REVIEW

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Inositol depletion, GSK3 inhibition and bipolar disorder

Wenxi Yu¹ & Miriam L Greenberg^{*,1}

Valproic acid and lithium are widely used to treat bipolar disorder, a severe illness characterized by cycles of mania and depression. However, their efficacy is limited, and treatment is often accompanied by serious side effects. The therapeutic mechanisms of these drugs are not understood, hampering the development of more effective treatments. Among the plethora of biochemical effects of the drugs, those that are common to both may be more related to therapeutic efficacy. Two common outcomes include inositol depletion and GSK3 inhibition, which have been proposed to explain the efficacy of both valproic acid and lithium. Here, we discuss the inositol depletion and GSK3 inhibition hypotheses, and introduce a unified model suggesting that inositol depletion and GSK3 inhibition are inter-related.

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Bipolar disorder (BD) is a severe psychiatric illness affecting about 2% of the world population. BD patients suffer from recurring cycles of mania and depression, which greatly hamper interpersonal relationships and career success. The mortality rate of BD patients is 15–20% higher than that of the general population [1]. Approximately 15% of BD patients commit suicide [2]. Lithium and valproic acid (VPA) are among the most widely used and best-studied mood stabilizers [3,4]. However, these and other major antibipolar therapies cause serious side effects and have limited efficacy [5]. Thus, there is a great demand for more effective antibipolar drugs. Efforts to develop new treatments for BD are hampered by the lack of knowledge of the therapeutic mechanisms of the current drugs. Several hypotheses have been proposed to elucidate the mechanisms underlying the mood-stabilizing effects of the drugs. In this review, we focus on the controversies and connections characterizing two current hypotheses of the therapeutic mechanisms of lithium and VPA – inositol depletion and GSK3 inhibition – and suggest that the two mechanisms may be related.

Inositol depletion hypothesis

• Inositol metabolism

Myo-inositol is the precursor of all inositol lipids and inositol phosphates. Eukaryotic cells obtain inositol by three routes. Inositol is taken up from the surrounding environment by inositol transporters [6,7]. In the absence of exogenous inositol, it is synthesized *de novo* from glucose-6-phosphate (G6P) in a two-step reaction. G6P is first converted to inositol-3-phosphate by myo-inositol-3-phosphate synthase (MIPS), which is encoded by *ISYNA1* and *INO1* in human and yeast cells, respectively [8–11]. The second step is the conversion of inositol-3-phosphate to inositol,

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¹Department of Biological Sciences, Wayne State University, Detroit, MI 48202, USA

*Author for correspondence: Fax: +1 313 577 6891; mgreenberg@wayne.edu

which is catalyzed by IMPase [12]. Inositol is also obtained by recycling inositol phosphates [13]. The levels of inositol in brain are significantly higher than in blood and other tissues [14], suggesting that high levels of inositol are critical for normal brain function. Although brain cells can take up inositol from the blood, uptake is slowed by the blood–brain barrier [15,16], suggesting that inositol *de novo* synthesis and the recycling of inositol phosphates are the main sources of inositol in brain [17].

Inositol is an essential substrate for the synthesis of phosphatidylinositol (PI), from which are derived the phosphatidylinositol phosphates. Seven known phosphatidylinositol phosphates are derived from PI, including PI(3)P, PI(4)P, PI(5)P, PI(3,4)P₂, PI(3,5)P₂, PI(4,5)P₂ and PI(3,4,5)P₃ [18]. Phosphoinositides are signaling molecules that mediate cell growth, proliferation, apoptosis, insulin action and many other cellular events [19]. It is not surprising, therefore, that perturbation of phosphoinositide metabolism is associated with many disorders [18]. Upon receptor-mediated activation of phospholipase C (PLC), PI(4,5)P₂ is cleaved to form inositol-1,4,5-triphosphates (IP₃) and 1,2-diacylglycerol (DAG) [20]. IP₃ can be recycled to myo-inositol by a series of dephosphorylations catalyzed by inositol polyphosphate phosphatase and IMPase [21]. Alternatively, IP₃ can be phosphorylated sequentially to form IP₄, IP₅ and IP₆ by inositol phosphate kinases [13,21]. These molecules convey signals for a variety of cellular processes, although the functions of inositol phosphates are not fully understood [22,23]. Inositol phosphates can be further phosphorylated on existing phosphate groups to form pyrophosphates [24,25], whose functions are involved in the regulation of gene expression, vesicular tracking and DNA repair [26–28].

Many inositol-containing molecules function as metabolic sensors that regulate neuronal function and neurotransmission [13]. For example, IP₃ is a second messenger that activates the release of calcium from cellular storage [20]. Calcium signaling regulates neuronal differentiation, apoptosis and exocytosis [29]. Many receptors in the central nervous system activate PLC-dependent cleavage of PIP₂ and increase IP₃/calcium release [30]. Perturbation of intracellular inositol metabolism has been associated with BD, Alzheimer's disease, diabetes and cancer [18]. Therefore, maintaining stable inositol homeostasis is critical for normal cellular function [13,31].

• Altered inositol levels in BD

A correlation has been observed between BD and altered levels of inositol in brain. Altered myo-inositol and phosphoinositide levels have been observed in brains of living BD patients using magnetic resonance spectroscopy [32–34]. Higher myo-inositol signals were detected in brains of BD patients during the manic phase [35]. Conversely, significantly lower levels of myo-inositol were identified in the frontal cortex of BD patients during the depressive phase [36]. Frontal cortex samples from postmortem BD patients also exhibited decreased myo-inositol levels [37]. Furthermore, myo-inositol levels were reduced in cerebrospinal fluid obtained from affective depression patients [38]. Interestingly, dietary supplementation of inositol (12 g/day for 4 weeks in one study) led to significant efficacy for the treatment of depression [39,40]. Inositol also alleviated depression in animal models [41,42]. These studies suggest that abnormal brain inositol levels may play a role in mood disorders.

• VPA & lithium inhibit inositol synthesis

Despite the fact that lithium has been used for more than 60 years for the treatment of BD, the therapeutic mechanism of the drug remains unknown [3]. Similarly, the mechanism underlying VPA efficacy is not understood [43]. Lithium was shown to be an uncompetitive inhibitor of IMPase, which catalyzes the conversion of inositol-3-phosphate to myo-inositol [44–46]. Berridge *et al.* hypothesized that inhibition of inositol synthesis by lithium leads to decreased PI synthesis and subsequent attenuation of PI signaling [46]. These pivotal studies laid the foundation for the inositol depletion hypothesis as a potential therapeutic mechanism of action of lithium. In support of the hypothesis, studies in animal models suggested that the mood-stabilizing effect of lithium is correlated with inhibition of inositol synthesis. Lithium reduced myo-inositol levels in rat brain [47]. Inositol levels in rat cerebral cortex decreased 30% by 6 h after lithium injection, and the reduction of inositol persisted for 24 h. In addition, VPA and lithium treatment led to a reduced intracellular concentration of IP₃ [17,48,49]. Lithium-induced inositol depletion resulted in reduction of PIP₃ [49,50]. Inositol-deficient diet augmented the effect of lithium in behavioral studies [51]. These studies support the hypothesis that mood-stabilizing drugs suppress PI signaling via affecting

inositol metabolism. Inositol depletion also affects other cellular functions that are associated with psychiatric illness. Inositol depletion and VPA treatment altered PI(3,5)P₂ homeostasis and perturbed vacuolar ATPase function in yeast; while similar studies have not yet been carried out in mammalian cells, these functions are important for neurotransmission [52]. VPA and lithium prompted synapse formation between hippocampal neurons, which could be reversed by pretreatment with exogenous inositol [53]. Inositol depletion resulted in defective craniofacial development and brain function in a mouse model [54].

Understanding how inositol synthesis is regulated is of obvious importance to elucidating drug-related mechanisms of inositol depletion. Surprisingly, regulation of inositol synthesis in mammalian cells has not been well studied. In contrast, inositol synthesis has been well characterized in the yeast *Saccharomyces cerevisiae* [55–57]. In this yeast, both lithium and VPA were shown to inhibit inositol synthesis. Lithium reduces intracellular inositol levels in yeast, as in human cells, by inhibiting IMPase [58,59]. Interestingly, VPA was also shown to perturb inositol metabolism in yeast [59]. VPA depletes inositol by a different mechanism from that of lithium. Vaden *et al.* first discovered that VPA causes decreased levels of intracellular inositol-3-phosphate and inositol in yeast [59], consistent with inhibition of MIPS, which catalyzes the synthesis of inositol-3-phosphate from G6P. Indeed, VPA was shown to cause a 35% decrease of MIPS enzymatic activity *in vivo* at a drug concentration used therapeutically (0.6 mM). Subsequent studies showed that VPA also inhibited human MIPS expressed in yeast cells [60]. In contrast to direct inhibition of IMPase by lithium, inhibition of MIPS activity is indirect and not observed *in vitro*. Indirect inhibition of MIPS by VPA is also observed in human brain [61]. Consistent with inositol starvation, chronic VPA treatment significantly decreased PI synthesis and increased CDP-DAG levels in yeast [62].

Both yeast and human MIPS are phosphoproteins, and phosphorylation of MIPS has been shown to regulate activity of both enzymes [63,64]. Three phosphorylation sites were identified and mapped to Ser-184, Ser-296 and Ser-374 in yeast MIPS and the corresponding sites Ser-177, Ser-279 and Ser-357 in human MIPS. VPA was shown to increase phosphorylation of yeast MIPS [64]. The simultaneous mutation of both

Ser-184 and Ser-374 to Ala resulted in a four-fold increase in MIPS enzyme activity and decreased sensitivity of cells to VPA [64]. Although inhibition of MIPS by VPA is indirect, VPA directly or indirectly affects PKA, AKT (also known as protein kinase B), GSK3 and PKC signaling pathways [65–67]. Therefore, it is plausible that inhibition of MIPS by VPA may be an indirect outcome of affecting these kinases.

VPA-mediated perturbation of inositol metabolism was also reported in animal studies. VPA and lithium caused similar levels of inositol depletion in rat brain [68]. Acute VPA treatment reduced inositol levels in mouse frontal cortex tissue [61]. Inositol reversed the inhibitory effects of VPA and lithium on the collapse of sensory neuron growth cones and the increase in growth cone area in rat ganglia cells [17]. These studies indicate that inositol depletion is a common outcome of structurally disparate antibipolar drugs.

• Inositol depletion & PKC

PKC, a target of lithium and VPA that is associated with BD, is affected by inositol. PKC comprises a family of serine/threonine kinases that are ubiquitous in mammalian tissues [69]. It is highly enriched in brain, where its activity affects numerous cellular processes, including neurotransmission, secretion, cell proliferation and localization of extracellular receptors [70]. Several PKC isoforms are activated by DAG, the signaling molecule generated from the cleavage of PIP₂ [71]. Numerous studies associate PKC with the pathophysiology and treatment of BD. Serotonin-induced PKC translocation was altered in platelets obtained from BD patients during the manic phase [72]. The ratio of membrane-bound to cytosolic PKC activities was shown to be elevated in BD patients and decreased after lithium treatment [72]. Alteration of PKC levels and activities were also reported in a study of postmortem BD brain [73]. Furthermore, a genome-wide association study identified diacylglycerol kinase η (*DGKH*) as a risk gene in the etiology of BD [74]. *DGKH* catalyzes the metabolism of DAG to phosphatidic acid. Because DAG is a necessary cofactor for many isoforms of PKC, *DGKH* is thought to attenuate PKC [75]. A study of postmortem brain tissue demonstrated increased expression of *DGKH* in the prefrontal cortex of BD patients [76], suggesting that altered expression of *DGKH* is involved in the pathogenesis of BD.

Taken together, these findings suggest a close association between perturbation of the PKC pathway and BD.

Interestingly, both lithium and VPA were shown to reduce PKC levels [77]. Rats chronically treated with lithium exhibited a decrease in membrane-associated PKC α in hippocampus [78]. The coadministration of myo-inositol reversed the decrease in PKC α and PKC ϵ levels in hippocampus samples of rats treated with lithium [79]. Chronic treatment with VPA also reduced levels of PKC α and PKC ϵ in rat cells [80]. Tamoxifen, a strong inhibitor of PKC widely used for the treatment of breast cancer, exhibited significant efficacy in reducing manic symptoms, although it was not effective for BD patients during the depression phase [81].

In summary, perturbation of PKC activity is closely associated with the etiology of BD. It is tempting to speculate that downregulation of PKC by lithium and VPA induces inositol depletion, which may exert therapeutic effects by altering downstream signaling pathways.

• Discrepancies of the inositol depletion hypothesis

Some studies do not support the inositol depletion hypothesis. First, reduction of mouse brain inositol levels alone did not lead to mood stabilization [82]. Mice heterozygous for the null allele of the *SMIT1* gene exhibited a 33–37% decrease in inositol in brain tissue, which was greater than the 22–25% decrease observed in lithium-treated mouse brain. However, only lithium-treated mice, but not *SMIT*^{+/−} mice, exhibited a significant decrease in immobility in the forced swim test [82,83]. A second argument against the inositol depletion hypothesis is that inositol depletion does not necessarily lead to decreased PI signaling. Phosphatidylinositol levels were compared in WT and *SMIT*^{+/−} mouse fetal brain. Although *SMIT*^{+/−} mice exhibited a 92% decrease in intracellular inositol, phosphatidylinositol levels were not significantly changed [84]. However, it is possible that 8% of WT levels of inositol remaining in *SMIT*^{+/−} mice may be sufficient to accommodate normal PI signaling. Third, it is not clear how inositol depletion can effectively treat both mania and depression. Studies by Cheng *et al.* suggested a mechanism whereby VPA and other mood stabilizing drugs may exert their dual action by maintaining stable PI signaling [85]. Inositol depletion may attenuate PI signaling in manic patients, while VPA-induced

inhibition of prolyl oligopeptidase may increase PI signaling in depressed patients [85].

In summary, while many common outcomes of antibipolar drugs are well explained by inositol depletion, some studies are not consistent with the hypothesis.

GSK3 inhibition hypothesis

• GSK3 activation is inhibited by lithium

In 1996, Klein and Melton reported that lithium is an inhibitor of GSK3 β in *Xenopus* [86]. Subsequent studies showed that lithium inhibited GSK3 β in *Drosophila*, cultured mammalian cells and rat brain [87–93]. Lithium can directly inhibit GSK3 by competing with the cofactor magnesium for binding to the enzyme [94] and/or by prompting inhibitory serine phosphorylation through multiple mechanisms, including PKA, PI3K/AKT, PKC pathways and autoregulation of GSK3 [95–98]. These findings led to the hypothesis that GSK3 inhibition may be the therapeutic mechanism of mood-stabilizing drugs.

GSK3 was first identified as a protein kinase that phosphorylates and inactivates glycogen synthase [99,100]. As a serine/threonine kinase, GSK3 is involved in the regulation of many cellular functions that affect cell fate determination, cell survival and signal transduction [101–103]. Two isoforms of GSK3 exist in mammalian cells, GSK3 α and GSK3 β [104,105]. These isoforms exhibit 98% identity in the amino acid sequences of their kinase domains [105]. GSK3 activity is regulated by the PI3K/AKT pathway, which phosphorylates GSK3 α and GSK3 β on serine-21 and serine-9, respectively, inhibiting their enzymatic activities [106,107]. GSK3 β , the predominant form in brain, regulates more than 40 proteins in many cell signaling pathways, some of which play a role in BD as well as Alzheimer's disease and cancer [102,103,108,109].

To determine if GSK3 β inhibition may account for the therapeutic effect of mood-stabilizing drugs, behavioral efficacies of reduction of intracellular GSK3 β and lithium treatment were compared [110]. Mice chronically treated with lithium exhibited significantly decreased immobility in the forced swim test, a positive antidepressive effect [111]. Interestingly, mice with heterozygous deletion of GSK3 β , in which GSK3 β levels are significantly decreased, also exhibited decreased immobility time in the forced swim test, as well as other behavior changes similar to those seen in lithium-treated

mice [110]. Behavior changes observed in lithium-treated and *GSK3 β ^{+/-}* mice were reversed by overexpression of GSK3 β , indicating that GSK3 β is a target of lithium [112]. Consistent with inhibition of GSK3 as a therapeutic mechanism, mood-stabilizing effects were reported for many GSK3 inhibitors. Rodents treated with GSK3 inhibitors AR-A014418 and L803-mts exhibited reduced immobility time in the forced swim test [113–115]. In addition to the antidepressive effect, GSK3 inhibitors also reduced manic behaviors in animal studies. A variety of GSK3 inhibitors reduced hyperactivity in mouse models of mania [114,116]. In addition, mice with heterozygous deletion of *GSK3 β* also exhibited attenuated amphetamine-induced hyperactivity [116]. These findings suggest that reduced GSK3 activity contributes to alleviation of manic behavior in animal models.

• Inhibition of GSK3 by VPA

VPA was shown to inhibit GSK3 β in some [117–120] but not all studies [121]. Chen *et al.* demonstrated that VPA inhibits GSK3 in both *in vivo* and *in vitro* assays [117]. The activities of GSK3 α and GSK3 β , assayed *in vitro* by incorporation of ³²P into CREB phosphopeptides, decreased in the presence of VPA in a concentration-dependent manner. *In vivo* assays using β -catenin degradation as an indicator of GSK3 β activity showed significant increases in β -catenin levels in cytosol and nucleus of VPA-treated neuronal cells, consistent with GSK3 inhibition [117]. Phosphorylation of tau protein by human GSK3 β was also decreased in the presence of VPA in a concentration-dependent manner [119]. Furthermore, VPA treatment inhibited phosphorylation of MAP1B by GSK3 β *in vivo* in neuronal cells [118]. However, in contrast to the finding of Chen *et al.*, VPA did not inhibit GSK3 β *in vitro* in the study of Hall *et al.*, suggesting that inhibition of GSK3 β by VPA may be indirect [118]. The inhibitory effect of VPA on GSK3 β was further supported by the finding of increased levels of GSK3 β serine-9 phosphorylation in VPA-treated human neuroblastoma cells [122]. In addition, similar to lithium, VPA exerted behavioral effects as a result of disrupting the AKT/GSK3 signaling pathway [123].

While the findings described above suggest that VPA inhibits GSK3 β , this outcome is not universally supported. In the report of Phiel *et al.*, VPA did not affect the phosphorylation of tau protein by GSK3 β in neuronal

cells or the *in vitro* phosphorylation of glycogen synthase peptide-2 [121]. In addition, increased β -catenin levels were observed in dorsal root ganglia cultures treated with lithium but not VPA [17]. Similar results were obtained in other studies [124,125]. The variety of model systems and methodologies used in these studies likely contribute to the discrepancy in results, and further investigation is necessary to understand the effect of VPA on GSK3.

• HDAC inhibition & GSK3 activity

Therapeutic concentrations of VPA have been shown to inhibit HDAC [126–128]. Interestingly, HDAC inhibitors exhibit antidepressive effects in mouse models of depression [129,130]. HDAC inhibition greatly affects the transcription profile and alters cellular signaling, consequences that could potentially account for the therapeutic effects of VPA [131]. For example, long-term treatment with the HDAC inhibitor Cpd-60 led to significantly increased levels of *SGK1* expression in mouse brain [130]. Upregulation of *SGK1* was also reported in rodents treated with lithium and other antidepressants [132,133]. *SGK1* encodes a protein kinase that phosphorylates and inhibits GSK3 β [134]. As discussed previously, the activity of GSK3 is negatively regulated by the AKT signaling pathway. VPA increased the activation-associated phosphorylation of AKT and the inhibition-associated phosphorylation of GSK3 β [122]. Two other HDAC inhibitors, TSA and sodium butyrate, also mimicked the effect of VPA in activating AKT and inhibiting GSK3 β , suggesting that HDAC inhibition promotes GSK3 β inhibition via the AKT pathway [122]. Although the discrepancy remains as to whether VPA directly affects GSK3 activity, as discussed above, these findings suggest that VPA indirectly inhibits GSK3 β by inhibiting HDAC, which may underlie the antidepressant effects of the drug.

• Neurotrophic effects of GSK3 inhibition

Many studies have reported a loss of neuronal and glial cells in the brains of BD patients [135–138], possibly due to increased apoptosis [139]. GSK3 is a proapoptotic enzyme, the activation of which facilitates apoptosis [140,141]. Therefore, GSK3 inhibition may exert its neurotrophic and antibipolar effects by decreasing apoptosis of neuronal cells in the brain. The reduction of intracellular GSK3 β levels was shown to protect neurons from amyloid- β -induced neurotoxicity [142],

while overexpression of GSK3 β induced caspase-3-dependent apoptosis in mouse neuronal cells [143]. G-CSF is a neuroprotective growth factor that antagonizes apoptosis by inhibiting GSK3 β [144]. The connection between altered GSK3 activity and apoptosis was further supported by the finding that several selective GSK3 inhibitors and antibipolar drugs, including VPA and lithium, also provided significant protection from apoptotic cell death [67,145–147]. In a mouse model, VPA was shown to exert an anti-apoptotic effect by upregulating B-cell lymphoma 2 (Bcl-2) expression [148]. VPA and lithium increased intracellular Bcl-2 levels in human neuronal cells [149]. VPA also inhibited apoptosis in human endothelial cells by preventing Bcl-2 ubiquitination [150]. Furthermore, lithium prompted neural precursor cell proliferation via GSK-3 β -NF-AT signaling [151]. GSK3 inhibition exhibited neuroprotective effects against excitotoxicity [152]. These studies suggest that VPA and lithium may exert their therapeutic effects by promoting survival and proliferation of neuronal cells as a consequence of GSK3 inhibition.

In addition to promoting anti-apoptotic signaling, inhibition of GSK3 β leads to activation of the Wnt pathway and upregulation of β -catenin [153–155]. As a transcription factor, β -catenin plays an important role in regulating neuronal connectivity, which is critical for

diverse neuronal functions [156]. Some evidence suggests that increased intracellular β -catenin is a potential therapeutic strategy of BD treatment. L803-mts, a selective GSK3 β inhibitor with antidepressive efficacy, caused elevated β -catenin expression in mouse hippocampus [113]. Overexpression of β -catenin in mouse brain and lithium treatment induced similar behavior changes, including decreased immobility time in the forced swim test [157]. Furthermore, overexpression of β -catenin inhibited amphetamine-induced hyperlocomotion, mimicking the antimanic effect of lithium. A recent study using induced pluripotent stem cell lines derived from BD patients indicated abnormal neurogenesis and expression of genes that are critical for Wnt signaling [158]. The proliferation defect in BD-induced pluripotent stem cells was rescued by GSK3 inhibition. Together, these findings suggest that the therapeutic efficacy of GSK3 β inhibition in BD may occur by upregulating β -catenin. Targeting Wnt/ β -catenin signaling may be a promising strategy for BD treatment.

In summary, GSK3 may play a pivotal role in the therapeutic mechanisms of BD therapy. Altered GSK3 activity and protein levels were observed in BD patients. Decreasing GSK3 by genetic ablation or treatment with inhibitors mimics the mood-stabilizing effect of antibipolar drugs in animal behavior studies.

Figure 1. A potential GSK3 phosphorylation site in MIPS. Both human and yeast MIPS contain a potential GSK3 phosphorylation site (serine-279 of human MIPS and serine-296 of yeast MIPS) within a six-amino acid region of identity [64].

Figure 2. Model: dual effects of VPA and lithium on inositol depletion and GSK3 inhibition contribute to mood stabilization. GSK3 is required for optimal *de novo* synthesis of inositol in yeast. VPA (A) indirectly inhibits MIPS, the rate limiting enzyme of inositol *de novo* synthesis, possibly by inhibiting GSK3, thereby reducing intracellular inositol. Lithium (B) depletes inositol by inhibiting IMPase, and inhibits GSK3 by multiple mechanisms. In addition to inhibition of MIPS activity, GSK3 inhibition may also affect metabolism of G6P, the substrate for inositol *de novo* synthesis. Inositol depletion leads to perturbation of numerous cellular functions, some of which are associated with mood stabilization. Inhibition of GSK3 affects cells in numerous ways, some of which are neurotrophic and may contribute to mood stabilization. VPA: Valproic acid.

In addition, several studies reported that VPA inhibits GSK3 enzymatic activity. These findings suggest that GSK3 inhibition, similar to inositol depletion, is a common effect of structurally disparate mood-stabilizing drugs.

A unified model of inositol depletion & GSK3 inhibition

While inositol depletion and GSK3 inhibition may appear to be unrelated, we suggest that they may constitute components of a single mechanism.

Studies have shown that GSK3 is required for optimal inositol biosynthesis in yeast [159]. Yeast cells lacking *GSK3* (*gsk3Δ* cells) exhibit multiple features of inositol depletion: intracellular inositol levels in *gsk3Δ* cells are 70% lower than in WT cells; the growth rate of *gsk3Δ* cells in inositol-free medium is significantly slower than that of WT cells and the mutant exhibits decreased MIPS enzymatic activity [159]. These findings indicate that *GSK3* is required for optimal inositol homeostasis in yeast. As discussed above, MIPS activity is regulated by phosphorylation. Interestingly, a potential GSK3 phosphorylation site has been identified in yeast MIPS (Figure 1). Mutation of this residue results in alteration of MIPS enzymatic activity, suggesting a potential regulatory mechanism of MIPS by GSK3 through phosphorylation [64]. Strikingly, a sequence identical to the putative yeast GSK3 phosphorylation site is present in human MIPS [64]. Mutation of this site in the human enzyme affected MIPS activity similar to the yeast mutant enzyme. We speculate that regulation of MIPS activity by phosphorylation of this site may be a conserved mechanism of regulation of inositol synthesis. Interestingly, inositol synthesis in neuronal cells was shown to affect GSK3 activity. Knock down of the *ISYNA1* gene, which encodes MIPS in mammalian cells, led to inactivation of GSK3α by increasing inhibitory phosphorylation of serine-21 [160], suggesting that GSK3 and inositol synthesis may be coordinately regulated.

GSK3 may also regulate inositol synthesis by affecting metabolism of G6P, which is the substrate for inositol *de novo* synthesis. GSK3 controls the conversion of glucose to glycogen by regulating glycogen synthase activity [99]. The inhibition of GSK3 in hepatic cells reduces expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase, which regulates gluconeogenesis [161]. In addition, expression of phosphoglucomutase 2 (PGM2), which catalyzes the interconversion of glucose-1-phosphate and G6P [162], requires GSK3 activity [163]. Interestingly, lithium inhibits PGM2 [164], whereby it may also affect intracellular G6P production. These findings suggest that GSK3 may regulate inositol synthesis by controlling the availability of G6P. It will be of great importance to determine if VPA, lithium and GSK3 inhibition affect the rate of glucose uptake and G6P production.

The model shown in Figure 2 unifies both inositol depletion and GSK3 inhibition in the

following hypothesis. VPA induces inositol depletion by decreasing MIPS activity through inhibition of GSK3. As a major component of intracellular signaling molecules, inositol is involved in the regulation of PI synthesis, protein secretion and many other cellular functions [18,19,165]. Alteration of inositol metabolism affects expression of hundreds of genes and causes numerous cellular consequences [31,166], among which are those that may lead to mood stabilization. VPA-induced GSK3 inhibition also exerts neurotrophic effects by reducing apoptosis of neuronal cells through upregulation of anti-apoptotic factors and by upregulation of β-catenin. Lithium also causes dual effects of GSK3 inhibition as well as inositol depletion by inhibition of IMPase. The inter-relationship between inositol depletion and GSK3 inhibition may contribute to the therapeutic effects of VPA and lithium.

Conclusion

Although the therapeutic mechanisms of VPA and lithium are not understood, both inositol depletion and GSK3 inhibition are common outcomes of treatments by these structurally dissimilar drugs and may play a role in their therapeutic effects. We speculate that VPA- and lithium-induced GSK3 inhibition may inhibit MIPS enzymatic activity by mediating the inhibitory phosphorylation of MIPS, the rate-limiting enzyme of *de novo* inositol synthesis, resulting in depletion of intracellular inositol.

Future perspective

The studies summarized in this review indicate that both inositol depletion and GSK3 inhibition are common outcomes of treatment with VPA and lithium. Interestingly, the finding that GSK3 is required for optimal inositol synthesis and the identification of putative GSK3 phosphorylation sites in the inositol biosynthetic enzyme MIPS suggest that VPA-induced inositol depletion may result from GSK3 inhibition. This suggests that drug-induced inositol depletion and GSK3 inhibition are mechanistically related in a single unifying hypothesis.

How these biochemical outcomes affect mood is not understood. Both inositol depletion and GSK3 inhibition alter many downstream pathways, including inhibition of PKC and HDAC, as discussed above, as well PI synthesis, expression of anti-apoptotic factors and regulation of β-catenin, among others. Identifying the

therapeutically relevant targets of inositol depletion and GSK3 inhibition is critically important for the development of effective therapeutics to treat BD.

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EXECUTIVE SUMMARY

Inositol depletion hypothesis – valproic acid & lithium exert their therapeutic effects by depleting inositol

- Bipolar disorder (BD) patients exhibit altered levels of inositol in brain.
- Inositol depletion is a common outcome of structurally disparate antibipolar drugs. Valproic acid (VPA) and lithium, two widely used antibipolar drugs, deplete intracellular inositol by inhibiting inositol *de novo* synthesis. VPA inhibits enzymatic activity of myo-inositol-3-phosphate synthase (MIPS), the rate-limiting enzyme of inositol synthesis, possibly by affecting phosphorylation status of MIPS. Lithium is an uncompetitive inhibitor of IMPase, which catalyzes inositol recycling as well as the second step of inositol *de novo* synthesis.
- Perturbation of the PKC pathway is associated with BD. Lithium and VPA have been shown to downregulate the PKC pathway, suggesting that these drugs may exert their therapeutic effects by altering PKC signaling pathways.
- While many studies support inositol depletion as a therapeutic mechanism of antibipolar drugs, this hypothesis is not universally accepted.

GSK3 inhibition hypothesis – VPA & lithium exert their therapeutic effects by inhibiting GSK3

- Inhibition of GSK3 results in multiple neurotrophic effects, which may account for therapeutic efficacy of VPA and lithium.
- Lithium is an inhibitor of GSK3.
- Many studies suggest that VPA inhibits GSK3 activity, although some findings argue against this outcome. VPA may indirectly inhibit GSK3 β by inhibiting HDAC, which underlie the antidepressant effects of the drug.

Unifying hypothesis – the therapeutic effects of VPA & lithium derive from inositol depletion mediated by GSK3 inhibition

- GSK3 is required for optimal *de novo* synthesis of inositol in yeast.
- Both human and yeast MIPS contain a potential GSK3 phosphorylation site.
- VPA may cause inositol depletion by inhibiting GSK3.
- Inositol depletion leads to perturbation of numerous cellular functions, some of which are associated with mood stabilization. Inhibition of GSK3 leads to neurotrophic effects that contribute to mood stabilization.

Conclusion

- Both inositol depletion and GSK3 inhibition are supported by many studies as outcomes of antibipolar drugs. However, the underlying therapeutic mechanisms of these drugs are not known.
- Although inositol depletion and GSK3 inhibition hypotheses appear to be unrelated, they may constitute components of a single mechanism in which inositol depletion results from GSK3 inhibition.

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