Glycogen Synthase Kinase-3: A Target for Novel Bipolar Disorder Treatments

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The enzyme glycogen synthase kinase-3 (GSK-3) is a direct target of lithium. While originally recognized as an important molecule in a limited number of cellular processes, with unclear significance for the treatment of bipolar disorder, recent evidence suggests it has critically important cellular functions in the adult brain. GSK-3 has an essential role in a number of signaling pathways and regulates the function of a diverse number of proteins, notably transcription factors and cytoskeletal elements. The most important functions of the enzyme in regard to bipolar disorder may be critical effects on cellular resilience and neuronal plasticity. There is tremendous interest in GSK-3 inhibitors as novel therapeutic agents, and selective, small-molecule compounds are rapidly being developed for a broad range of other maladies including diabetes, Alzheimer's disease, stroke, and inflammatory conditions. In this perspectives article, we provide an overview of the molecular targets of lithium, focusing on GSK-3–regulated signaling pathways and the important functions of GSK-3 that may have relevance for the treatment of bipolar disorder. We conclude with a discussion of the GSK-3 inhibitors furthest in development and the clinical trials that may emerge.

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In recent years, there has been a growing appreciation that bipolar disorder—once regarded as a remitting disorder with a generally favorable long-term outcome—can be a devastating and life-threatening illness. A number of studies and analyses have now shown that for a large percentage of patients, outcome is quite poor, with high rates of relapse, chronicity, lingering residual symptoms, subsyndromes, cognitive and functional impairment, and psychosocial disability.[1–5] Furthermore, suicide is estimated to be the cause of death in approximately 15% of individuals with severe bipolar disorder,[6,7] and in addition to suicide, many other deleterious health-related effects are increasingly being recognized.[3,8–10] The costs associated with disability and premature death represent an economic burden of billions of dollars annually in the United States alone.[3,11] It is thus not altogether surprising that the Global Burden of Disease study identified bipolar disorder as the sixth leading cause of disability worldwide and as an illness that is likely to represent an increasingly greater health, societal, and economic problem in the coming years.[12]

The discovery of lithium's efficacy as an antimanic agent just over 50 years ago revolutionized our ability to treat patients with bipolar disorder.[13] Perhaps the single most important finding about the efficacy of lithium that has influenced American psychiatry is the fact that lithium, given over long periods of time, reduces the frequency and severity of subsequent mood episodes in bipolar disorder.[14] This recurrent pattern of illness has been eloquently argued to be central to the illness and hence should be in the forefront of our framing questions about etiology and treatment.[6] After more than 50 years, lithium continues to be one of the mainstays of treatment for this disorder, both for the acute manic phase and as prophylaxis for recurrent manic and depressive episodes.[15] However, lithium is clearly far from the perfect medication, and increasing evidence suggests that a significant number of patients do not respond adequately and/or are intolerant of its side effects. Similarly, other mood stabilizers such as valproate and carbamazepine are ineffective or intolerable for a significant proportion of patients. The recognition of the significant morbidity and mortality of patients with severe mood disorders, as well as the growing appreciation that a significant percentage of patients respond poorly to existing treatments, has made the task of discovering new

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therapeutic agents that are both efficacious and have few side effects increasingly more important.

In recent years, there has been an increase in the number of options available for the treatment of recurrent mood disorders with a parallel and unprecedented increase of interest in the treatment of bipolar disorder by pharmaceutical companies, clinicians, researchers, and the general public. A number of newer pharmacologic agents are rapidly being introduced into the market and used by clinicians for the management of bipolar patients[15]; however, almost without exception, all of the new agents are simply central nervous system (CNS)-penetrant drugs that have been developed for other disorders, most notably epilepsy and schizophrenia. Many of these agents have little clinical evidence to support their use. Almost as rapidly as the anticonvulsants are introduced into the market for the management of epilepsy and atypical antipsychotic drugs are introduced for the treatment of schizophrenia, these drugs are used by clinicians for the treatment of bipolar disorder. This practice has undoubtedly resulted in large part from the appalling dearth of pharmacologic agents specifically developed for the treatment of bipolar disorder. While the ever-increasing number of agents in our therapeutic armamentarium has markedly increased our ability to treat a larger number of patients symptomatically, there is a clear need to develop agents that are more specific for treating the fundamental core aspects of bipolar disorder.

In this perspectives article, we review the exciting recent data, which suggest that an enzyme—glycogen synthase kinase—may represent a therapeutically relevant target for lithium's actions. Furthermore, because this important signaling molecule may also be involved in the pathophysiology and/or treatment of Alzheimer's disease and stroke, there is concerted effort in industry and academia to develop novel, selective, brain-penetrant glycogen synthase kinase-3 (GSK-3) inhibitors. Selective GSK-3 inhibitors may not only represent truly novel classes of drugs for bipolar disorder, but may also help unravel the complexities and underlying pathophysiology of this disorder. We would like to emphasize that excellent reviews have been published recently that deal with many aspects of the biochemical functions of GSK-3, and the interested reader is referred to these.[16–19] We focus on those aspects of the function of GSK-3 that are most relevant for bipolar disorder and have tailored this review for clinical psychiatrists, since GSK-3 inhibitors may represent a new lead in the development of agents specifically for the treatment of bipolar disorder.

HOW DOES LITHIUM EXERT ITS THERAPEUTIC EFFECTS?

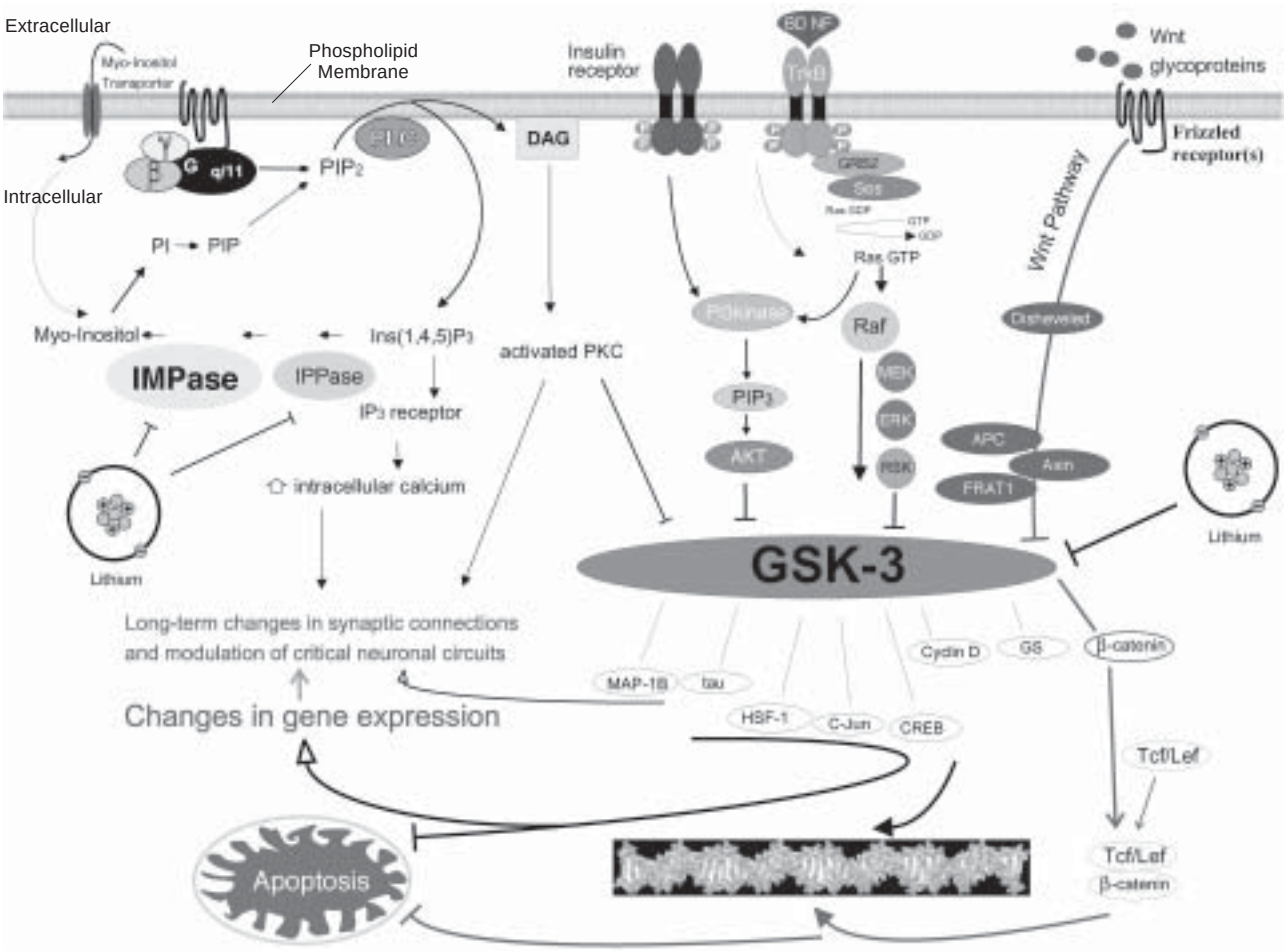
Despite decades of research, there is no compelling evidence that lithium binds to an extracellular "lithium receptor," and the preponderance of the data suggest that lithium exerts its effects by directly targeting intracellular enzymes.[20,21] Lithium has a hydrated ionic radius very similar to that of magnesium, a necessary cofactor for many enzymes. It has been found that lithium inhibits some enzymes through competition for this required cofactor.[22–25] The mechanism of the therapeutic action of lithium is not known. However, since the therapeutic effects of lithium are only observed after days or weeks of treatment, many investigators feel that initial inhibitory effects on enzyme(s) produce a cascade of critical genomic, cellular, and neuronal circuit-level changes, which ultimately treats the underlying pathology and thus results in modulation of the diverse constellation of signs and symptoms seen in the disorder.[21,26–28]

Although lithium is documented to have some degree of inhibition of a number of enzymes,[29] only a few are significantly inhibited at therapeutic lithium concentrations (0.6–1.2 mM).[20,21,30] In mammalian systems, lithium inhibits a group of at least 4 related phosphomonoesterases (the best-known of which is inositol monophosphatase [IMPase]),[24] the metabolic enzyme phosphoglucomutase,[31–33] and GSK-3.[34,35]

The phosphomonoesterases are a group of magnesium-dependent, lithium-sensitive phosphatases that includes inositol polyphosphate 1-phosphatase (IPPase), IMPase, fructose 1,6-bisphosphastase 1-phosphatase (FBPase), and bisphosphate nucleotidase (BPNase, also referred to as 3'-phosphoadenosine-5'-phosphate [PAP] phosphatase). Of these enzymes, IMPase has received the greatest attention as a therapeutically relevant target of lithium; readers interested in a more thorough discussion of lithium's effects on FBPase and BPNase are referred to recent review articles.[21,30]

IMPase is an important "recycling enzyme" in the phosphoinositide signaling pathway. This second messenger system, once activated (by, for example, various muscarinic, serotonergic, or glutamatergic receptors), yields 2 second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 is then sequentially dephosphorylated ("recycled") to yield inositol (in particular a form known as *myo*-inositol), which can be utilized to form the starting material of the phosphoinositide signaling pathway. The enzyme that removes the last phosphate group from IP_3 to yield *myo*-inositol is the lithium-inhibitable enzyme IMPase[36] (Figure 1). The finding that lithium inhibits IMPase[37,38] led to the heuristic inositol depletion hypothesis, which posited that lithium, via inhibition of IMPase, would decrease the availability of *myo*-inositol and thus ultimately the amount of phosphatidylinositol 4,5-bisphosphate (PIP_2) (the starting material for the signaling pathway), thereby putting a "brake" on this pathway.[39] It is further suggested that the brain is uniquely susceptible to inositol depletion since it has limited capacity to derive inositol.[39] IPPase, mentioned above as an-

Figure 1. Cellular Pathways Mediated by Glycogen Synthase Kinase-3 (GSK-3) and/or Lithium



GSK-3 functions as an intermediary in a number of signaling pathways including neurotrophic signaling pathways, the insulin/PI_3 kinase pathway, and the Wnt pathway. Its function while active in many of these pathways is pro-apoptotic. Inhibiting GSK-3, for example with lithium, is generally anti-apoptotic. Many GSK-3 targets are transcription factors (β-catenin, c-Jun, HSF-1, CREB) and cytoskeletal elements (tau, MAP1B) (see Table 1). The influence of neurotrophic factors (such as BDNF) on cell survival is mediated—in part—by activation of the MAP kinase cascade. In this pathway, activation of neurotrophic factor receptors (Trks, TrkB for BDNF) results in activation of the MAP kinase cascade via several intermediate steps. The resultant activation of the small guanosine triphosphate–binding protein Ras (via adapter proteins GRB2 and Sos) leads to activation of a cascade of serine/threonine kinases. These include Raf, MEK, and MAP kinase (also referred to as extracellular signal-regulated kinase, or ERK). One target of ERK is Rsk, a kinase that can phosphorylate and deactivate GSK-3. Ras can also activate PI_3 kinase, a kinase that is activated by insulin signaling as well, and inactivates GSK-3 via phosphorylation. In this pathway, GSK-3 inhibition activates glycogen synthase. GSK-3 is also an important intermediary in the Wnt signaling pathway. Via the frizzled family of receptors, Wnt-secreted glycoproteins activate disheveled. Disheveled activation results in inhibition of GSK-3 via interactions within a complex that contains the proteins APC, axin, and FRAT1. Under normal conditions, phosphorylation of β-catenin by GSK-3 results in its degradation by ubiquitin. Following GSK-3 inhibition, nondegraded (nonphosphorylated) β-catenin binds to Lef/Tcf transcription factors, targeting transcription of specific genes. GSK-3 can be inhibited by at least the following 5 different mechanisms, which are relevant for the development of novel GSK-3 inhibitors (see Table 2). (1) Lithium appears to be competitive for a magnesium binding site on GSK-3.[23,25] (2) In the Wnt pathway, GSK-3 is inhibited by interacting with specific proteins that are part of a larger protein complex. Proteins that inhibit GSK-3 in this manner are axin and FRAT1.[59,60] (3) In the other cellular pathways that inhibit GSK-3 activity, GSK-3 is inactivated by phosphorylation of one of its serine residues (by kinases Akt, P90Rsk, P70 S6, PKC, and PKA).[17,18] (4) Most small-molecule synthetic inhibitors compete with ATP for a binding site. These include indirubins,[128] SmithKline Beecham compounds SB-415286 and SB-21676,[89,131–133] and Chiron compounds.[153] (5) Recently, synthetic small-molecule GSK-3 inhibitors, which are not ATP competitive, have been developed that compete with substrate binding.[134,135] The upper left portion of the figure depicts lithium's actions on the phosphoinositol signaling pathway. Activation of some G proteins induces phospholipase C hydrolysis of PIP_2 to DAG and IP_3. DAG activates PKC. IP_3 binds to the IP_3 receptor that also functions as a calcium channel in the cell. This interaction results in the release of intracellular calcium reservoirs from the endoplasmic reticulum; calcium is an activator of many enzymes and plays a prominent role in many cellular signaling events. IP_3 is recycled back to PIP_2 by the enzymes IMPase and IPPase, both of which are inhibited by lithium.[36] The inositol depletion hypothesis suggests that lithium exerts its therapeutic actions by depleting free inositol and thus dampening the activation of downstream signaling pathways in neurons.[39]

Abbreviations: APC = adenomatous polyposis coli, ATP = adenosine triphosphate, BDNF = brain-derived neurotrophic factor, CREB = cyclic AMP response element binding protein, DAG = diacylglycerol, ERK = extracellular signal-regulated kinase, FRAT1 = frequently rearranged in advanced T-cell lymphomas 1, G = G protein, GDP = guanosine diphosphate, GRB2 = growth factor receptor-bound protein 2, GS = glycogen synthase, GTP = guanosine triphosphate, HSF-1 = heat shock factor-1, IMPase = inositol monophosphatase, $Ins(1,4,5)p_3$ and IP_3 = inositol 1,4, 5-triphosphate, IPPase = inositol polyphosphate 1-phosphatase, MAP1B = microtubule-associated protein 1B, MEK = MAP kinase kinase, P = phosphate groups, PI = phosphoinositide, PI_3 kinase = phosphatidylinositol 3-kinase, PIP = phosphatidylinositol phosphate, PIP_2 = phosphatidylinositol 4,5-bisphosphate, PIP_3 = phosphatidylinositol 3,4,5-trisphosphate, PKC = protein kinase C, PLC = phospholipase C, Rsk = ribosomal protein S6 kinase, Trk = tyrosine receptor kinase.

other lithium-inhibitable enzyme, acts prior to IMPase in the inositol recycling pathway, and inhibition of IPPase would most likely have similar effects[36,40] (Figure 1). While lithium has been shown to decrease free inositol levels in brain sections in vitro and in the brains of rodents treated chronically with lithium,[40,41] far less consistent effects have been observed for PIP_2, and those positive results that exist suggest a small effect.[40,42] Lithium has also been shown to decrease *myo*-inositol in human subjects; however, the *myo*-inositol reduction was observed after only 5 days of treatment, suggesting that any decrease in PIP_2 signaling occurs prior to clinically relevant effects that generally occur after longer treatment.[43] Perhaps more relevant as direct mediators of a clinical response may be the effects of lithium on downstream targets of phosphoinositol signaling (that may be due to inhibition of IMPase/IPPase). Lithium (and valproate) has been shown in cultured cells and in the rodent brain to cause selective reductions of protein kinase C (PKC) isozymes; in the case of lithium, this reduction appears to result from the depletion of inositol.[44] PKC is a major downstream target of the phosphoinositide signaling pathway (Figure 1). The selective lithium-induced inositol-dependent reduction of PKC isoenzymes[44] led to a "proof-of-concept" study in which tamoxifen—a PKC inhibitor at high concentrations—was examined in an open-label clinical trial; initial results are promising and have led to the initiation of a larger double-blind, placebo-controlled trial.[45]

The inositol depletion hypothesis remains a viable supposition.[39] However, it is likely that true validation will only come from the successful development of brain-penetrant small-molecule inhibitors of IMPase.[46] Past industry efforts have attempted to develop a brain-penetrant IMPase inhibitor by altering the primary substrate of IMPase, inositol monophosphate.[47] Compounds with sufficient inhibition were developed but thus far have failed to advance through clinical trials because they are highly charged[48] or extremely lipophilic,[49] both of which limited the bioavailability in the brain (see Atack[47] for review). The published crystal structure and modeling studies of IMPase may help in the development of novel inhibitors.[50,51]

GLYCOGEN SYNTHASE KINASE-3

A Newer Target For Lithium

Approximately 7 years ago, developmental biologists working with *Xenopus* oocytes made the novel observation that lithium inhibited GSK-3 in a noncompetitive manner.[34] It currently appears to be the only kinase significantly directly inhibited by lithium at therapeutic concentrations.[29,34,35] When this intriguing observation was made in 1996, GSK-3 was only well recognized as an important enzyme in a limited number of cellular processes—namely in the Wnt signaling pathway and as an inhibitor of glycogen synthase (acting as an important intermediary in the insulin signaling pathway; Figure 1).[17] Since then, however, evidence of the role of GSK-3 in regulating important CNS functions, and also dysfunction, has increased at a tremendous rate, leading to the possibility of clinical trials of selective GSK-3 inhibitors in classical neurodegenerative disorders (e.g., Alzheimer's disease, stroke) and in bipolar disorder (see discussion in the final section describing the development of GSK-3 inhibitors).

The K_i for lithium's inhibition of GSK-3 was initially reported by 2 laboratories as 2 mM[34] or between 1 and 2 mM.[35] Therapeutic plasma lithium concentrations in patients generally range from 0.6 to 1.2 mM, whereas human brain concentrations may be lower.[52] However, lithium is now known to inhibit GSK-3 through competition for magnesium,[23,25] and the aforementioned studies estimated lithium's ability to inhibit GSK-3 in the presence of 10 mM magnesium, which is considerably higher than physiologic levels. The use of such nonphysiologic conditions results in an underestimation of lithium's ability to inhibit GSK-3 in vivo. While brain intracellular magnesium concentrations are not precisely known, they are estimated to be between 0.2 mM and 1.2 mM.[53–57] This range suggests that the concentration of lithium required to significantly inhibit GSK-3 is considerably lower than 1 to 2 mM. In support of such a contention, it has recently been demonstrated that chronic lithium at therapeutic concentrations (~0.6 mM in plasma) significantly increases protein levels of a direct GSK-3 target, β-catenin, in the rat brain.[58] Inhibition of GSK-3 leads to an upregulation of β-catenin,[59,60] and thus β-catenin is often quantitated as a readout of in vivo GSK-3 inhibition. Interestingly, lithium has also been shown to increase phosphorylation of GSK-3 at the inhibitory serine site in treated cells and in the brains of mice.[61–67] Recent work suggests that this finding is specific to lithium's direct inhibition of GSK-3 (as opposed to modulation of other enzymes or signaling pathways) and that this action may be mediated by the GSK-3–dependent protein phosphatase-1/inhibitor-2 complex.[64]

What Is It?

GSK-3 is a regulator of a number of cellular signaling pathways that are active both during development and in adult organisms. As we discuss later, the most critical function of GSK-3 relevant for the treatment of bipolar disorder may be its role in regulating apoptosis (programmed cell death) in neurons. In humans, GSK-3 is found in 2 nearly identical forms, α and β. These forms share a nearly identical amino acid sequence in their catalytic domain, generally appear to be regulated by the same signaling pathways, and have similar specificity in regard to targets. Both forms are constitutively active in cells and are deactivated by phosphorylation of a serine residue

or by interactions among proteins within the Wnt signaling pathway.[17,18] It is this constitutive activity—and intracellular signal-mediated inactivation—that makes GSK-3 unique among kinases; the majority of kinases are quiescent and inactive in cells and are activated by specific signals (often phosphorylation).

GSK-3 was named based on its originally described function as a kinase that inactivates glycogen synthase. Following insulin receptor stimulation, phosphatidylinositol 3-kinase (PI_3) and Akt (also protein kinase B) are activated, resulting in the phosphorylation and concomitant inactivation of GSK-3. Inactivated GSK-3 no longer phosphorylates glycogen synthase, allowing the formation of glycogen from glucose[17,18] (Figure 1). GSK-3 is also a key modulator of signaling in the Wnt signaling pathway.[59,60] In mammals, secreted Wnt glycoproteins bind to the frizzled family of receptors. Wnt pathway activation results in dissociation of GSK-3 from β-catenin, preventing β-catenin phosphorylation that serves as a mark for its degradation. The nonphosphorylated, and thus nondegraded, β-catenin is then able to enter the nucleus and act as a transcription factor in concert with Tcf (also called Lef) transcription factors to regulate gene transcription[59,60] (Figure 1). Interestingly, dissociation of GSK-3 from β-catenin does not appear to require phosphorylation-mediated deactivation, but rather critical interactions within a protein complex (GSK-3, adenomatous polyposis coli [APC], frequently rearranged in advanced T-cell lymphomas 1 [FRAT1], axin, and dishevelled).

In addition to regulation by Akt and Wnt signaling, other kinases including p70 S6 kinase, Rsk, PKC, and cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA) appear to deactivate GSK-3 by phosphorylation of a serine residue (Figure 1). Signals from growth factors such as brain-derived neurotrophic factor (BDNF) are propagated via both Rsk (via the extracellular signal-regulated kinase [ERK] mitogen-activated protein [MAP] kinase pathway)[68–72] and Akt (PI_3 kinase pathway).[73] As recently reviewed by Frame and Cohen,[16] GSK-3 has multiple targets within the cell that can be grouped into 6 categories: transcription factors, enzymes that regulate metabolism, proteins bound to microtubules, scaffolding proteins, components of the cell division cycle machinery, and proteins involved in cell adhesion (Table 1). The effect of GSK-3 on numerous transcription factors such as c-Jun, heat shock factor-1 (HSF-1), and nuclear factor of activated T cells has drawn considerable interest and is particularly noteworthy.[19] Generally, but not exclusively, GSK-3 activity results in suppression of the activity of transcription. Conversely, inactivation of GSK-3—for example, by lithium—appears to activate these transcription factors.[19] As is clear (Figure 1), GSK-3 has a central role as a multifunctional mediator of many cellular processes and regulates the function of many important cellular targets. In the next section, we describe the data suggesting that impairments in the regulation of cellular resilience and neuroplasticity may underlie the pathogenesis and/or pathophysiology of mood disorders. This section is followed by a description of the data suggesting that lithium (and GSK-3 inhibition) is involved in regulating neuronal death and cellular resilience to injury.

Table 1. Targets of Glycogen Synthase Kinase-3[a]

Transcription factors	Proteins bound to microtubules
C/EBPβ	APC
HSF-1	MAP1B
c-Jun	MAP2
Myc	Human dynamin-like protein
NFATc	Kinesin light chain
C/EBPα	Tau
CREB	Scaffolding proteins
MITF	Axin
β-Catenin	Presenilin-1
Regulation of metabolism	Components involved in cell division
eIF2B	Cyclin D
Glycogen synthase	Human ninein
ATP citrate lyase	Proteins involved in cell adhesion
Cyclic AMP–dependent kinase	β-Catenin
Pyruvate dehydrogenase	DF3/MUC1
	Neural cell-adhesion protein

[a]The table shows many of the targets of GSK-3 (see Frame and Cohen[16] for a complete review). Some targets have only been validated in vitro and thus require in vivo validation.[16–19] It is believed that GSK-3 plays a central role in regulating apoptosis and cell viability, which may be relevant to the treatment of severe mood disorders, as enhancing cellular resilience and neuroplasticity may underlie some of the effects of mood stabilizers and antidepressants.[151,152] Generally, the active state of GSK-3 increases apoptosis and decreases the activity of multiple transcription factors. Thus, inhibiting GSK-3 generally activates transcription factors and is neuroprotective.

Abbreviations: APC = adenomatous polyposis coli, ATP = adenosine triphosphate, C/EBP = CCAAT/enhancer binding protein, CREB = cyclic AMP response element binding protein, eIF2B = eukaryotic translation initiation factor 2B, HSF-1 = heat shock factor-1, MAP1B = microtubule-associated protein 1B, MAP2 = microtubule-associated protein 2, MITF = microphthalmia-associated transcription factor, NFATc = nuclear factor of activated T-cells.

FUNCTIONAL RELEVANCE OF GSK-3 INHIBITION IN THE TREATMENT OF BIPOLAR DISORDER

Is Modulation in Neuronal Plasticity and Cellular Resilience Relevant to the Pathophysiology of Bipolar Disorder?

A growing body of data suggests that severe mood disorders are associated with impairments of structural plasticity and resilience and, furthermore, that these changes are not simply the deleterious consequences of repeated affective episodes per se. There is now evidence from a variety of sources demonstrating regional reductions in CNS volume,[74–77] as well as reductions in the numbers and/or sizes of glia and neurons in discrete brain areas in severe, recurrent mood disorders.[78,79]

Functional imaging studies have revealed multiple abnormalities of regional cerebral blood flow and glucose metabolism in limbic and prefrontal cortical

(PFC) structures in mood disorders.[74–77] These abnormalities implicate limbic-thalamic-cortical and limbic-cortical-striatal-pallidal-thalamic circuits, involving the amygdala, orbital and medial PFC, and anatomically related parts of the striatum and thalamus in the pathophysiology of mood disorders. Structural imaging studies have demonstrated reduced gray matter volumes in areas of the orbital and medial PFC, ventral striatum, and hippocampus and enlargement of the third ventricle in patients with mood disorders relative to healthy controls.[74–77] Also consistent is that white matter hyperintensities are found at an increased rate in the brains of patients with bipolar disorder compared with control populations.[80] Complementary postmortem neuropathologic studies have shown abnormal reductions in cortex volume, glial cell counts, and/or neuronal densities/sizes in the subgenual PFC, orbital cortex, and dorsal anterolateral PFC.[78,79]

Many of these clinical findings are preliminary reports and, while extremely interesting, require further replication. Thus, it is not presently known whether this evidence of neuronal deficits constitutes developmental abnormalities that may confer vulnerability to abnormal mood episodes, compensatory changes to other pathogenic processes, the sequelae of recurrent affective episodes per se, or other factors that are difficult to control in patient populations. Understanding these issues will depend on extensive replication and experiments that delineate the onset of such abnormalities within the illness course and determine whether they antedate depressive episodes in individuals at high familial risk for mood disorders.[81]

The recent data suggesting neurotrophic effects of lithium in cellular paradigms, animal models, and the brains of patients suggest the possibility that treatment may prevent, or possibly even reverse, impairments in structural plasticity and cellular resilience. In support of this hypothesis, Drevets and colleagues have reanalyzed data from 1997 describing an ~40% reduction in subgenual PFC gray matter volumes in the brains of patients with mood disorders.[82] They found that the decrease in volume was restricted to patients who were not receiving lithium or valproic acid, that patients receiving these drugs had a significantly larger volume than other patients, and that the subgenual PFC volumes of patients chronically on lithium or valproic acid were not statistically different from control subjects (W. Drevets, personal communication; article in preparation).

GSK-3 Plays a Critical Role in Neuroprotection

GSK-3 appears to be a major protein involved in regulating apoptosis in neurons. Overall, the data suggest that active GSK-3 facilitates apoptosis while inhibition of GSK-3 attenuates cellular apoptosis. For example, increased activity of GSK-3 in a number of cell types increases apoptosis or is correlated with apoptosis in neuronal cells.[83–87] Conversely, decreasing GSK-3 activity with a protein inhibitor, antisense nucleotides, an inactivated form of GSK-3, or administration of lithium prevents these effects.[83–87] GSK-3 activity also increases in the rat cortex following focal cerebral ischemia.[62] A recently developed class of maleimides (novel GSK-3 inhibitors, discussed later in the section on GSK-3 inhibitors)[88] has also been shown to protect both central and peripheral neurons in culture from cell death.[89] Thus, accumulating evidence in neurons suggests that GSK-3 has a paramount role in regulating apoptosis and that inhibition or down-regulation of GSK-3 has the effect of preventing cell death. Recent work also shows that a novel inhibitor of GSK-3 is able to induce neuronal differentiation in embryonic stem cells.[90]

Research on the role of Wnt glycoproteins and Wnt signaling in the adult nervous system is still in its infancy, but there is evidence that they may play an important part in synaptic plasticity and neuronal survival.[91–95] Furthermore, recent research on the role of β-catenin in brain development has found that up-regulation of this protein is sufficient to cause the formation of gyri and sulci in the mouse brain, a finding observed only in higher mammals and suggestive of an important role in higher mammalian cognitive functions.[96] Another recent study, consistent with these results, reports that overexpression of GSK-3 (which would be expected to decrease β-catenin levels) in the neonatal mouse results in smaller overall brain volume.[97] However, in this latter study it should be noted that increasing GSK-3 would have other effects in addition to decreasing β-catenin.

Preclinical evidence both in cell culture and in animal models shows that lithium also has neuroprotective properties.[19,98,99] Lithium has been demonstrated to protect against the deleterious effects of glutamate, *N*-methyl-D-aspartate receptor activation, aging, serum or nerve growth factor deprivation, low potassium, ouabain (a glycoside; inhibits the sodium potassium adenosinetriphosphatase), thapsigargin (mobilizes intracellular calcium), 1-methyl-4-phenylpyridinium, and β-amyloid in vitro (reviewed in Manji et al.[98] and Chuang et al.[99]). More importantly, lithium's neurotrophic and cytoprotective effects have also been demonstrated in the rodent brain in vivo. In a rat model of stroke using middle cerebral artery occlusion, lithium markedly reduced neurologic deficits and decreased brain infarct size when given prior to[100] or following[101] middle cerebral artery occlusion. In a rat Huntington's disease model, lithium significantly reduced brain lesions resulting from infusion of quinolinic acid, an excitotoxin.[102] Lithium also enhances hippocampal neurogenesis in the adult rodent hippocampus[103,104] and in primary cell culture.[105]

On the basis of these preclinical data suggesting possible neurotrophic actions of lithium, the effects of lithium on neuroimaging measures related to neuronal viability

were addressed in longitudinal studies using magnetic resonance spectroscopy (MRS) to quantitate *N*-acetylaspartate (NAA, a putative marker of neuronal viability that is decreased in many neurologic diseases) levels and magnetic resonance imaging (MRI) to quantitate total brain gray matter. Measurements of bipolar patients were made at baseline (medication free, after a minimum 14-day washout) and then repeated after 4 weeks of lithium treatment at therapeutic doses. It was found that chronic lithium increased NAA concentration in the human brain in vivo.[106] It was additionally noted that there was an ~0.97 correlation between lithium-induced NAA increases and regional voxel gray matter content, suggesting that possible neurotrophic effects of lithium may be restricted primarily to brain gray matter. In a second longitudinal study, brain tissue volumes were examined using high-resolution 3-dimensional MRI and validated by quantitative brain tissue segmentation methodology to identify and quantify the various components by volume, including total brain white and gray matter content.[107] This study revealed that chronic lithium significantly increases total gray matter content in the human brain of patients with bipolar disorder. No significant changes were observed in brain white matter volume or in quantitative measures of regional cerebral water content, thereby providing strong evidence that the observed increases in gray matter content are most likely due to neurotrophic effects as opposed to any possible cell swelling and/or osmotic effects associated with lithium treatment. Recently, the gray matter findings have been corroborated in a cross-sectional MRI study[108] and the NAA findings have been corroborated in cross-sectional MRS.[109] Lithium's inhibition of GSK-3 may be, at least in part, responsible for both the preclinical and clinical neurotrophic actions of lithium.

GSK-3 May Also Regulate Circadian Rhythms

Recent evidence suggests that lithium may also regulate circadian cycles through inhibition of GSK-3. A great deal of evidence—hormonal, physiologic, and behavioral—suggests that dysregulation of circadian rhythms is present in patients with both bipolar disorder and unipolar depression.[110] For example, severe disruption in sleep-wake patterns is often observed in bipolar disorder (i.e., decreased need for sleep during manic periods or an increased or decreased ability to sleep during depressive episodes). Furthermore, treatment modalities that most likely modulate circadian rhythms, such as sleep deprivation, clearly have efficacy in the treatment of mood disorders.[111,112] For a complete review of the role of circadian factors in the pathogenesis, pathophysiology, and treatment of bipolar disorder, we refer the reader to a number of excellent reviews.[6,80,110–113]

It has been known for some time that lithium treatment has an effect of lengthening the circadian period in diverse species, ranging in complexity from individual cells to humans, suggesting that there is a single evolutionarily conserved target for this action.[114] However, the cellular target for lithium's effect on circadian cycles has remained unknown. Of relevance may be a recent preclinical finding in *Drosophila* suggesting that GSK-3 is an important mediator of the circadian cycle. Martinek et al.[115] identified the *Drosophila* orthologue of GSK-3β, SHAGGY, as a component of the circadian cycles in this species. Activation of this *Drosophila* GSK-3 appears to advance the entry of the TIMELESS protein into the nucleus, an action, based on the central role of TIMELESS in advancing the circadian day, that would be expected to advance the circadian cycle. Indeed, overexpression of SHAGGY significantly lengthened the *Drosophila* free-running circadian cycle.[115] Additionally, gene targeting resulting in a decrease in SHAGGY activity increases circadian period length,[115] precisely the effect (increase in circadian period) that has been noted in numerous species, including *Drosophila*, after treatment with lithium.[114] While there are many differences between the molecular components of circadian cycles in mammals and *Drosophila* (including the lack of a true TIMELESS orthologue), there are also many similarities.[116–118] It is therefore interesting to speculate that GSK-3 has a similar general—and evolutionarily conserved—action in the function of the mammalian circadian clock.[93] This putative function of GSK-3 thus represents a possible molecular target for the effect of lithium on circadian cycles.

Together, the clinical and preclinical data suggest that lithium may regulate circadian cycles via its effects on GSK-3 and that these effects may represent an important mechanism by which this monovalent cation brings about long-term stabilization of mood.

THE DEVELOPMENT OF SMALL-MOLECULE, SELECTIVE, CNS-PENETRANT GSK-3 INHIBITORS: RAPID PROGRESS

The data reviewed thus far clearly suggest that selective GSK-3 inhibitors may have considerable utility in the treatment of bipolar disorder. Validation of any potential therapeutic value of inhibiting GSK-3 will come from clinical trials of novel, brain-penetrant inhibitors. It is noteworthy that many pharmaceutical companies are actively developing these molecules. Much of the emphasis is for the treatment of diabetes, Alzheimer's disease, stroke, and inflammation.[16–18,119] It is beyond the scope of this review to describe in complete detail the reasons for these interests; however, the interested reader is referred to the following references.[16–18,119] Briefly, GSK-3's role as a kinase that deactivates glycogen synthase may be useful in lowering blood sugar and treating diabetes.[120] A former name for GSK-3 was tau kinase, and phosphorylation of tau by GSK-3 may advance the formation of

Table 2. Mechanisms by Which Glycogen Synthase Kinase-3 (GSK-3) Is Inhibited

Endogenous	Exogenous
Prevention of activating phosphorylation at tyrosine site	Magnesium competitive
Unidentified inhibitors	Lithium
Inhibitory phosphorylation	ATP competitive
A number of signaling pathways act on a GSK-3 serine site in this manner (ie, PKC/Akt/Rsk1)	Most novel compounds developed thus far belong to this class
Protein complex inhibitors	Substrate competitive
Act within Wnt signaling pathway (ie, FRAT1, axin peptides)	Design based on peptide substrates (ie, glycogen synthase)
	Synthesized small molecules

Abbreviations: ATP = adenosine triphosphate, FRAT1 = frequently rearranged in advanced T-cell lymphomas 1, PKC = protein kinase C, Rsk1 = ribosomal protein S6 kinase-1.

neurofibrillary tangles.[121] Recent evidence also suggests a role of GSK-3 in the formation of amyloid-β peptides in cell culture,[122,123] and also in transgenic mice expressing an amyloid-β peptide mutation.[123] Thus, active GSK-3 may play a role in both the formation of hyperphosphorylated tau and amyloid deposition, two pathologic processes associated with the development of Alzheimer's disease. GSK-3's general effects on apoptosis and regulation of NF-κB may have utility for the treatment of stroke and inflammation.[124] Recent evidence also suggests a role for GSK-3 inhibitors in inhibiting ischemic damage in a model of cardiac reperfusion injury.[125] It is likely that any small-molecule, brain-penetrant inhibitors that are developed for these maladies will also undergo clinical trials for the treatment of bipolar disorder.

GSK-3 Inhibitors Currently Under Development

Lithium is an inhibitor of GSK-3 activity[34,35] that appears to exert its actions by competition for a magnesium binding site.[23,25] In the cell, GSK-3 is inhibited either by phosphorylation of one of its serine residues (by the kinases Akt, Rsk, PKA, p70 S6 kinase, and PKC, among others)[17,18] or by protein inhibitors (namely axin or FRAT1) as part of a large multimeric protein complex within the Wnt signaling pathway.[59,60] While the mechanisms involved are unclear, GSK-3 can also be activated by phosphorylation of a tyrosine site, and thus inhibition of this process would be expected to inhibit GSK-3 activity (Table 2, Figure 1).

As mentioned, specific, brain-penetrant GSK-3 inhibitors are actively under development by a number of pharmaceutical companies. In this regard, Dorronsoro and colleagues[126] recently reviewed the exponential increase in the development of GSK-3 inhibitors over the last 4 years (they report more than 45 patents). A number of drug compounds, including the general classes of hymenialdisines, paullones, indirubins, maleimides, and thiadiazolidinones, have been developed as GSK-3 inhibitors; the majority of these appear to exert their actions by being competitive with ATP (Table 2). For a complete review, see Dorronsoro et al.[126] and Martinez et al.,[127] both of which are excellent references. We selectively review some of these compounds here in more detail.

Indirubin is an active ingredient of danggui longhui wan, which is a traditional Chinese herbal medicine. Recently, indirubin was identified as a potent inhibitor of GSK-3 with significant inhibition in the nanomolar range.[128] Indirubins inhibit GSK-3 by competing with ATP binding to the catalytic site.[128] Of the novel GSK-3 inhibitor compounds under development, indirubins are the only group for which there are published data on blood-brain barrier penetration and toxicologic data in animals and humans. In this regard, certain indirubins appear to readily pass the blood-brain barrier (reviewed in Ma and Yao[129]). Based on reasonable toxicity studies, indirubin has been approved in China for clinical trials to treat chronic myelocytic and chronic granulocytic leukemia; however, while currently in drug trials, indirubins are not entirely specific for GSK-3, also inhibiting cyclin-dependent kinases (the rationale for their use in leukemia).[130]

Maleimides are also potent and direct inhibitors of GSK-3 that show significant inhibition in the nanomolar range.[131] SmithKline Beecham identified a number of maleimide derivatives as potent inhibitors of GSK-3.[132] The maleimide derivatives SB-415286 and SB-216763 are structurally distinct compounds that inhibit GSK-3 activity by competing for the ATP binding site.[89,132] These compounds have been utilized in some of the preclinical studies described earlier in this review; for example, they are potent promoters of the survival of cerebellar granule neurons following survival factor withdrawal, or inhibition of PI_3 kinase activity,[89] and thus have represented a valuable tool in elucidating the role of GSK-3 in cell signaling pathways.[133]

The newly developed small thiadiazolidinones represent the first ATP-noncompetitive GSK-3 inhibitors reported to date.[134] A wide range of thiadiazolidinone compounds inhibit GSK-3 at micromolar concentrations.[134] The fact that these compounds represent noncompetitive inhibitors may prove particularly useful.

It has been suggested that inhibitors of the substrate binding site may provide substrate specificity to GSK-3–mediated phosphorylation.[17,127] In other words, most GSK-3 inhibitors compete with ATP for a binding site on GSK-3 and therefore block the phosphorylation of every GSK-3 substrate, not just those involved in, for instance, insulin signaling and tau phosphorylation. Thus, medications that inhibit GSK-3 in a substrate-specific manner may have utility in the treatment of various disease processes that may be modulated by specific actions of GSK-3. In this regard, the protein glycogen synthase has been utilized to develop phosphorylated peptide inhibitors of GSK-3, which increased glycogen synthase

activity in cell culture and improved glucose tolerance test performance of normal mice and in a diabetic mouse model.[135] The recent discovery of the way in which substrates bind to GSK-3 may assist in the development of substrate-specific inhibitory compounds.[136] Future medications are also expected to take advantage of interactions of GSK-3 in large complexes such as those that regulate Wnt signaling (Figure 1).

As a consequence of the multiple functions of GSK-3 in cells, inhibition of GSK-3 has the potential to affect a broad range of cellular processes including cell growth, gene transcription, and glycogen metabolism; thus, in addition to achieving selectivity for GSK-3 inhibition, it is critical to avoid widespread side effects in a potentially therapeutic treatment. In this regard, GSK-3 inhibitors that are not substrate-specific may require narrow dosing in patient trials. Furthermore, there are some specific possible side effects for these promising drugs. For example, in the heart, active GSK-3 appears to suppress cardiac hypertrophy.[137,138] There is also a concern that specific GSK-3 inhibitors may have carcinogenic properties (due to up-regulation of the Wnt pathway, which is common in human cancers).[16–18,119,127,139] However, epidemiologic studies on the effect of lithium on cancer risks are strongly negative to date,[140] and preliminary rodent studies within our group and by others do not suggest a major effect.[141–143] Nevertheless, careful testing in animals will need to be accomplished prior to human trials of more potent GSK-3 inhibitors. In summary, a large number of compounds with GSK-3–inhibitory properties are rapidly being developed, offering the promise of GSK-3 inhibitors that are highly potent and specific. Such novel compounds will permit the opportunity to investigate the utility of GSK-3 inhibition in the treatment of bipolar disorder. While lithium is known most commonly for its efficacy in treating mania, it possesses antidepressant properties as both a monotherapy[6,144] and an adjunct therapy[145]; thus, any forthcoming clinical trials will have to address potential antimanic and antidepressant (not to mention prophylactic) properties.

CONCLUDING REMARKS

As discussed, all of the pharmacologic agents that are currently available for the treatment of bipolar disorder have been obtained by serendipity or by testing an already approved drug for another indication in patients with bipolar disorder (e.g., anticonvulsants and antipsychotic drugs). This dearth in development of novel medications is largely due to the lack of understanding of the pathophysiology of bipolar disorder combined with a lack of knowledge regarding the true cellular targets of mood-stabilizing medications.[21] The inhibitory effect of lithium on GSK-3 provides us with a unique opportunity to test a novel hypothesis regarding a potential therapeutic target.

GSK-3 has a number of functions within the nervous system that evidence suggests may be critically important for the treatment of bipolar disorder. A multiplicity of cellular processes are ascribed to GSK-3. Many of these cellular processes are undoubtedly involved in regulating the cellular and neuronal circuits involved in the regulation of complex neurologic functions with relevance to the clinical phenotype of bipolar disorder, the sleep, appetite, and energy changes that make up the neurovegetative profile of bipolar disorder and endophenotypes common in patients with bipolar disorder.[80,146] Thus far, studies of GSK-3 in postmortem brains of bipolar subjects have been negative.[93,147–150] However, a number of signaling pathways (with which GSK-3 is involved) have been associated with the disorder.[42]

A number of cellular actions of lithium have been identified over the past few decades. These include—among many others—increased activity of basal activator binding protein 1, regulation of PKC and its substrates, reduction of arachidonic acid turnover, and increased expression of the neuroprotective protein B-cell lymphoma/leukemia-2 (bcl-2) (see Gould et al.[21] for review). It is quite likely that many of these pathways may play important roles in treating various facets of this complex illness.[21] Eventually, the multiple effects of lithium and, more generally, other mood stabilizers on biological systems will be traced back to fewer initiating events. It is likely that by understanding the identity of the lithium target enzyme(s) that lead to these events, we will better understand the pathophysiology of bipolar disorder and be able to—using a hypothesis-driven approach—attempt to treat patients with a selective enzyme inhibitor(s). In this regard, a number of GSK-3 inhibitors are actively under development, and it is anticipated that these will undergo trials for the treatment of bipolar disorder.

Drug names: carbamazepine (Tegretol and others), tamoxifen (Nolvadex and others), valproic acid (Depakene and others).

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