

Glycogen Synthase Kinase-3: a Putative Molecular Target for Lithium Mimetic Drugs

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Despite many decades of clinical use, the therapeutic target of lithium remains uncertain. It is recognized that therapeutic concentrations of lithium, through competition with the similarly sized magnesium cation, inhibit the activity of select enzymes. Among these is glycogen synthase kinase-3 (GSK-3). Recent preclinical evidence, including biochemical, pharmacological, genetic, and rodent behavioral models, supports the hypothesis that inhibition of GSK-3 may represent a target for lithium's mood-stabilizing properties. Specifically, it has been demonstrated that lithium administration regulates multiple GSK-3 targets *in vivo* and that multiple additional classes of mood-stabilizing and antidepressant drugs regulate GSK-3 signaling. Pharmacological or genetic inhibition of GSK-3 results in mood stabilizer-like behavior in rodent models, and genetic association studies implicate GSK-3 as a possible modulator of particular aspects of bipolar disorder including response to lithium. Furthermore, numerous recent studies have provided a more complete understanding of GSK-3's role in diverse neurological processes strengthening the hypothesis that GSK-3 may represent a therapeutically relevant target of lithium. For example, GSK-3 is a primary regulator of neuronal survival, and cellular responses to glucocorticoids and estrogen may involve GSK-3-regulated pathways. While the preclinical evidence discussed in this review is encouraging, ultimate validation of GSK-3 as a therapeutically relevant target will require clinical trials of selective novel inhibitors. In this regard, as is discussed, there is a major effort underway to develop novel, specific, GSK-3 inhibitors.

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LITHIUM: PAST, PRESENT, AND FUTURE

Mood disorders such as bipolar disorder and unipolar depression are pervasive diseases typified by episodes of alterations in mood. Depressive episodes are characterized by a depressed mood, cognitive changes, psychomotor changes, and a host of neurovegetative symptoms. In bipolar disorder, while there exist diverse presentations, patients typically alternate between (albeit not in a 1:1 manner) episodes of depression (mostly indistinguishable from unipolar depression) and episodes of mania, which is characterized by a hyperaroused state (either euphoric or dysphoric), increases in motor activity, racing thoughts, impaired judgment, decreased amount of sleep, and an apparent decreased need for sleep. Lithium has utility in the treatment of both bipolar disorder and unipolar depression.

While reported first in the scientific literature as a treatment for bipolar disorder over 50 years ago and in

widespread clinical use worldwide for over 30 years, the explanation of why the simple monovalent cation lithium is useful for the treatment of mood disorders has thus far been elusive. In 1949, John Cade documented in the medical literature lithium salts as useful for the treatment of 'psychotic excitement' (Cade, 1949). This was followed by the work of Mogens Schou and others during the next two decades eventually solidifying lithium's position as a valuable treatment for bipolar disorder—for the acute and maintenance treatment of both mania and depression.

A number of double-blind studies have confirmed lithium's efficacy both in the acute treatment of mania, and for mania prophylaxis (Schou, 2001). While in practice lithium is often considered not to be effective as monotherapy for bipolar and unipolar depression, multiple studies have suggested that lithium may be useful for both the acute and prophylactic treatment of depression (see Souza and Goodwin (1991) and Bauer and Mitchner (2004) for meta-analysis and review). Unfortunately, many of the studies addressing monotherapy antidepressant effects suffered from some methodological shortcomings, in particular low sample sizes and the use of crossover study designs. Furthermore, lithium's antidepressant effects may be modest, resulting in statistical significance but insufficient clinical antidepressant effects in many cases. However,

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lithium has clear utility as an adjunct antidepressant in treatment refractory patients (Heninger *et al*, 1983). Overall, in placebo-controlled trials, lithium has been found useful as an adjunct medication for 45% of patients (Bauer *et al*, 2003). Lithium also has antisuicidal effects, the molecular antecedents of which are of considerable interest (Baldessarini *et al*, 2001; Goodwin *et al*, 2003; Muller-Oerlinghausen, 2001). In spite of lithium's past success and proven efficacy in treatment of mood disorders, it is not the perfect medication.

It is increasingly being recognized that mood disorders such as bipolar disorder and depression represent pervasive diseases characterized not merely by isolated episodes of depression and/or mania but also by increased morbidity and mortality. This arises not only from a rate of suicide approaching 15 percent, but the existence of significant medical comorbidities, often limited social and economic functioning, and poor interepisode recovery (see Evans and Charney (2003) and accompanying issue of Biological Psychiatry). The entire current pharmacopia useful for the treatment of mood disorders (including lithium, anticonvulsants, antipsychotics, and antidepressants) often do not bring about full remission, and if they do, do so after a minimum of many weeks, if not months of treatment. Additionally, currently available medications often lack the ability to completely prevent breakthrough episodes, and, generally, have undesirable side effects even in the best efficacy scenarios (eg sexual side effects with selective serotonin reuptake inhibitors). In a critique of the efficacy of current antidepressants, Walsh *et al* (2002) found that compounds successful in double-blind placebo control studies for the treatment of depression are efficacious only about 50% of the time, whereas placebo groups generally see about a 30% response.

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 either do not respond fully to existing treatments or are intolerant of side effects has made the challenge of discovering novel therapeutic agents increasingly more prominent. Recent efforts to develop 'novel' medications for the treatment of mood disorders has relied primarily upon developing 'me too' drugs with a similar mechanism of actions to older medications (ie increasing synaptic monamine levels in depression) or testing drugs developed and approved for other indications (most generally antipsychotic and antiepileptic drugs) in hope that they may have efficacy in treating either bipolar mania or bipolar or unipolar depression.

However, it is without doubt that the development of medications, which share some targets of older antidepressants (SSRIs *versus* tricyclics), have altered the tolerability of drugs, and saved many lives. While development of a drug that mimics lithium's therapeutic target while not interacting with those targets that result in side-effects will similarly probably not cure bipolar disorder, it would likely revolutionize the tolerability, as SSRIs did for the treatment of depression in the 1980s. Further, understanding precisely how lithium acts, through clinical validation, will provide important clues regarding mood disorder pathophysiology. It is thus of critical importance to understand lithium's relevant therapeutic target.

At therapeutic concentrations, lithium inhibits a small number of enzymes through competition for a magnesium-binding site (see Gould *et al* (2004c) for a review). While it is possible that this molecule has more than one initial direct target by which it exerts its mood-stabilizing effects, the most parsimonious explanation is that a single initial target is most relevant (akin to William of Ockham's razor—*Pluralitas non est ponenda sine necessitate* [Plurality should not be posited without necessity]). Thereafter, many downstream effects (eg, modulation of diverse signaling pathways) may be of relevance.

For more than a decade, the most widely accepted target for lithium has been inhibition of inositol monophosphatase (IMPase) and related enzymes. Theoretically, inhibition of IMPase in the brain would prevent recycling of inositol, thus depleting free inositol that is required for phosphoinositol-mediated intracellular signaling cascades. Indeed, extensive preclinical and some clinical studies have provided support for this hypothesis (see Attack (2000) for a review). In studies that are widely replicated, Allison and Stewart (1971) first reported that acute lithium administration to rats resulted in a decrease in brain inositol. Moore *et al* (1999) found that 5–7 days of lithium administration to bipolar subjects resulted in a decrease of frontal lobe myo-inositol levels. However, studies have not been able to determine if this effect of lithium has therapeutic relevance. This is in large part due to the lack of novel blood-brain barrier penetrant IMPase inhibitors. Previous industry efforts have resulted in numerous compounds; however, the best studied have failed due to being either extremely hydrophobic or hydrophilic (Attack, 1997). Thus, it has not been possible to directly test for true clinical evidence of therapeutic efficacy. Research efforts remain underway, and may still someday result in a clinically testable hypothesis.

In 1996, it was discovered that lithium inhibited the enzyme glycogen synthase kinase-3 (GSK-3) (Klein and Melton, 1996; Stambolic *et al*, 1996). These findings raised the possibility that GSK-3 inhibition might play a role in the treatment of bipolar disorder. However, the past 2 years have seen the emergence of exciting new biochemical, pharmacological, genetic, and rodent behavioral studies, all of which support the hypothesis that inhibition of GSK-3 represents a therapeutically relevant target for lithium's mood-stabilizing properties. In this perspectives paper, we review and synthesize this new data, and discuss the implications for the development of truly novel agents for the treatment of mood disorders.

GSK-3: A PLURIPOTENT KINASE

GSK-3 was characterized in 1980 as a protein that phosphorylated and deactivated glycogen synthase (Embi *et al*, 1980). It is a ubiquitous kinase, found in both neurons and glia, localized to the cytoplasm, nucleus, and mitochondria (see Jope and Johnson (2004) for a review). It is generally considered constitutively active and found as two isoforms, α and β (51 and 47 kDa, respectively), that have 97% sequence homology in their catalytic domains, and generally (but not always) have similar biological effects. The constitutive activity arises from phosphorylation of tyrosines 279 or 216 (α and β , respectively), although there

is some evidence of active regulation of this site in the brain (Bhat *et al*, 2000). Determining the kinases most responsible for phosphorylation of this serine is an active area of research.

The primary mechanism of regulation involves inhibitory phosphorylation of their N-terminal serines (21 and 9, respectively; see Frame and Cohen (2001) for a review). Phosphorylation of this site is very dynamic, as a number of pathways and kinases converge here; dephosphorylation is regulated by protein phosphatase 1 (Bennecib *et al*, 2000; Tanji *et al*, 2002; Zhang *et al*, 2003). GSK-3 activity is additionally regulated by binding proteins; for example, in the Wnt signaling pathway, GSK-3 phosphorylation of β -catenin is regulated by proteins adenomatous polyposis coli (APC), the scaffold protein Axin, and frequently rearranged in advanced T-cell lymphomas 1 (FRAT1). Most substrates recognized by GSK-3 have the amino-acid sequence -S-X-X-X-S-, where the C-terminal serine is phosphorylated and

acts as a primer allowing GSK-3 to phosphorylate the N-terminal serine (Fiol *et al*, 1987). Phosphorylation of serine 9/21 appears to exert inhibitory effects on GSK-3 by interaction with its own binding site that interacts with the primed serine residues (Dajani *et al*, 2001; Frame *et al*, 2001).

One of the most notable qualities of GSK-3 is the vast number of signaling pathways that converge on this enzyme and, subsequently, an even greater number of biological targets (see Frame and Cohen (2001) and Grimes and Jope (2001b) for a review). However, the enzyme is perhaps best established in three pathways. As depicted in Figure 1, GSK-3 functions as a regulator of the Wnt signaling pathway where activation determines cell fate in embryonic development. GSK-3 is normally active in the Wnt pathway, where its primary target is β -catenin. β -Catenin has multiple roles in the cell; it interacts with cadherin localized to the cell membrane, and acts as a transcription factor in

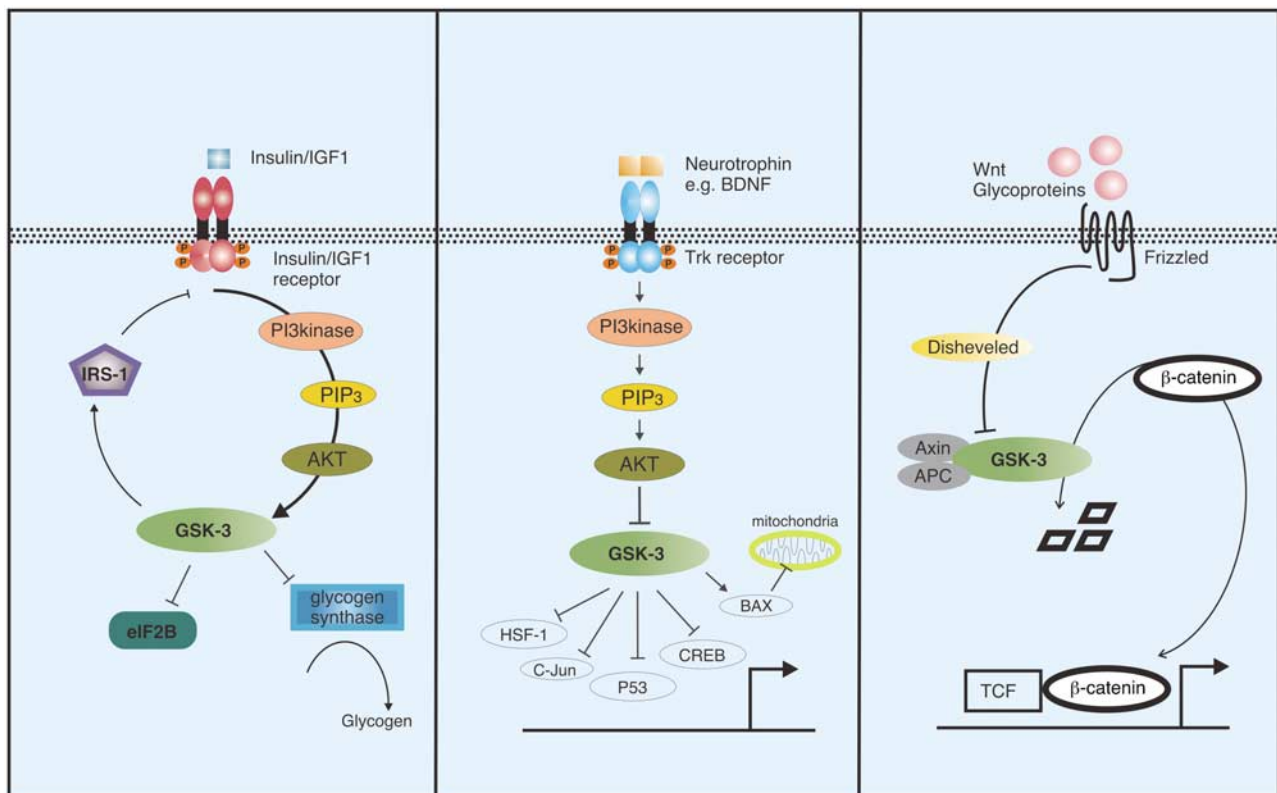


Figure 1 Glycogen synthase kinase-3 (GSK-3) is a component of diverse signaling pathways. These include insulin/insulin-like growth factor (IGF-1) signaling (left), neurotrophic factor signaling (center), and the Wnt signaling pathway (right). Insulin signaling through its tyrosine receptor kinase (Trk) receptor activates PI3kinase-mediated signaling, resulting in inhibition of GSK-3. Inhibition of GSK-3 activates glycogen synthase and eukaryotic initiation factor 2B (eIF2B) while inhibiting insulin receptor substrate-1 (IRS-1; an inhibitor of the insulin receptor). Insulin is generally thought to have minimal effects on CNS neurons; however, IGF1 interacting with its cognate receptor appears to have similar functions. Neurotrophins such as brain-derived neurotrophic factor (BDNF) act through Trk receptors A, B, and C to activate PI3kinase, AKT, and inhibit GSK-3. Many effectors have been implicated in GSK-3's neurotrophic effects including transcription factors (eg heat-shock factor-1 (HSF-1), C-Jun, P53, and cyclic AMP response element binding protein (CREB)) and recently the proapoptotic bcl-2 family member BAX. In the Wnt signaling pathway, secreted Wnt glycoproteins interact with the frizzled family of receptors and through disheveled mediated signaling inhibit GSK-3. Stability of this process requires the scaffolding proteins Axin and adenomatous polyposis coli (APC). Normally, active GSK-3 phosphorylates β -catenin leading to its ubiquitin-dependent degradation. However, when GSK-3 is inhibited in the Wnt pathway, β -catenin is not degraded, allowing for its interaction with (T-cell-specific transcription factor) TCF to act as a transcription factor. GSK-3's role in these pathways modulates energy metabolism, provides neuroprotection, and increases neuroplasticity (not mutually exclusive). Thus, our hypothesis is that lithium may act by enhancing these processes through inhibition of GSK-3. However, as detailed in the text, GSK-3 modulates a number of signaling pathways not detailed in the figure. It remains to be determined which pathway(s) may be most relevant to lithium's actions in the treatment of bipolar disorder.

concert with T-cell-specific transcription factor/lymphoid enhancer factor (TCF/LEF) in the Wnt pathway. Normally, active GSK-3 phosphorylates β -catenin leading to its ubiquitin-dependent degradation. However, when the Wnt pathway is initiated by secreted wnt glycoproteins binding to the frizzled family of transmembrane receptors, GSK-3 is inhibited. While the interaction is not fully understood, GSK-3 inactivation requires the disheveled protein (Figure 1). Inhibition of GSK-3 results in nonphosphorylated β -catenin; the levels of which increase resulting in increased β -catenin-driven gene expression (see Logan and Nusse (2004) for a current review).

In the insulin signaling pathway, insulin, through its tyrosine receptor kinase (Trk) receptor activates PI3kinase-mediated signaling resulting in Akt (protein kinase B)-mediated inhibition of GSK-3 (Figure 1; Eldar-Finkelman and Ilouz, 2003). Inhibition of GSK-3 activates the enzyme glycogen synthase (which contributes to the synthesis of glycogen and from where GSK-3's name derives) and eukaryotic initiation factor 2B (eIF2B) while inhibiting insulin receptor substrate-1 (IRS-1; an inhibitor of the insulin receptor) (Eldar-Finkelman and Ilouz, 2003). Although insulin is currently not known to exert more than minimal effects on CNS neurons, insulin-like growth factor 1 (IGF1) appears to have many similar functions (see Bondy and Cheng (2004) for a discussion of these effects).

In addition to mediating insulin's effects, PI3 kinase and Akt have distinct properties as mediators of the actions of neurotrophic molecules and this represents the most established pathway by which GSK-3 exerts its neurotrophic/neuroprotective effects. Neurotrophic factors are necessary for survival and functioning of neurons. Their actions increase cell survival both by providing necessary trophic support for growth and by exerting inhibitory effects on cell death pathways. The primary actions of neurotrophic factors are mediated via intracellular signaling pathways following binding to a Trk receptor. For instance, the most prevalent neurotrophic factor, brain-derived neurotrophic factor (BDNF), exerts its initial actions by interacting with TrkB. TrkB binding of BDNF initiates TrkB dimerization, which results in autophosphorylation of multiple tyrosine residues in its cytoplasmic domain, and thereafter modulation of intracellular signaling pathways including activation of PI-3 kinase, of which a primary target is Akt (Figure 1). Akt phosphorylates GSK-3, among many other targets. The precise downstream mechanisms that mediate GSK-3's actions in neurotrophic pathways are not fully understood, but are believed to include effectors such as p53 cyclic AMP response element binding protein (CREB), heat-shock factor-1, C-Jun, and Bax (Grimes and Jope, 2001b; Linseman *et al*, 2004; Watcharasit *et al*, 2002).

Other kinases that regulate GSK-3 include protein kinase C (PKC), protein kinase A (PKA), and ribosomal S6 kinase (p90RSK). In addition to these functions, GSK-3 has been implicated in amyloid β metabolism, control of cell division, regulation of neuronal growth cones, transport of intracellular organelles, and phosphorylation of cytoskeletal proteins tau and MAP1B, among other actions. Thus, the mechanisms of regulation and biological targets of GSK-3 are quite diverse (Figure 1). The precise mechanisms regulating these distinct pathways are an active area of research; however, it is likely that intracellular compart-

mentalization allows GSK-3 to accomplish all these functions without significant crosstalk among pathways (Jope and Johnson, 2004).

GSK-3: REGULATION BY LITHIUM AND DIVERSE THYMOLEPTICS

Lithium

In 1996 it was discovered that lithium was a direct inhibitor of GSK-3 (Klein and Melton, 1996; Stambolic *et al*, 1996). This effect was later identified to be through competition for magnesium (Gurvich and Klein, 2002; Ryves and Harwood, 2001). While other group I metal ions (sodium and potassium) do not inhibit GSK-3, the group II ion beryllium does inhibit its activity in a magnesium and ATP competitive manner (Klein and Melton, 1996; Ryves *et al*, 2002). More recently, zinc has also been identified as a GSK-3 inhibitor both directly (Ilouz *et al*, 2002) and indirectly through phosphorylation (An *et al*, 2005). Similar to lithium, zinc's direct actions appear to be via competition for magnesium binding (Hagit Eldar-Finkelman, February 2005, personal communication). While it was clear initially that lithium's actions on GSK-3 had major ramifications for the developmental effects of lithium, it was likewise apparent that GSK-3 inhibition could potentially have therapeutic relevance as well (Klein and Melton, 1996; Stambolic *et al*, 1996). However, it has only been most recently that extensive preclinical research is strongly supporting this hypothesis. Original *in vitro* measures of lithium's inhibitory potency found 50% inhibition at concentrations of 1–2 or 2 mM (Klein and Melton, 1996; Stambolic *et al*, 1996), a finding that suggests marginal inhibition at therapeutically relevant serum levels of 0.6–1.2 mM. However, these data may not accurately reflect *in vivo* potency, as the magnesium concentrations utilized were much higher than brain intracellular levels. Furthermore, through a 'feedback' mechanism, *in vivo* inhibition of GSK-3 results in an increase in inhibitory serine phosphorylation. This finding, originally observed by Chuang and co-workers (Chalecka-Franaszek and Chuang, 1999) has been replicated by a number of groups. Proposed mechanisms include GSK-3-regulated decreased activity of protein phosphatase 1 (Zhang *et al*, 2003), increased activity of PKC (Kirshenboim *et al*, 2004), or increased Akt activity (Chalecka-Franaszek and Chuang, 1999). Thus, moderate direct inhibition by lithium is amplified through an increase in serine 21/9 phosphorylation. Munoz-Montano *et al* (1997) originally reported that i.p. injections of lithium could cross the blood-brain barrier in 7-day-old rats. This has been followed by overwhelming evidence to support the statement that therapeutic concentrations of lithium (where rodents had lithium blood levels similar to those observed in patients) result in biologically meaningful GSK-3 inhibition in the mammalian brain (Table 1).

In addition to lithium's direct effects, additional diverse thymoleptics (medications with favorable effects on mood) including valproate, electroconvulsive seizures (ECS; an animal model of electroconvulsive therapy), MAOIs, fluoxetine, imipramine, clozapine, risperidone, and haloperidol regulate GSK-3 indirectly (Table 2). Further, as described in the GSK-3 functions section, estrogen has

Table 1 Evidence for Lithium Inhibition of Brain GSK-3 at Therapeutic Doses

| Method of administration | Description | Reference |
|--|--|---|
| 4 weeks in mouse chow | Increased GSK-3 α/β serine 21/9 phosphorylation in the cortex, hippocampus, and striatum | (Roh <i>et al</i> , 2005; De Sarno <i>et al</i> , 2002) |
| Gastric gavage for 3 weeks | Decreased A-beta levels in APP-Swedish transgenic mice | (Phiel <i>et al</i> , 2003) |
| 4 weeks of once daily i.p. injections | Increased β -catenin surrounding intracranial injection site | (De Ferrari <i>et al</i> , 2003) |
| 4 weeks in mouse chow | Decreased tau aggregation in mutant tau transgenic mice | (Perez <i>et al</i> , 2003) |
| 9 days of lithium in rat chow | Increased β -catenin levels in frontal cortex | (Gould <i>et al</i> , 2004a) |
| 7 days in mouse chow | Increased β -catenin in hypothalamus; increased TCF/LEF-driven gene expression in amygdala and hippocampus | (O'Brien <i>et al</i> , 2004) |
| Single oral gavage or 7 months in mouse chow | Decreased A-beta production in PDAPP mice; decreased A-beta production and plaques in GSK-3 transgenic mice | (Su <i>et al</i> , 2004) |

Only those studies where blood lithium levels were documented to be within the human therapeutic range (serum levels 0.6–1.2 mM) are included.

mood-modulating effects and regulates GSK-3. It should be pointed out that many diverse drugs have effects on GSK-3, which could suggest nonspecificity of this effect. To the best of our knowledge, few studies have reported the effects of nonthymoleptic medications in their models. This will clearly be necessary to determine the specificity of the effects observed.

Valproate

Extensive literature has shown that the mood stabilizer valproate (valproic acid) has regulatory effects on GSK-3. However, both direct and indirect effects may be relevant. Chen *et al* (1999) originally reported that valproate inhibited GSK-3 α and β *in vitro*. This finding has also been replicated by two independent groups (Grimes and Jope, 2001a; Kim *et al*, 2005; Werstuck *et al*, 2004). However, other groups have not been able to replicate this *in vitro* finding (Phiel *et al*, 2001; Williams *et al*, 2002; Williams *et al*, 2005). Hall *et al* (2002) have found significant inhibition, but only at concentrations above therapeutic levels. It is noteworthy that Werstuck *et al* (2004) identified derivatives of valproate that were even more potent inhibitors of GSK-3, suggesting that valproate metabolites may potentially interact with GSK-3 *in vivo*. However, it should also be noted that another group was unable to find direct inhibitory effects of valproate metabolites (Williams *et al*, 2005). It is possible that these contradictory studies of the effects of valproate on GSK-3 may be due to minor differences in substrates or assay conditions. Definitive studies to examine the direct effects of valproate on GSK-3 are clearly warranted.

While the direct effects of valproate on GSK-3 are a matter of debate, valproate exerts effects consistent with inhibition of GSK-3 in cell culture and animal models. Valproate treatment of SH-SY5Y cells at concentrations relevant to treatment results in activation of Akt and an increase in GSK-3 inhibitory phosphorylation (De Sarno *et al*, 2002). Valproate results in a decrease in phosphorylation of MAP1B in developing neurons, an effect that parallels GSK-3 inhibition (Hall *et al*, 2002). Valproate is also an activator of the Wnt signaling pathway, as judged by

its effect of increasing β -catenin in cell culture (Chen *et al*, 1999; Phiel *et al*, 2001) and in the rat brain following 9 days of administration (Gould *et al*, 2004a). Acute valproate administration to mice prevents hypoxia-induced decreases in phosphorylation of GSK-3 (Roh *et al*, 2005). Relatedly, Li *et al* (2002) found that another mood-stabilizing anti-convulsant, lamotrigine, similar to lithium and valproate, attenuates staurosporine and heat-shock-induced caspase-3 activity in a cell line overexpressing GSK-3 β . Thus, valproate clearly has inhibitory effects on GSK-3-regulated pathways, but it is debatable whether this is direct, indirect, or both direct and indirect.

Antidepressants

An analysis of the ability of the serotonergic system to regulate GSK-3 was recently reported by Li *et al* (2004). This group utilized a number of different mechanisms (including the administration of common antidepressant medications), and Western analysis, to show that acute increases in serotonin levels modulate GSK-3 β in the mouse brain.

Administration of the serotonin release stimulator and reuptake inhibitor, *d*-fenfluramine increased GSK-3 serine 9 phosphorylation in the prefrontal cortex, hippocampus, and striatum. The monoamine oxidase inhibitor clorgyline had no effect when administered on its own, but enhanced GSK-3 β phosphorylation when administered prior to *d*-fenfluramine. Similar results were observed with both fluoxetine and imipramine in the prefrontal cortex (Li *et al*, 2004). These imipramine Western blot findings were recently confirmed by immunohistochemistry and extended to show that administration of imipramine to mice prevents hypoxia-induced decreases in GSK-3 phosphorylation (Roh *et al*, 2005).

Electroconvulsive Seizures

Electroconvulsive therapy (ECT) is generally considered the most effective treatment for depression. Although often not as well appreciated, ECT is an efficacious treatment for mania as well (Mukherjee *et al*, 1994). Electroconvulsive seizure (ECS), an animal model of ECT, has been shown to

Table 2 Diverse Classes of Mood Stabilizers and Antidepressants have Effects on GSK-3

| Drug or manipulation | Involvement in mood disorders | Effects on GSK-3 | Study design | References (see text for complete references) |
|----------------------|--|---|--|--|
| Lithium | Primary treatment for bipolar disorder | Direct inhibitor of GSK-3 by competition for magnesium | <i>In vitro</i> , cell culture, <i>in vivo</i> | (Klein and Melton, 1996) |
| Valproate | Primary treatment for bipolar disorder | Some studies suggest direct inhibition; many studies suggest indirect inhibition | <i>In vitro</i> , cell culture, <i>in vivo</i> | (De Samo et al, 2002; Gould et al, 2004a; Chen et al, 1999) |
| Lamotrigine | Treatment for depression and bipolar disorder | Attenuates staurosporin-stimulated-caspase-3 activity in neuroblastoma cells overexpressing GSK-3 | 7 days treatment in cell culture at 0.2 and 0.3 mM | (Li, 2002) |
| Antidepressants | Treatment for depression (unipolar and bipolar) | Fluoxetine, imipramine, and clorgyline increase inhibitory phosphorylation in the mouse brain | Acute injections in mice | (Roh et al, 2005; Li et al, 2004) |
| Antipsychotics | Treatment for bipolar disorder and schizoaffective disorder | Clozapine (cell culture and rats), dopamine (mice), haloperidol, risperidone (rats) increase inhibitory phosphorylation; haloperidol, clozapine, and risperidone and clozapine increase β -catenin and GSK-3 protein levels in rats | Clozapine studies were acute to 24 h cell culture; rodent studies were variable long-term treatments | (Emamian et al, 2004; Kang et al, 2004b; Alimohamad et al, in press) |
| ECS | Model of ECT, the most efficacious therapy for severe refractory depression; also a successful treatment for refractory mania | Increases inhibitory phosphorylation of GSK-3 and β -catenin in rats | Single (p-GSK-3) and multiple (β -catenin) administrations to rats | (Roh et al, 2003; Madsen et al, 2003) |
| Estrogen | Times of estrogen fluctuation are associated with the onset of mood symptoms; regulation of estrogen is sometimes therapeutic | Increases inhibitory phosphorylation of GSK-3 in rats; estrogen receptor α interacts with β -catenin | 1 h following estrogen administration to ovariectomized rats; interaction studies were performed in cell culture, rat brain, and <i>Drosophila</i> | (Kouzmenko et al, 2004; Cardona-Gomez et al, 2004) |
| Amphetamine | Can precipitate mania in susceptible individuals; used as a lithium responsive rodent model of mania | Increases inhibitory phosphorylation of GSK-3 in the brain after 15 min; decreases inhibitory phosphorylation after 90 min; acute behavioral effects prevented by GSK-3 inhibition | Studies were performed in mice | (Svenningsson et al, 2003; Beaulieu et al, 2004) |
| Zinc | Shown in a preliminary double-blind study to be an effective adjunct treatment for depression (Nowak et al, 2003a). Antidepressant-like effect in the FST and olfactory bulbectomy rodent models | Direct inhibitor of GSK-3 by competition for magnesium | <i>In vitro</i> and cell culture | (Ilouz et al, 2002) |

See text for discussion and complete references.

alter phosphorylation of GSK-3. Roh *et al* (2003) found that a single administration of ECS to rats resulted in an increase in brain GSK-3 β phosphorylation after 10 min. In a follow-up study, this group provided evidence that this phosphorylation may occur in an Akt-dependent manner (Kang *et al*, 2004a). Madsen *et al* (2003) studied the effects, in rats, of once daily ECS on expression of the GSK-3 target β -catenin. They found that 10 days of ECS increased β -catenin levels in the subgranular zone of the hippocampal dentate gyrus, and that β -catenin strongly colocalized with BrdU (a marker of cell proliferation). These data led the authors to postulate that the increase in β -catenin may be responsible for ECS-induced increased neurogenesis (Madsen *et al*, 2003; Malberg *et al*, 2000). They also found that ECS increased the levels of Wnt-2, a ligand that activates β -catenin-dependent Wnt signaling through inhibition of GSK-3 (Madsen *et al*, 2003).

Antipsychotics

In addition to their use in the treatment of psychosis antipsychotics (both typical and atypical) have utility for the treatment of bipolar disorder, and an increasing number of these drugs are FDA approved for this indication. The effect of clozapine on GSK-3-mediated signaling pathways has been examined in cell culture. Kang *et al* (2004b) found that similar to antidepressants, ECS, lithium, and valproate, clozapine administration increases inhibitory (serine 9) phosphorylation of GSK-3 β . This finding was coincident with an increase in nuclear β -catenin levels. While this study did not address the effects of clozapine *in vivo*, chronic treatment of mice with haloperidol increased serine 9 phosphorylation of GSK-3 β (Emamian *et al*, 2004). The authors postulated that this was via an AKT-dependent mechanism, as the same treatment increased AKT phosphorylation. Chronic treatment may be required, as a study by another group found that a single injection of haloperidol to mice did not increase phosphorylation of GSK-3 β in the frontal cortex 1 h following administration (Li *et al*, 2004). Most recently, Alimohamad *et al* (2005) found that administration of haloperidol, risperidone, or clozapine increased levels of β -catenin, GSK-3 total protein, and GSK-3 β serine 9 phosphorylation in regions of the rat brain. They additionally examined the effects of a single, and 28 daily injections of haloperidol and risperidone, finding that only the chronic administration paradigm resulted in increases (Alimohamad *et al*, 2005). The initial Western blot findings were confirmed with immunohistochemistry while additionally identifying increased nuclear β -catenin immunoreactivity. The protein increases were also found to occur following administration of the D2/D3 receptor antagonist raclopride. Coupled with their previous results, this finding suggests that the mechanism of action involves inhibition of the D2 receptor (Alimohamad *et al*, 2005).

RECENT SUPPORT FROM ANIMAL BEHAVIORAL MODELS AND GENETIC STUDIES

Recent evidence suggests that many of the behavioral effects of lithium in rodent models may be due to inhibition of

GSK-3. Lithium has long been documented to attenuate amphetamine hyperactivity in rodents, having become one of the most popular and reproducible models of antimanic drug efficacy (see Einat *et al* (2003) for a discussion). The hyperactivity is attenuated by antipsychotic drugs as well as anticonvulsants often utilized for the treatment of bipolar disorder (Lamberty *et al*, 2001). It also has validity in the sense that amphetamine commonly precipitates manic episodes in susceptible individual who suffer from bipolar disorder—an effect that may be attenuated by lithium (Huey *et al*, 1981; van Kammen *et al*, 1985; Van Kammen and Murphy, 1975). Further, the motoric changes in activity present in bipolar disorder are among the most defining characteristics of the illness, ranging from near catatonic immobility to the profound hyperactivity of manic states. Similarly, loss of motivation is one of the central features of depression and anhedonia/extreme hedonia are among the most defining characteristics of bipolar depression and mania, respectively. In this context, it is noteworthy that the midbrain dopamine systems are known to play critical roles in regulating not only motoric activity but also motivational and reward circuits. On these grounds it is perhaps surprising that the dopaminergic system has not traditionally received as much attention as serotonergic or noradrenergic mechanisms in studies addressing the pathophysiology of bipolar disorder and depression.

Beaulieu *et al* (2004) have recently found in mice that amphetamine induced increases in activity is mediated, at least in part, through a GSK-3 dependent mechanism. This group reported that amphetamine administration to mice resulted in a reduction in the phosphorylation of GSK-3 α and GSK-3 β in the striatum, an effect opposite to lithium administration. They additionally reported that the endogenous hyperactivity found in dopamine transporter (DAT) knockout mice was attenuated by administration of lithium, in addition to five other GSK-3 inhibitors. Furthermore, in mice lacking one copy of GSK-3 β amphetamine hyperactivity was decreased. Pharmacological inhibition of GSK-3 also attenuates amphetamine hyperactivity in rats (Gould *et al*, 2004b).

O'Brien *et al* (2004) identified another lithium-dependent behavior in mice; they found that lithium treatment results in an antidepressant-like effect (increased activity) in the forced swim test (FST). Hagit Eldar-Finkelman and co-workers have found similar results following intraventricular injection of lithium to mice (unpublished personal communication, February 2005). The FST, developed by Porsolt *et al* (1977) is widely utilized as a behavioral measure of antidepressant efficacy. It has recently been scrutinized due to the fact that the measure has been validated with monoaminergic acting antidepressants and as such has the potential to miss antidepressants with novel mechanisms. Furthermore, a number of disparate neurobiological mechanism could lead to increased activity. However, the specificity of the effect of lithium is supported by studies utilizing alternate GSK-3 inhibitors. Specifically, Kaidanovich-Beilin *et al* (2004) reported antidepressant-like effects in the FST paradigm following intracerebral ventricle injections in mice of L803-*mts* (a peptide substrate-competitive inhibitor). Gould *et al* (2004b) similarly found antidepressant-like effects in the FST following peripheral administration of AR-A014418, a selective ATP-binding site

inhibitor, to rats. Furthermore, O'Brien *et al* (2004) examined the behavioral effects of knocking out a single copy of GSK-3 β , observing in these animals the same antidepressant-like behavior in the FST induced by pharmacological inhibition of GSK-3.

Supportive evidence comes from behavioral studies of elemental zinc, another inhibitor of GSK-3 β (Ilouz *et al*, 2002). Zinc has antidepressant-like properties in the FST and olfactory bulbectomy model in rats (Krocza *et al*, 2001; Krocza *et al*, 2000; Nowak *et al*, 2003b), and low (ineffective) doses of zinc combined with ineffective doses of antidepressants likewise have antidepressant-like effects in preclinical models (Krocza *et al*, 2001; Szewczyk *et al*, 2002; Wyska *et al*, 2004). Furthermore, in a recent placebo-controlled clinical study, adjunctive treatment with Zinc had antidepressant effects (Nowak *et al*, 2003a). These data suggest the possibility that zinc may exert these effects through inhibition of GSK-3; however, zinc has other biological effects, including antagonism of NMDA receptors (see Quiroz *et al* (2004) for a review), that require consideration.

Recent genetic evidence is also supportive. GSK-3 α and β are mapped to human 19q13.1–13.2 and 3q13.3–21.1, respectively (Hansen *et al*, 1997; Shaw *et al*, 1998), both of which have been marginally linked to bipolar disorder (Badenhop *et al*, 2002). Within a population of nonbipolar subjects, Russ and co-workers studied sequence variations of the GSK-3 β gene and promoter. They did not identify any sequence variations in the gene; however, they reported five promoter single-nucleotide polymorphisms (SNPs), two of which were present at a rate over 10% (18 and 41). One of these common promoter polymorphisms, (–50 T/C), has been studied by Benedetti and co-workers. They reported that this polymorphism is associated with response to lithium in a group of 88 bipolar disorder type I patients (Benedetti *et al*, 2005). Specifically, the carriers of the T/T genotype improved less on lithium than either the T/C or C/C groups.

This group has additionally studied other factors that may be associated with the –50T/C polymorphism. In a sample of 185 Italian bipolar patients, they identified an earlier age of onset associated with the T genotype ($P=0.0047$) (Benedetti *et al*, 2004a). A follow-up study of another group of patients confirmed the age of onset effect of the T genotype (Benedetti *et al*, 2004b). They further identified a second association with an antidepressant response to sleep deprivation. A total of 60 bipolar depressed patients were administered a night of total sleep deprivation followed by a night of undisturbed sleep. Patients with the C/C genotype had better acute effects to total sleep deprivation, and a subsequent higher relapse following one night of recovery sleep. These findings led Benedetti *et al* (2004b) to speculate that the C/C genotype may characterize a milder form of the disorder, with later age of onset and more responsivity to treatment. An initial family-based association study (transmission disequilibrium test) by this same group was negative (Tubazio *et al*, 2004), which could be interpreted as suggesting that GSK-3 polymorphisms may be associated more strongly with bipolar disorder endophenotypes (Gottesman and Gould, 2003). This supports a hypothesis that there exists a genotype–environment interaction (Benedetti *et al*, 2004b; Lenox *et al*, 2002).

It is important to keep in mind that the general caveats of genetic association studies are fraught with poor reproducibility due to population stratification artifacts. The associations to response to lithium and sleep deprivation both require replication in a distinct patient group. Further, while Benedetti and co-workers were able to reproduce their age of onset findings in a separate sample, it is likely that the second sample was derived from a similar population.

FUNCTIONS OF GSK-3 RELEVANT TO MOOD STABILIZATION

It is not simply the finding that GSK-3 is inhibited by lithium (and other thymoleptics), which makes it of such interest. GSK-3 is involved in many biological processes that could have relevance for the treatment of mood episodes. These functions include (but are not limited to) regulation of monoaminergic signaling, neuroprotection (or enhancing neuroplasticity or cellular resilience), regulation of circadian rhythms, modulating estrogen and glucocorticoid activity, or regulation of brain metabolism. As the underlying neurobiology of bipolar disorder is currently unclear, it is difficult to eliminate any of these possibilities.

Regulation of Monoaminergic Signaling

Regulation of monoaminergic signaling has long been implicated in the mechanism of action of antidepressant medications, as well as the pathophysiology of mood disorders (Bunney and Davis, 1965; Schildkraut, 1965). This conclusion arose initially from observations that the antihypertensive reserpine, which decrease brain monoamine levels, could precipitate depression and that MAOIs and tricyclics increased synaptic monoamine levels. Rapid lowering of serotonin, via tryptophan depletion, predictably results in the onset of depression symptoms in remitted depressed patients being treated with medications (Charney, 1998). Thus, *in toto*, the data suggests that while other factors are undoubtedly also involved, the monoaminergic systems do play important roles in the pathophysiology and treatment of mood disorders.

Recent data have suggested that intracellular responses to both serotonergic and dopaminergic inputs are modulated by GSK-3. As discussed in the section on the effects of antidepressants on GSK-3, increasing serotonin levels, through a variety of pharmacological mechanisms, results in inhibitory phosphorylation of mouse frontal cortex, hippocampal, and striatal GSK-3 β as witnessed by increases in serine 9 phosphorylation (Li *et al*, 2004; Roh *et al*, 2005). The effect appears to be through 5HT1A receptors. An antagonist of this receptor, WAY100635, attenuates GSK-3 β inhibition and 8-OH-DPAT (5HT1A agonist) was able to increase serine 9 phosphorylation (Li *et al*, 2004). Conversely, administration of the 5HT2 receptor antagonist LY53857 resulted in an increase in phosphorylation. Further experiments utilizing both LY53857 and serotonin agonists further supported the finding that the effects of serotonin agonism on phosphorylation of GSK-3 serine 9 are mediated by 5HT1A receptors, while activation of the 5HT2 receptor attenuates this effect (Li *et al*, 2004). These findings are particularly noteworthy since these are precisely the same serotonergic system changes (*viz.* enhancement of 5HT1A throughput and antagonism/down-

regulation of 5HT₂ receptors) that have been most implicated in the mechanisms of action of a variety of antidepressant treatments (Blier and Abbott, 2001).

GSK-3 has also been implicated as being effected by dopaminergic signaling. Beaulieu *et al* (2004) showed that phosphorylated striatal GSK-3 α and β levels decreased 90 min following injection of amphetamine, an effect that was not observed at 30 min, and paralleled increases in Akt phosphorylation. The action on GSK-3 appears to be independent of the effects of cyclic AMP. In dopamine transporter knockout mice, administration of α MTP to deplete catecholamine synthesis or raclopride (D₂ receptor antagonist) increased phosphorylation. However, this effect was not observed with the D₁ antagonist. Experiments in mice have conversely shown that amphetamine increases serine 9 phosphorylation after 15 min in the frontal cortex and striatum, suggesting that the effects may be time dependent (Svenningsson *et al*, 2003). The findings that serotonin, dopamine, and amphetamine regulate GSK-3 provides an important link between a critical signaling molecule and neurochemical brain circuits likely involved in the pathophysiology of bipolar disorder.

Neuroprotection

An extensive literature has found that lithium exerts neurotrophic/neuroprotective effects *in vitro*, in animal models, and perhaps even in humans. Lithium has been found to protect neuronal cell cultures against many varied agents including excessive glutamate, A beta, serum deprivation, colchicine, low potassium, and prion related protein, among many others (see Chuang *et al* (2002) and Manji *et al* (1999) for a review). Supporting the notion that these cell culture observations have true physiological relevance, lithium is neuroprotective in many animal models including ischemia, Huntington's disease, HIV gp120 envelope protein injection (a model of HIV-associated dementia), and radiation (see Chuang *et al* (2002) and Manji *et al* (1999) for a review). Proof from well-designed epidemiological and clinical studies will be required to validate whether these actions are clinically relevant for the treatment of mood disorders. In this regard, prospective studies designed to address this question found that 4 weeks of lithium treatment increases human gray matter (Moore *et al*, 2000b) and levels of the neuronal marker N-acetyl aspartate (NAA) (Moore *et al*, 2000a); both of which are consistent with the neurotrophic/neuroprotective effects that have been observed in preclinical models.

It has further been hypothesized that the neuroprotective effect of lithium may be related to clinical efficacy of the drug (Manji *et al*, 1999). Methodologies to investigate structural and functional deficits within the human brain have exponentially increased in recent years allowing for a more complete and intensive analysis of potential deficiencies in the brains of patients suffering from neuropsychiatric disorders and it is now possible to discern relatively subtle differences in cerebral blood flow, receptor density, or in macroscopic brain structure (Seibyl *et al*, 2004). Likewise, postmortem studies of the brain have been enhanced with technological advances that allow for relatively unbiased observations of cell density, morphology, and size (Lopez *et al*, 2004). The functional and

morphometric findings in mood disorders have been extensively reviewed elsewhere (Drevets, 2000; Manji *et al*, 2001; Manji *et al*, 2000; Rajkowska, 2002).

In brief, the brains of patients with mood disorders show both macroscopic and microscopic changes that separate them from healthy individuals. While these findings are not yet diagnostic, many are now becoming consistent. These include studies utilizing structural imaging, which report a decrease in frontal and temporal gray matter and an increase in ventricular size in patients with mood disorders (Drevets, 2000). Many studies also report a decrease in the size of other neuronal structures, including the hippocampus and portions of the basal ganglia (Drevets, 2000; Rajkowska, 2002). White matter hyperintensities (WMH) on T₂-weighted MRI are also observed at a greater incidence in the brains of mood disorder patients, especially bipolar disorder (Altshuler *et al*, 1995). Functional imaging has also revealed multiple abnormalities of regional cerebral blood flow and glucose metabolism in these same limbic and prefrontal cortical structures (Drevets, 2000; Manji *et al*, 2001).

Recent postmortem studies using unbiased stereology techniques and additional improved technologies (Lopez *et al*, 2004) have identified a number of changes including a decreased size and/or density of neurons, and decreased number and density of glial cells in regions of the anterior cingulate cortex, orbital frontal cortex and dorsal lateral prefrontal cortex, as well changes in the hippocampus and amygdala (see Rajkowska (2002) for a complete review of these findings).

An extensive series of literature has also suggested that it is likely that lithium may exert some, if not the majority, of its neuroprotective effects through inhibition of GSK-3. (see Chuang *et al* (2002), Jope and Bijur (2002) and Manji *et al*, 1999) for a review). Specifically, in most systems over activity of GSK-3 is proapoptotic, while inhibiting GSK-3 is antiapoptotic. For example, increased activity of GSK-3 in a number of cell types increases apoptosis, or is correlated with apoptosis in neuronal cells (Bijur *et al*, 2000; Bijur and Jope, 2001; Hetman *et al*, 2000; Maggirwar *et al*, 1999; Pap and Cooper, 1998; Takashima *et al*, 1993). Conversely, decreasing GSK-3 activity either with a protein inhibitor, antisense nucleotides, utilizing an inactivated form of GSK-3, or with administration of lithium or alternate GSK-3 inhibitors prevents these effects (Bijur and Jope, 2001; Hetman *et al*, 2000; Maggirwar *et al*, 1999; Pap and Cooper, 1998; Takashima *et al*, 1993). GSK-3 activity also increases in the rat cortex following focal cerebral ischemia (Bhat *et al*, 2000).

Thus, both neuroimaging and postmortem investigations suggest that the pathology of mood disorders may involve cell- and structural-based impairments in function and plasticity, consequences that perhaps could be modified by the neurotrophic effects of medications (Manji *et al*, 2000). Given GSK-3's position as a primary regulator of apoptosis, it is likely that the neuroprotective effects of lithium are due, at least in part, to GSK-3 inhibition (Jope and Bijur, 2002; Manji *et al*, 1999).

Neurohormone Regulation: Estrogen and Glucocorticoids

A great deal of evidence suggests that gonadal steroids, and in particular estrogen, may play a role in the pathophysio-

logy and/or treatment of mood disorders. For example, while major depressive disorder affects 10% of men, it affects up to two to three times as many women, a figure that is only accurate following the onset of puberty. Times of estrogen hormone fluctuation, such as during the postpartum and premenstrual periods, are associated with mood changes (mania and depression) (Bloch *et al*, 2003; Payne, 2003). However, little evidence suggests that absolute hormonal levels of progesterone and estrogen are different from controls (Bloch *et al*, 2003). This supports a notion that downstream effects of these hormones, such as receptor desensitization or abnormal signaling cascades, are responsible for the abnormal mood states. Furthermore, recent studies have described the role of gonadal steroids (especially estrogen) in neuroprotection, cellular resilience, and neural plasticity—effects similar to those observed with mood stabilizers (Lee and McEwen, 2001; Payne, 2003; Ramirez *et al*, 2001).

It is thus noteworthy that estrogen has been shown to regulate GSK-3. Estrogen administered to adult ovariectomized rats increased hippocampal serine GSK-3 phosphorylation (Cardona-Gomez *et al*, 2004). This group further reported that the effects occurred in primary hippocampal cultures, and that GSK-3 was colocalized in a complex that included estrogen receptor α (ER α) and β -catenin. Kouzmenko *et al* (2004) similarly immunoprecipitated ER α and β -catenin, finding that this interaction was enhanced by the addition of estrogen. They further identified that ER α and β -catenin could act in concert to drive gene expression using both TCF/LEF-binding element (TBE; topflash construct) and estrogen response element (ERE) reporting vectors. Using the chromatin immunoprecipitation (ChIP) assay they also identified interactions of ER α and β -catenin antibodies with genes containing TBE and ERE, respectively. These data are consistent with a recent finding, albeit in non-neuronal tissue, that concomitant lithium and estrogen administration to ovariectomized mice results in an increase in estradiol-induced proliferative and morphogenetic changes in the uterus (Gunin *et al*, 2004).

Current theories suggest that stress and reactivity to stress, via modulation of the hypothalamic pituitary adrenal (HPA) axis, may be a major pathogenic mechanism underlying susceptibility to recurrent mood disorders and a likely contributor to the neuroimaging and postmortem abnormalities observed. This pathway represents the primary 'stress' pathway in humans and other mammals, whereas the lateral ventricular nucleus of the hypothalamus releases corticotropin-releasing hormone/factor (CRH/F), which stimulates the production of adrenocorticotropin releasing hormone (ACTH) by the pituitary. This latter hormone stimulates the production of glucocorticoids by the adrenal gland. Cortisol is considered a primary stress hormone of the body, having varied effects on metabolism, neurovegetative behaviors of organisms, and a multitude of effects on the functions of neurons and neuronal systems. Smith and Frenkel (2004) reported an interaction between glucocorticoids and β -catenin. Their studies, in osteoblast-like cultures, showed that dexamethasone administration resulted in a decrease in β -catenin levels and inhibition of TCF/LEF-mediated transcription. Ohnaka *et al* (2005) have reported similar results. They found that Wnt3a-stimulated increases in β -catenin in primary cultured human osteo-

blasts were attenuated by dexamethasone administration. If these studies hold true in the brain, they may provide an interesting nexus between the HPA axis and β -catenin/GSK-3.

Regulation of Circadian Rhythms

Circadian rhythm abnormalities have been extensively studied in mood disorders for decades. Converging evidence suggests that abnormal circadian rhythms play a role in bipolar disorder (Bunney and Bunney, 2000; Healy and Waterhouse, 1995; Klemfuss, 1992; Wehr and Wirz-Justice, 1982): (1) circadian abnormalities (period shortening) are well documented in patients with bipolar disorder, (2) nonpharmacological treatments (such as sleep deprivation, adjustment of sleep cycles, and light therapy) are often helpful in the treatment of mood disorders, (3) Sleep deprivation is a highly efficacious—but short-term—treatment for the depressive phase of bipolar disorder, (4) sleep deprivation (albeit more than just a circadian manipulation) is a common precipitant of the manic phases of bipolar disorder, and finally (5) there exists an extensive body of data describing lithium's effects on circadian rhythms. Lithium has been shown in numerous studies to modify the phase and period of circadian rhythms in a variety of species, ranging from unicellular organism and insects to mice and even humans (see Klemfuss (1992) for a complete review). Most consistently, lithium has been shown to lengthen the circadian period across species. This finding, combined with the previously mentioned reports of alternative methods that modify circadian cycles proving effective in the treatment of mood disorders, led to the suggestion that some of the therapeutic effects of lithium may also arise, at least in part, from its effect on the circadian cycle length (Wehr and Wirz-Justice, 1982).

Data suggesting that GSK-3 may represent lithium's target responsible for alterations in circadian cycles derived initially from *Drosophila* studies. Martinek *et al* (2001) identified the *Drosophila* orthologue of GSK-3 β , SHAGGY, as a component of the circadian cycles. Overexpression of SHAGGY lengthened the *Drosophila* free-running circadian cycle (Martinek *et al*, 2001). Additionally, a decrease in SHAGGY activity resulted in an increase in circadian period length (Martinek *et al*, 2001), the effect (increase in circadian period) that has been noted in numerous species, including *Drosophila*, after treatment with lithium (Klemfuss, 1992; Padiath *et al*, 2004).

It should be noted, however, that while there are many similarities between the molecular components of circadian cycles in mammals and *Drosophila*, there are many differences as well (Reppert and Weaver, 2000). Beginning to address this, Iwahana *et al* (2004) investigated the effects of lithium on GSK-3 in the mouse suprachiasmatic nucleus (SCN). They found that the serine 9 phosphorylation of GSK-3 and total GSK-3 α (β was found only at a low level) protein levels were regulated at certain points during the circadian cycle, and that lithium administration had inhibitory effects on their levels and activity. These effects were coincident with an affect of lengthening of the circadian cycle, and appeared specific for the SCN, as similar effects were not observed in the arcuate nucleus. As mentioned in an earlier section response to sleep depriva-

tion has been associated with a GSK-3 β promoter polymorphism (Benedetti *et al.*, 2004b).

Additional studies in mammals are eagerly awaited. However, GSK-3 represents a possible molecular target for the actions of lithium on circadian cycles, and even if the circadian effects of lithium are not relevant to the treatment of mood disorders, it still may remain a valuable *in vivo* marker of GSK-3 inhibition.

Metabolic Effects

Another consideration is that GSK-3's role in the regulation of cellular energy metabolism may be of importance. As described, GSK-3 was initially named based upon its role in the phosphorylation of glycogen synthase, thereby acting as a critical regulator of insulin's effects on the insulin/PI3kinase/Akt pathway. Inactivation of GSK-3 leads to synthesis of glycogen, activation of the insulin receptor through decreased phosphorylation of IRS-1, and decreased phosphorylation and activation of eukaryotic initiation factor (eIF)-2B (Cohen and Goedert, 2004; Eldar-Finkelman and Krebs, 1997; Welsh and Proud, 1993). While insulin may not have a major action on most brain areas, IGF-1 is active and, in many ways, mimics the effects of insulin (Bondy and Cheng, 2004). Lithium has been noted to, among other metabolic effects, increase glycogen concentrations in the rodent brain (Plenge, 1976). However, lithium has inhibitory effects on metabolic enzymes fructose 1,6-bisphosphatase (FBPase) and phosphoglucomutase (PGM) that should be considered (Gould *et al.*, 2004c).

A number of metabolic changes have been noted in the brains of patients with mood disorders. These include elevation of resting cerebral blood flow and glucose metabolism in the amygdala and left medial thalamus (Drevets, 2000). However, a decrease in cerebral blood flow and metabolism has been noted in the caudate and the subgenual prefrontal cortex (see Drevets (2000) for a complete review of these data). The utilization of glucose additionally represents a potential role in neuroprotection. For example, work by Sapolsky and colleagues has strongly implicated efficiency of glucose utilization and uptake with the susceptibility of neurons to harmful stimuli (neuronal resilience) (see Sapolsky (1996) for review). GSK-3's role in these processes may be of relevance and, although not extensively investigated, GSK-3 inhibition may at least in part mediate neuroprotective effects through effects on cellular metabolism.

THERAPEUTIC IMPLICATIONS

Thus, precisely how GSK-3 inhibition could be exerting actions in the treatment of bipolar disorder is a matter of considerable interest. While it is possible that multiple effects of inhibition of this enzyme may be relevant (or that different effects may have a role in antidepressant vs antimanic actions), the most parsimonious explanation suggests a single biological process or pathway that would be targeted to exert therapeutic effects. Given the large number of cellular targets of GSK-3, a number of prospects exist. As discussed in this review, these include neuropro-

TECTIVE effects, modulation of circadian rhythms, modulation of monoaminergic-mediated signaling events, response element binding of neurohormones and β -catenin, and metabolic effects. GSK-3's role in these processes may provide a link between seemingly diverse processes regulated by lithium (or that are abnormal in bipolar disorder).

Additionally discussed, diverse thymoleptics including lithium, valproate, ECS, antidepressants, antipsychotics, and estrogen regulate GSK-3 directly or by modulating phosphorylation (Table 2). Thus, GSK-3 may represent a common pathway on which these medications converge, and perhaps, a target for novel medications. Of course, in the framework of the development of novel therapeutics, this discussion is moot unless the potential exists to clinically test the hypothesis that inhibiting GSK-3 has thymoleptic properties. Indeed, true validation—especially considering the less than ideal animal models for bipolar disorder and depression—will only arise from clinical trials of brain penetrant and selective novel GSK-3 inhibitors.

Fortunately, there exists major pharmaceutical interest in the development of novel, potent inhibitors of GSK-3, and most large companies have a GSK-3 inhibitor development program (see Cohen and Goedert (2004) for a review). The primary impetus for these inhibitors is generally not for the treatment of mood disorders, but rather for the treatment of other diseases, especially Alzheimer's disease and diabetes (Cohen and Goedert, 2004). However, regardless of the reason for development, GSK-3 inhibitors are actively being developed and will expectantly be available for bipolar disorder treatment trials in the future. The majority of these molecules act by inhibiting the interaction between ATP and GSK-3. Many of these inhibitors have had some effects on other kinases, most commonly cyclin-dependent kinases (CDKs). However, more recent efforts have reported increased specificity. There are additionally attempts to develop inhibitors that are non-ATP competitive. For example, Eldar-Finkelman and co-workers have reported the development of L803-mts, a substrate competitive inhibitor that is active in rodent models of both diabetes and depression (Kaidanovich-Beilin *et al.*, 2004; Plotkin *et al.*, 2003). Competition for substrate, and through other non-ATP competitive approaches, has the potential to inhibit GSK-3 action in some pathways but not others. As would be expected from a molecule with such a vast array of intracellular functions, inhibition of GSK-3 could be predicted to result in deleterious side effects (Cohen and Goedert, 2004). Most concerning may be the role of β -catenin and the Wnt signaling pathway in promoting tumor growth. Current epidemiological evidence and 50 years of clinical use does not implicate long-term lithium use with an increased risk of any type of cancer (Cohen *et al.*, 1998). Furthermore, lithium administration to mice with a mutation in the APC protooncogene protein does not result in an increase in the number of colonic tumors (Gould *et al.*, 2003).

For far too long, the accepted outcome of treatment for mood disorder has been incomplete remission in some patients who are responsive to the current pharmacopia. As clinical researchers, we cannot continue to be accepting of the status quo. The ultimate goal must be to strive for full remission in all patients. To reach this goal, truly novel

medications must be developed. It is likely that future strategies will account for disease heterogeneity, subclassifying and subsequently treating mood disorders based upon endophenotypes (Gottesman and Gould, 2003). Understanding the complex neurobiology of these disorders will be a major advancement. This will certainly not be fully complete until the majority of susceptibility genes have been identified. However, in the interim, advances in understanding targets of current medications will allow the ability to test novel compounds for efficacy in proof of concept studies. This approach to medication development should ultimately yield novel medications for the treatment of severe mood disorders.

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