and this might not be detected with the available assays for inositol and inositol phosphates.

The inositol depletion hypothesis has also been invoked to explain the profound effects of lithium on developing organisms (29, 75). Indeed, teratogenic doses of lithium inhibit IMPase in vivo in *Xenopus* embryos (71) and cause a 30% reduction in inositol levels (76), while in *Dictyostelium*, lithium lowers intracellular inositol by 20% (77). In spite of these small changes in inositol levels, coinjection

of inositol can prevent this teratogenic effect of lithium in *Xenopus*, providing additional support for the inositol depletion hypothesis (75).

Although the inositol depletion hypothesis is an attractive model for explaining the effects of lithium in some settings, a closer examination of this hypothesis casts doubt on whether inhibition of IMPase is sufficient to explain the developmental effects of lithium (71, 78). Alternative inhibitors of IMPase (bisphosphonates) that are 1000-fold more potent than lithium (79) have no effect on the development of *Xenopus* embryos despite complete inhibition of IMPase in vivo (71). This observation suggests that inhibition of IMPase is not sufficient for the developmental effects of lithium in *Xenopus*. Why then does coinjection of inositol with lithium reverse the developmental effects of lithium in *Xenopus* (as above)? Elevated levels of inositol or inositol phosphates may have unexpected and indirect effects on other lithium-sensitive pathways; in support of this, raising the level of inositol in *Xenopus* blocks dorsal axis induction by agents other than lithium that are unlikely to act through depletion of inositol, as discussed below (78). In addition, agonist-induced increases in IP_3 can be blocked in slime molds by disruption of the gene encoding phospholipase C (although IP_3 is still detectable at basal levels), yet this mutation has no effect on *Dictyostelium* development (80), despite the dramatic effect of lithium treatment. Furthermore, *Dictyostelium* has at least three forms of IMPase, two of which are insensitive to lithium, which may explain the modest effect of lithium on inositol levels (20% reduction) in this organism (77). Taken together, these observations suggest that the developmental effects of lithium are mediated through a target distinct from IMPase, although the possibility remains that inhibition of IMPase is necessary but not sufficient for lithium action in these settings.

Phosphomonoesterases Other Than IMPase

In addition to IMPase, a number of structurally related phosphomonoesterases have been described that require metal ions (especially magnesium) and are inhibited by lithium (70). Biochemical and crystallographic analyses of three of these, IMPase, inositol polyphosphate 1-phosphatase (IPPase), and fructose 1,6-bisphosphatase (FBPase), have revealed similar tertiary structures and a consensus sequence (D-X_n-EE-X_n-DP(i/l)D(s/g/a)T-X_n-WD-X_{11}-GG) that is involved in metal ion binding, which plays a role in catalysis, and that most likely interacts with lithium ions (70, 81–84). A number of other phosphomonoesterases contain this consensus sequence, including Hal2p and Tol1 (from budding yeast and fission yeast, respectively), SAL1 (from Arabidopsis), the bacterial protein cysQ, and related bisphosphate 3′ nucleotidases (BPntase) from mammals (70, 85–90), defining a large family of lithium-sensitive enzymes.

IPPase catalyzes the removal of the 1-phosphate from inositol 1,4-bisphosphate or 1,3,4-trisphosphate to form inositol 4-phosphate or inositol 3,4-bisphosphate (83, 91). An in vivo correlation between lithium action and inhibition of IPPase is found in *Drosophila* neuromuscular junctions, where lithium exposure phenocopies mutations in IPPase, both of which lead to a similar defect in synaptic

transmission due to increased vesicle release (92). The fact that lithium inhibits IPPase in vitro offers a likely explanation for the effects of lithium in this setting. It would be interesting to test whether overexpression of IPPase or expression of a lithium-resistant form of IPPase prevents the effect of lithium on the *Drosophila* neuromuscular junction and in other settings.

HAL2 was identified in *Saccharomyces cerevisiae* as a gene that, when overexpressed, confers tolerance to high-salt conditions (93). (*HAL2* is identical to *MET22*, which is required for methionine synthesis (94).) Hal2p removes the 3′ phosphate from adenosine 3′, 5′ bisphosphate (pAp) and is inhibited by lithium at submillimolar concentrations (IC50 = 0.1 mM (86)). Although lithium toxicity in yeast requires 100–200 mM LiCl in the growth medium (93, 95), it is not clear what the intracellular concentration is under these conditions. Inhibition of Hal2p by salt (lithium or sodium) or loss-of-function mutations in *HAL2* leads to the accumulation of pAp, which in turn causes inhibition of sulphotransferases and RNA processing enzymes such as exoribonuclease Xrn1p (95, 96). Hal2p was recently crystallized, and this structure, combined with genetic analysis, led to the identification of residues important in binding metal ions and in mediating inhibition by monovalent cations (96). In fact, mutations in Hal2p were identified that reduced sensitivity to monovalent cations; corresponding mutations were generated in human IMPase and were found to reduce sensitivity to lithium almost ninefold (96). It will be fascinating to test whether transgenic expression of lithium-resistant IMPase (or other phosphomonoesterases, if possible) confers resistance to lithium in model organisms or cell lines.

BPntase (also identified as RnPIP) is a bisphosphate 3′-nucleosidase with activity toward pAp and other bisphosphate nucleotides, as well as toward 3′ phosphoadenosine 5′ phosphosulfonate (85, 89, 90). BPntase/RnPIP is dependent on magnesium and is uncompetitively inhibited by lithium with a low Ki (157–300 μM). Since BPntase/RnPIP is structurally related to Hal2p and SAL1, which are involved in salt tolerance in yeast and plants, Speigelberg et al have proposed that inhibition of BPntase might account for lithium-induced nephrogenic diabetes insipidus observed in mammals, a conditioned characterized by poor concentration of urine with consequent polyuria. BPntase/RnPIP also remove the 1-phosphate from inositol 1,4 bisphosphate (85, 89, 90), similar to SAL1 (88).

In summary, a large family of lithium-sensitive phosphomonoesterases has been described in a broad range of organisms; these enzymes offer attractive targets to consider as potential targets of lithium action, and in a few cases are associated with lithium effects in vivo, particularly with IPPase in *Drosophila*, where loss of function is phenocopied by lithium, and with Hal2p in *S. cerevisiae*, in which overexpression prevents the effects of high lithium or sodium concentrations.

Glycogen Synthase Kinase-3

A novel hypothesis to explain the effects of lithium action on embryonic development, glycogen synthesis, and hematopoiesis has been proposed based on the

observation that lithium is a direct inhibitor of glycogen synthase kinase-3 (GSK-3) (71). GSK-3 was first identified as a negative regulator of glycogen synthesis that phosphorylates and inhibits glycogen synthase (97, 98). Two isozymes of GSK-3, GSK-3α and GSK-3β, have been identified (99). Although published work has focused on GSK-3β, GSK-3α has similar but not identical biochemical properties (98 but see also 100). GSK-3β is expressed broadly in eukaryotes, including protozoans such as yeast and *Dictyostelium*, higher plants such as *Arabidopsis*, rice, and corn, invertebrates such as *Caenorhabditis elegans*, *Drosophila*, and sea urchins, and vertebrates including *Xenopus*, mice and humans. In mammals, GSK-3β is expressed in early embryos and in most adult tissue types, including brain (98, 99).

GSK-3β is inhibited by lithium with a Ki (1–2 mM) within the effective range for lithium action (71). Lithium does not inhibit other protein kinases tested, including casein kinase II, protein kinase A, $p34^{cdc2}$, MAP kinase, and protein kinase C. Lithium is an uncompetitive inhibitor of GSK-3β with respect to substrate (71), as seen with IMPase and other phosphomonoesterases but appears to be competitive with respect to magnesium, which may explain its mechanism of inhibition (LJ Conrad & PS Klein, unpublished data). Lithium inhibition of GSK-3β, as well as GSK-3α, has subsequently been confirmed in vitro (101). Lithium also inhibits GSK-3β in vivo, as demonstrated in a number of systems by the reduced phosphorylation of known GSK-3 substrates such as tau protein, MAP-1B, and others (62, 101–106). Furthermore, lithium inhibits GSK-3 derived from diverse species, from *Dictyostelium* to mammals (33, 62, 101).

How then does this inhibition of GSK-3 explain the mechanism of lithium action in developmental settings? Strong support for this hypothesis comes from genetic disruption of GSK-3β in *Dictyostelium* (GSKA). Loss of GSKA in *Dictyostelium* alters cell fate so that presumptive spore cells are diverted to a stalk cell fate (107). The phenotype described by Harwood et al (107) is remarkably similar to the effect of lithium described by Maeda 25 years earlier (28), and thus, lithium phenocopies loss of GSKA in this setting. This was confirmed by demonstrating that lithium inhibits GSKA (62, 108).

In higher eukaryotes, GSK-3β is a negative regulator of the Wnt signaling pathway (Figures 3, 4), which plays a central role in the patterning of early embryos (109–113), in the regulation of cell proliferation (114–116), and in neuronal signal transduction in adults (27). In the absence of Wnt signaling, GSK-3β is found in a complex with several other proteins, including the adenomatous polyposis coli protein (APC), axin, protein phosphatase 2A (PP2A), dishevelled (dsh), and β-catenin (117). This multiprotein complex promotes GSK-3β-mediated phosphorylation of β-catenin, targeting β-catenin for ubiquitination and degradation via the proteosome pathway. Upon activation of Wnt signaling, GSK-3β is inhibited (118–120), allowing β-catenin protein to accumulate (121). Accumulated β-catenin translocates to the nucleus where it forms a transcriptionally competent complex with members of the Tcf/Lef family of DNA binding factors, resulting in the transcription of Wnt target genes (109, 110).

Perturbations that interfere with GSK-3β–mediated phosphorylation of β-catenin, such as null mutations in the GSK-3β gene, known as *zeste-white-3* or *shaggy*

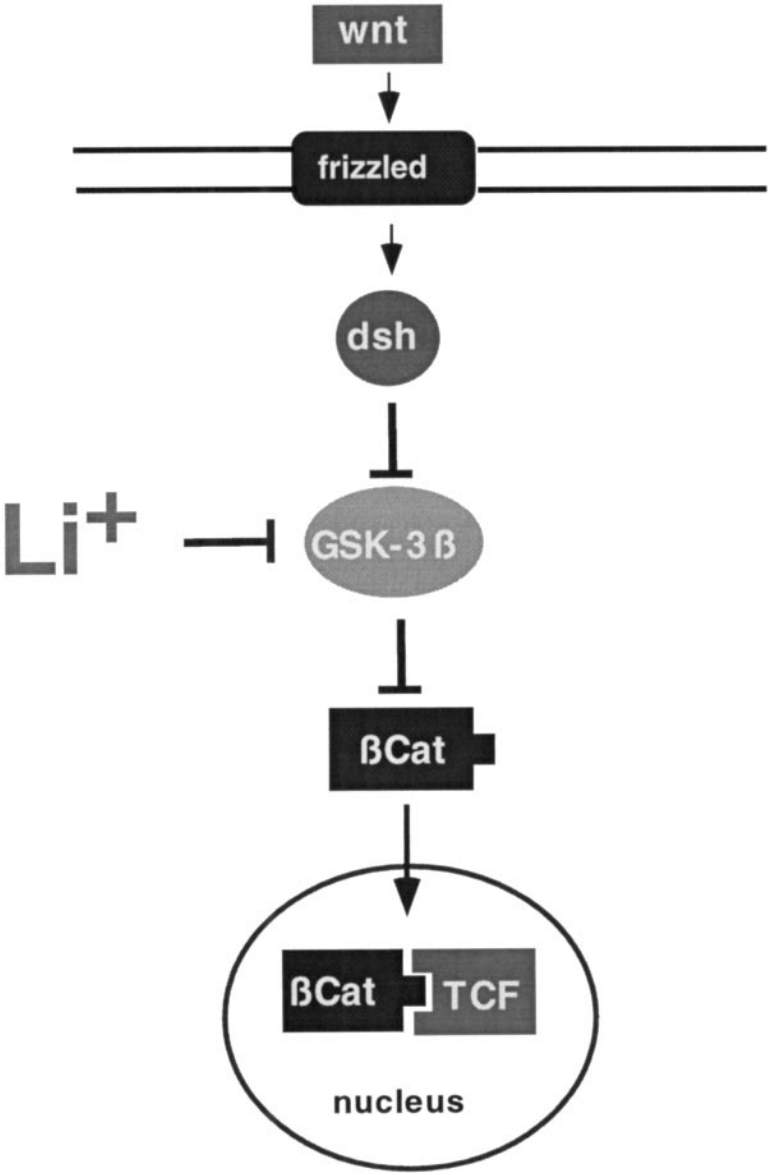

Figure 3 The Wnt signaling pathway. Wnts are a family of secreted glycoproteins involved in embryonic patterning, axonal remodeling, regulation of cellular proliferation, stem cell development, and numerous other processes. In the absence of Wnt, GSK-3β phosphorylates β-catenin, resulting in its rapid degradation. Binding of Wnt to the frizzled receptor leads to inhibition of GSK-3β and stabilization of β-catenin, which accumulates in the nucleus where it interacts with Tcf/Lef DNA binding factors to activate downstream target genes. Lithium activates Wnt signaling by inhibiting GSK-3β directly. (Arrows indicate positive effect and horizontal or inverted T indicates inhibitory effect.)

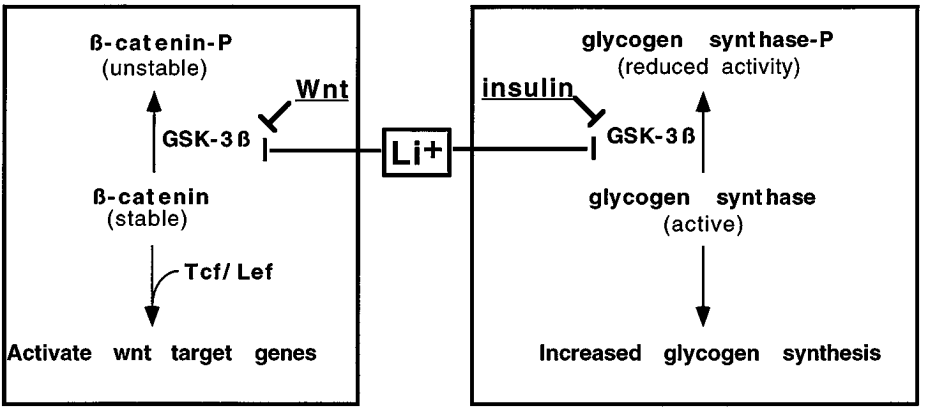

Figure 4 Lithium activates β-catenin and glycogen synthase by inhibiting GSK-3β. GSK-3β negatively regulates β-catenin and glycogen synthase. Shown on the left, inhibition of GSK-3β by lithium results in the stabilization of β-catenin and the activation of Wnt target genes. Shown on the right, GSK-3β reduces the activity of glycogen synthase. Activation of the pathway by insulin or treatment of cells with lithium results in the inhibition of GSK-3β, leading to an increase in glycogen synthesis.

in *Drosophila* (122–124), or dominant inhibitory forms of GSK-3β (125–127), cause constitutive activation of Wnt signaling. Thus, agents such as lithium that inhibit GSK-3β are predicted to mimic Wnt signaling (71), and this has been observed in numerous settings: Lithium leads to accumulation and nuclear localization of β-catenin protein in *Xenopus* oocytes and embryos (62, 128, 129), sea urchin embryos (33, 34, 130, 131), and cultured mammalian epithelial cells and neurons (101, 118, 132). This effect on β-catenin in turn activates Wnt-dependent genes, leading, for example, to duplication of the dorsal axis in *Xenopus* (30, 133–135) or axonal remodeling in mouse cerebellar cells (27, 106, 136), as well as activation of Tcf/Lef-dependent transcriptional reporters (131, 137, CJ Phiel & PS Klein, unpublished data). Lithium also mimics Wnt signaling in the induction of epithelial differentiation of embryonic nephrogenic mesenchyme (35, 138, 139), stimulation of cellular proliferation (36, 114), inhibition of adipocyte differentiation (140), and the stimulation of hematopoiesis (44, 141, 142).

While the inhibition of GSK-3β and stabilization of β-catenin by lithium are consistent with the developmental phenotypes, they are correlations, and one could argue that they are independent of each other. However, the developmental effects of lithium are clearly dependent on activation of downstream Wnt signaling since dominant negative Tcf/Lef blocks the vegetalizing effect in sea urchins (131), and depletion of β-catenin similarly blocks the dorsalizing effect of lithium in *Xenopus* embryos (J Heasman, personal communication).

Two reports have suggested that the effects of lithium on early vertebrate development are mediated through pathways independent of β-catenin (143, 144).

Using a cell-free system derived from *Xenopus* embryos, Nelson & Gumbiner (143) found no change in endogenous or exogenous β-catenin levels after exposure to lithium and argued that the dorsalizing effects of lithium may involve a different pathway. These observations are at odds with reports showing stabilization of exogenous β-catenin by lithium in oocytes, embryos, and cell-free extracts (62, 129, 145); it is not yet clear which variables in the assays account for this difference. Furthermore, treatment of preimplantation mouse embryos results in a range of dorsal-anterior patterning defects in postimplantation embryos that are not associated with changes in β-catenin protein levels or nuclear localization (144), which again raises the possibility that lithium can perturb embryonic development by disrupting more than one pathway. It would be interesting to test the bisphosphonate IMPase inhibitors in these specific assay systems.

As discussed above, lithium causes a 30% reduction in inositol levels in *Xenopus* embryos and coinjection of inositol with lithium prevents lithium-induced dorsalization in *Xenopus*, observations that have provided strong support for the inositol depletion hypothesis. However, inositol also reverses dorsalization caused by dominant inhibitory GSK-3β, which functions as an activator of Wnt signaling, raising the possibility that injected inositol may have an indirect effect in this assay (62). Since elevated GSK-3β activity rescues the effect of dominant inhibitory GSK-3β (125–127) and of lithium (PS Klein, unpublished data), inositol could indirectly activate GSK-3β. While neither inositol nor inositol 1-phosphate affects GSK-3β activity in vitro (PS Klein, unpublished data), recent data have shown that exposure of cells to high concentrations of glucose and hexosamines can increase GSK-3β activity significantly (146). A similar response to high levels of inositol or inositol phosphates could provide an indirect mechanism for inositol rescue of lithium effects on GSK-3β regulated pathways.

As mentioned previously, GSK-3β was initially described as an inhibitor of glycogen synthase, which is regulated by the insulin signaling pathway (97, 98, 147, 148). Insulin signaling activates the protein kinase Akt/PKB, which directly phosphorylates and inhibits GSK-3β, alleviating its inhibition of glycogen synthase (Figure 4). Again, alternative agents that inhibit GSK-3β, such as lithium, would then be predicted to activate glycogen synthase (71), as has been observed (37–41).

Disruption of the GSK-3β gene in mice leads to fetal hepatic necrosis but does not appear to activate Wnt signaling (100). GSK-3α may compensate for the loss of GSK-3β, at least with respect to Wnt signaling. This hepatic degeneration in GSK-3β null mice is consistent with increased sensitivity to tumor necrosis factor (TNF) seen in mice lacking the transcription factor NF-κB, and thus Hoeflich et al (100) proposed that GSK-3β is required for NF-κB regulation of TNF responses. In this respect, lithium again phenocopies loss of GSK-3β because when fibroblasts derived from GSK-3β null mice are exposed to lithium, it also sensitizes their response to TNF (149) and inhibits activation of NF-κB-dependent transcription.

If the effects of lithium are indeed manifested through inhibition of GSK-3β, then alternative inhibitors of GSK-3β should mimic lithium action. Dominant inhibitory GSK-3β mimics lithium action, but these mutated forms of GSK-3 do

not inhibit endogenous GSK-3 activity per se (150). However, two alternative inhibitors of GSK-3β have been described through two hybrid screens for GSK-3 interacting genes (151, 152). GSK-3 binding protein (GBP), a homologue of a previously novel protein named FRAT (frequently rearranged in advanced T-cell lymphoma (152)), has been shown to bind GSK-3β in vivo, to inhibit its enzymatic activity in the tau phosphorylation assay, and to cause dorsalization when overexpressed in *Xenopus* embryos, similar to lithium (152, 153). Mutations that prevent binding to GSK-3 prevent in vivo inhibition and dorsalization of embryos (152).

In addition, the axis inhibitor gene axin (154) was found by two-hybrid screens in numerous laboratories (155–163) to act as a scaffolding protein that binds GSK-3β and β-catenin. This binding is required for GSK-3β phosphorylation of β-catenin (157). Axin thus facilitates phosphorylation and degradation of β-catenin. While full-length axin does not alter the enzymatic activity of GSK-3β, fragments of axin that bind GSK-3β are potent inhibitors of its enzymatic activity and mimic the effects of lithium in *Xenopus* embryos (151) as well as in mammalian cells (164; PS Klein, unpublished data). The GSK-3 interacting domain of axin has been mapped to a 25-amino acid sequence that is well conserved among vertebrate axins (although less well conserved in *Drosophila* axin), and this short peptide still binds GSK-3, inhibits its activity in the tau phosphorylation assay, causes stabilization of β-catenin, and activates Wnt-dependent gene expression (151).

Valproic acid, a well-known antiepileptic agent that is also a highly effective treatment for BPD, has been reported to inhibit GSK-3β-mediated phosphorylation of a peptide substrate in vitro (165) and can cause accumulation of β-catenin protein. If valproic acid can be shown to inhibit GSK-3β in vivo, this would provide exciting additional support for the hypothesis that GSK-3β is an important target in the therapy of BPD.

In summary, the evidence in support of GSK-3β as a relevant target of lithium action, at least in development and in glycogen synthesis, includes (*a*) in vitro inhibition, (*b*) in vivo inhibition, (*c*) lithium phenocopying GSK-3β loss of function, (*d*) alternative inhibitors of GSK-3β mimicking lithium action, (*e*) downstream inhibition of Wnt signaling blocking the effects of lithium, and (*f*) while lithium-resistant forms of GSK-3β have not been identified, simple elevation of the levels of GSK-3β also appears to reverse the effects of lithium.

CONCLUDING REMARKS

A Role for GSK-3β in Alzheimer's Disease?

A prominent pathological feature in Alzheimer's disease (AD) is the presence of neurofibrillary tangles that are primarily composed of hyperphosphorylated tau protein arranged in paired helical filaments (166–168). GSK-3β is one of several protein kinases that can phosphorylate tau in vitro and appears to phosphorylate tau at the same sites in vivo and in paired helical filaments (169–176). Phosphorylation

of tau has been shown by numerous laboratories to be inhibited by lithium (78, 101–104). While it is not clear whether hyperphosphorylation of tau plays a causative role in AD or is a consequence of the disease process, it is intriguing to consider that lithium, through inhibition of GSK-3β, could prevent the accumulation of hyperphosphorylated tau protein and neurofibrillary tangles.

Furthermore GSK-3β and β-catenin have been reported to interact with presenilins 1 and 2 (177–183). Mutations in *presenilin-1* and *-2* have been identified in hereditary forms of early-onset AD (184–186). Conflicting reports suggest a number of possible roles for presenilin in the regulation of β-catenin and neuronal survival (187–190). If presenilins are confirmed to regulate β-catenin in vivo and if modulation of β-catenin protein levels plays a role in the pathogenesis of AD, then clearly the use of lithium, which inhibits GSK-3β and thereby increases the level of β-catenin, could have important consequences in patients with AD.

Lithium and BPD

While a number of direct targets of lithium have been identified, the target responsible for the therapeutic effect of lithium in the treatment of BPD is still unknown. Furthermore, lithium could regulate multiple targets to achieve its dramatic mood-stabilizing effect. Recent studies have identified alternative inhibitors of IMPase (79), BPntase (85), and GSK-3β (151, 152), which could be used in animal models (191, 192) of lithium-sensitive behaviors to determine which potential target is responsible for the behavioral effects of lithium.

Conditional gene disruption and transgenic expression of these genes in mice will also prove highly useful in deciphering the relevant targets of lithium action in neuropsychiatric settings as well as in other settings. In addition to identifying alternative therapies for BPD, this analysis could lead to new agents that mimic other lithium actions, for example stimulating hematopoiesis in neutropenic patients or enhancing the response to extracellular signals such as insulin and TNF. An understanding of the mechanisms of lithium action should also provide important insights into the pathogenesis of BPD and other disorders of mood.

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