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Search for a common mechanism of mood stabilizers

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Abstract

Manic-depression, or bipolar affective disorder, is a prevalent mental disorder with a global impact. Mood stabilizers have acute and long-term effects and at a minimum are prophylactic for manic or depressive poles without detriment to the other. Lithium has significant effects on mania and depression, but may be augmented or substituted by some antiepileptic drugs. The biochemical basis for mood stabilizer therapies or the molecular origins of bipolar disorder is unknown. One approach to this problem is to seek a common target of all mood stabilizers. Lithium directly inhibits two evolutionarily conserved signal transduction pathways. It both suppresses inositol signaling through depletion of intracellular inositol and inhibits glycogen synthase kinase-3 (GSK-3), a multifunctional protein kinase. A number of GSK-3 substrates are involved in neuronal function and organization, and therefore present plausible targets for therapy. Valproic acid (VPA) is an antiepileptic drug with mood-stabilizing properties. It may indirectly reduce GSK-3 activity, and can up-regulate gene expression through inhibition of histone deacetylase. These effects, however, are not conserved between different cell types. VPA also inhibits inositol signaling through an inositol-depletion mechanism. There is no evidence for GSK-3 inhibition by carbamazepine, a second antiepileptic mood stabilizer. In contrast, this drug alters neuronal morphology through an inositol-depletion mechanism as seen with lithium and VPA. Studies on the enzyme prolyl oligopeptidase and the sodium *myo*-inositol transporter support an inositol-depletion mechanism for mood stabilizer action. Despite these intriguing observations, it remains unclear how changes in inositol signaling underlie the origins of bipolar disorder.

Keywords: Lithium; Valproate; Carbamazepine; GSK-3; Inositol phosphate signaling; Manic-depression

1. Introduction

Carl Lange discovered lithium as a treatment for manic-depression in the 1880s [1]. Its initial use declined for lack of a credible therapeutic mechanism—it was originally linked to the idea that manic-depression, now known as bipolar affective disorder, and a number of other medical conditions arose through a buildup of uric acid [2]. Lithium was re-discovered in 1949 by John Cade [3], and since then has been used as an effective treatment for both acute and long-term phases of bipolar affective disorder. It is estimated that 1 in 1000 people may be taking lithium in the United States. Although clearly beneficial (the suicide rate of untreated patients runs at higher than 15%), lithium treatment is not without problems. These include potential serious side-effects and individuals who fail to respond [4]. Although perhaps not as effective as lithium on both depression and mania, some antiepileptic drugs have also been found to have mood-stabilizing properties, but again these are not without complications [5,6]. Despite an intense research effort from academic and clinical institutions and the pharmaceutical industry, the development of better mood stabilizers has been slow. Probably, the greatest barrier to progress is still our lack of a molecular understanding of the mechanisms that underlie the origin and treatment of this disorder.

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Abbreviations: DRG, dorsal root ganglia; GSK-3, glycogen synthase kinase-3; IMPase, inositol monophosphatase; InsP, inositol phosphate; Ins(1,4,5)P_3, inositol 1,4,5-trisphosphate; IPP, inositol polyphosphate 1-phosphatase; PAP, 3′-phosphoadenosine 5′-phosphate; PAPS, 3′-phosphoadensosine 5′-phosphosulphate; PI3 kinase, phosphatidylinositol 3-kinase; PI(4,5)P_2, phosphatidylinositol (4,5) bisphosphate; PLC, phospholipase C; PO, prolyl oligopeptidase; RnPIP, *Rattus norvegicus* 3′-phosphoadenosine 5′-phosphate and inositol 1,4-bisphosphate phosphatase; and VPA, valproic acid.

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2. Lithium

In addition to its therapeutic uses, embryologists have long known lithium as a teratogen: a drug that affects patterning and proportion of cell types in the developing embryo. For example, in sea urchins, lithium causes vegetalization of the animal blastomeres [7] and in vertebrates, such as *Xenopus* and zebrafish, it causes expansion of the dorsal mesoderm and duplication of the dorsal axis [8,9]. These effects are not restricted to animals, as lithium treatment of *Dictyostelium*, a non-metazoan eukaryote, leads to mis-specification of spore and basal disc cell fate [10,11]. Despite the assumed clinical risk during pregnancy, there are surprisingly few reports of teratogenic effects of lithium on human development, although some reports have suggested an increased risk of heart defects [12].

A number of signal transduction pathways are affected by lithium treatment, but many of the reported effects appear to be indirect [13]. Currently, only two protein families have been demonstrated as direct cellular targets of lithium and are affected at close to therapeutic concentrations. IMPase is the best known member of a super-family of structurally related phosphomonoesterases [14]. Biochemical and structural studies of a number of family members, including IMPase, IPP, fructose 1,6-bisphosphatase, and the rat PAP phosphatase (RnPIP), have revealed a magnesium ion binding site within the active site of the enzyme that is targeted by lithium [15–17]. This magnesium ion is not required for direct hydrolysis of the phosphodiester bond, but is required for binding the cleaved product and phosphate. Lithium prevents product release and traps products in the active site; this results in an uncompetitive mode of inhibition [18].

Some lithium-sensitive phosphomonoesterases, including RnPIP and the *Saccharomyces cerevisiae* protein Hal2, dephosphorylate both PAP and inositol 1,4-bisphosphate, with in many cases the PAP phosphatase activity being the dominant enzyme activity [19]. PAP phosphatase catalyses the hydrolysis of PAP to form AMP [20]. Lithium inhibition, therefore, may lead to increased PAP, which is a potent inhibitor of enzymes that utilize PAPS, such as the sulphotransferases. Loss of PAP phosphatase genes in yeasts causes sulfur and methioinine auxotrophy, and inhibits RNA processing [21]. It is not yet clear whether blocking PAP phosphatase contributes to the overall therapeutic effects of lithium treatment.

Lithium also inhibits the GSK-3 family of serine/threonine protein kinases [22]. These are a highly conserved family of kinases that have been identified in all eukaryotic groups examined to date. In vertebrates, GSK-3 is found as two isoforms: GSK-3α and GSK-3β plus a splice variant of GSK-3β [23,24]. In other organisms, the number of genes varies from one in *Dictyostelium* to four in *S. cerevisiae*, and even more in plants [25]. Lithium has been demonstrated to inhibit GSK-3 both *in vitro* and *in vivo* [22]. Enzyme kinetic experiments suggest that this is also through competition for magnesium ion binding [26,27]. In contrast to phosphomonoesterases, lithium is a non-competitive inhibitor with respect to its protein substrates. The fact that lithium inhibits both of these enzyme groups through an interaction with magnesium suggests that this may be a general mechanism of lithium action. Lithium could, therefore, potentially target all magnesium binding proteins. However, those with low-affinity magnesium sites, of which only phosphomonoesterases and GSK-3 are known, may be sensitive to the concentrations used in both therapeutic and experimental conditions. In this commentary, we will further consider the consequences of IMPase and GSK-3 inhibition.

3. Lithium and InsP signaling

Over 20 years ago, it was shown that lithium perturbs InsP metabolism by inhibition of IMPase and IPP [28]. Inositol plays a role in signal transduction in two ways. The soluble InsPs bind to receptors in the cytoplasm. The most well-known example is Ins(1,4,5)P_3, which binds to a receptor on the surface of the endoplasmic reticulum to release calcium [29]. This then elicits a range of cell responses, including activation of protein kinases such as PKC. Other InsP species, particularly Ins(1,3,4,5)P_4 and $InsP_6$, have also been ascribed signal functions [30]. Inositol is also incorporated into phosphatidylinositol phosphates, present in the plasma membrane. Both PI(4,5)P_2 and a variety of phosphatidylinositides phosphorylated at the 3′ carbon form protein binding sites within the membrane and lead to protein translocation from the cytoplasm [31].

PI(4,5)P_2 is hydrolyzed by PLC to generate Ins(1,4,5)P_3 and diacylglycerol (DAG). PLC activity is directly regulated through ligand-stimulated receptors and can lead to acute changes in Ins(1,4,5)P_3 concentrations [32]. Ins(1,4,5)P_3 is then degraded by a 5′-phosphatase and IPP to form inositol monophosphate ($InsP_1$). The final phosphate is removed by IMPase to release free inositol, which then can be reincorporated into membrane PIPs. In addition to the recycling of inositol from InsPs, IMPase is also required for *de novo* synthesis of inositol from glucose-6-phosphate, which is isomerized by inositol-1-phosphate synthase to $InsP_1$ [33]. On the basis of these observations, Berridge *et al.* [34] proposed the inositol-depletion hypothesis, in which inhibition of IMPase by lithium reduces the pool of free inositol and therefore suppresses inositol-based signaling (Fig. 1). The uncompetitive inhibition of IMPase by lithium—where inhibition is dependent upon both the inhibitor and the substrate—means that lithium may preferentially act on those cells generating the most $InsP_1$.

There is little doubt that lithium can inhibit IMPase in brain slices [35,36], but the overall effect on Ins(1,4,5)P_3 varies between species; in some cases Ins(1,4,5)P_3 concentrations rise [37,38]. Lithium treatment, however, increases neurotransmitter release and may therefore increase Ins(1,4,5)P_3 synthesis through indirect effects

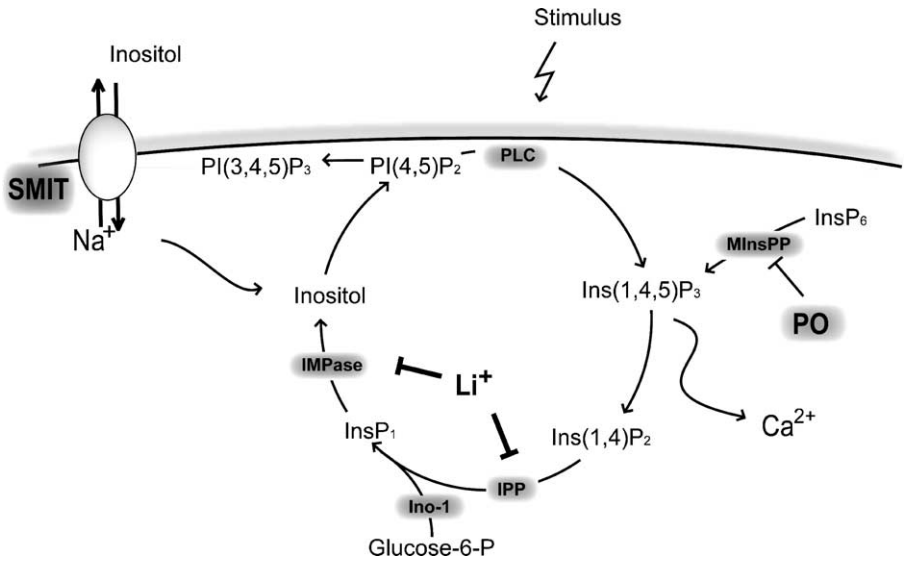

Fig. 1. An extended view of inositol-based signal transduction. Inositol is rate limiting for the synthesis of the phosphatidylinositol bisphosphate, $PI(4,5)P_2$. This membrane lipid is either further phosphorylated to $PI(3,4,5)P_3$ by PI3 kinase, or hydrolyzed to diacylglycerol and soluble $Ins(1,4,5)P_3$ by ligand-stimulated PLC enzymes. $Ins(1,4,5)P_3$ induces Ca^{2+} release from intracellular stores. The cellular $Ins(1,4,5)P_3$ concentration is reduced by phosphorylation to create higher order inositol phosphates or dephosphorylation to inositol. This latter pathway recycles inositol into the signal pathway. The enzymes inositol polyphosphate 1-phosphatase (IPP) and inositol monophosphatase (IMPase), which catalyse the final dephosphorylation steps, are lithium sensitive. Inositol is synthesized by isomerization of glucose-6-phosphate by the enzyme inositol synthase (Ino-1). As *de novo* synthesis involves an IP_1 intermediate, it is also blocked by lithium treatment. Environmental inositol is taken into the cell via a high-affinity sodium *myo*-inositol transporter (SMIT). Finally, inositol can be released from high order inositol phosphates. Multiple inositol polyphosphate phosphatase (MInsPP) dephosphorylates $InsP_6$ to $Ins(1,4,5)P_3$ and is regulated by prolyl oligopeptidase (PO).

[39]. Changes in whole brain biochemistry may be uninformative, and mood disorders could arise through region- or cell-specific changes in brain biochemistry. Indeed, brain scans indicate that changes in the metabolic activity correlated to mood disorders and treatment are restricted to specific brain regions [40,41]. At the subcellular level, lithium induces structural changes in the termini of peripheral neurons. In *Drosophila*, either lithium treatment or loss of IPP leads to ultrastructural changes in the neuromuscular junction and aberrant firing [42]. Lithium treatment of explants of DRG leads to increased spreading of the growth cone present at the axon terminus [43]. This effect is reversed by the addition of *myo*-inositol, compatible with an inositol-depletion mechanism.

Using reversal by inositol as a criterion, a number of brain physiological and behavioral effects of lithium have been ascribed to changes in InsP signaling. Lithium-induced suppression of rearing is well described in rats, and has been shown to be suppressed by intracerebroventricular injection of inositol [44,45]. A more defined effect is seen with limbic seizures induced by an injection of lithium followed by pilocarpine [46]. Again this effect can be suppressed by inositol. A number of observations, however, appear inconsistent with the inositol-depletion hypothesis. First, the reversal of lithium depletion would be expected to be specific for *myo*-inositol, which is the only stereoisomer utilized in the production of phosphatidylinositol. Whereas L-chiro-inositol fails to reverse the effects of lithium, in some cases epi-inositol does not [47,48]. Second, studies indicate that high daily intake of inositol, in the range of 6–12 g, may have positive effects on patients with depression [49,50]. These observations indicate a more complex cellular interaction with inositol (see Section 8). They may also be indicative of other mechanisms of lithium action.

4. Lithium and GSK-3

4.1. GSK-3 and Wnt signaling

GSK-3 was first identified as an enzymatic activity that phosphorylates and inactivates glycogen synthase [51]. This enzyme is a key target of the insulin response, which acts through GSK-3 inhibition. Consistent with its inhibition of GSK-3, lithium restores glycogen synthesis to hepatocytes from diabetic rats [52]. GSK-3, however, has many targets and functions within the cell [53] (Fig. 2). For example, GSK-3 phosphorylates a number of important transcription factors and cytoskeletal proteins. Among its targets is the protein β-catenin, which serves both functions, being a component of adherens junctions and a regulator of the LEF-1/TCF-3 family of transcription factors. GSK-3 phosphorylation of β-catenin leads to its degradation and hence blocks its function. The GSK-3 interaction with β-catenin appears to be insulated from other GSK-3-mediated events through their mutual binding to the protein Axin [54]. Function of the GSK-3–Axin–β-catenin complex is negatively regulated by the extracellular ligand Wnt, which when bound to its co-receptors Frizzled and LRP5 or 6 blocks

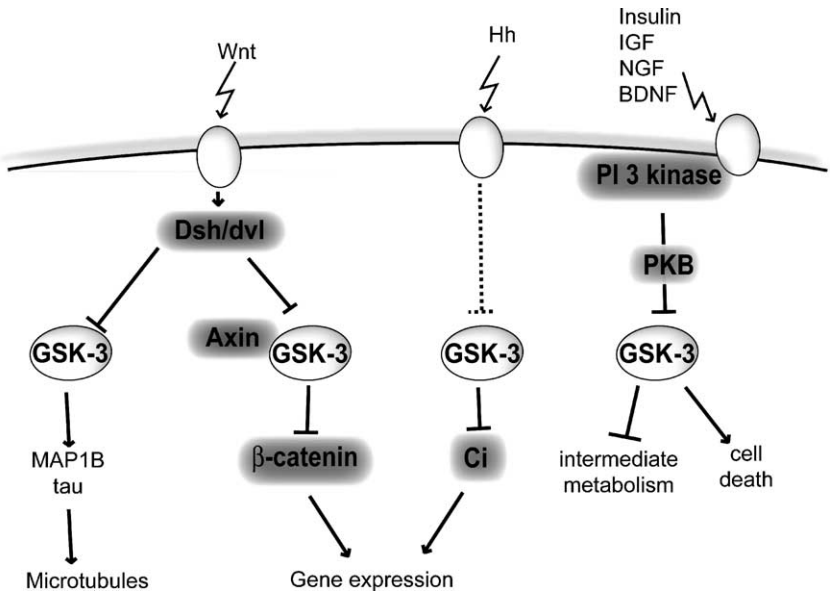

Fig. 2. Multiple roles for GSK-3. GSK-3 lies downstream of a number of apparently independent signal transduction pathways. In a complex with Axin, GSK-3 phosphorylates β-catenin, causing its degradation and preventing expression of genes, such as c-*myc* and cyclin D1. Cell stimulation by Wnt inhibits the action of GSK-3 on β-catenin, via a process that requires Dishevelled (Dsh or dvl). Wnt and dvl also regulate GSK-3 phosphorylation of MAP1B, in a β-catenin-independent manner (the role of Axin in this pathway is unknown). MAP1B and tau are both targets of GSK-3 and regulate microtubule behavior. In *Drosophila*, the cleavage of the transcription factor Cubitus interruptus (Ci) is regulated by GSK-3 phosphorylation. Ci cleavage is also regulated by Hedgehog (Hh) stimulation, although the interaction of this signal with GSK-3 has not been investigated, and leads to regulation of gene expression, including the cyclin D and cyclin E genes. Insulin, growth factors, and neurotrophic factors may activate PI3 kinase and inhibit GSK-3 through the action of PKB. This pathway regulates a number of cellular processes, such as intermediate metabolism. It has also been implicated in regulating apoptosis (cell death).

GSK-3 phosphorylation of β-catenin via a mechanism that requires the protein Dishevelled, known as Dsh or dvl, in mice [55,56]. Overexpression of β-catenin, Wnt, or Dsh in fertilized *Xenopus* oocytes leads to axis duplication as also seen following lithium injection [57].

In 1995, it was shown that injection of a dominant-negative GSK-3 causes axis duplication in *Xenopus* [58]. At the same time, the effect of GSK-3 gene disruption in the cellular slime mold *Dictyostelium discoideum* was published [25]. Disruption of the *Dictyostelium* GSK-3 homologue, *gskA*, removes all GSK-3 activity from the cells and leads to a specific pattern formation defect during its multicellular development. The same pattern defect had been reported in an earlier paper when *Dictyostelium* cells were developed in the presence of LiCl [10]. These observations suggested that lithium could exert its effects through the inhibition of GSK-3. Klein and Melton [22] showed that indeed lithium does inhibit GSK-3 activity. This inhibitory effect was also reported by Stambolic *et al.* [59], who further demonstrated that treatment of intact cells with lithium led to reduced GSK-3 activity *in vivo*, as assessed by stabilization of β-catenin and the decrease in the phosphorylation of another GSK-3 substrate, tau.

4.2. GSK-3 and the CNS

There are strong reasons to argue for a role of GSK-3 in the development and function of the CNS. Wnt protein signals are abundant during the development of the CNS, and persist into adulthood [60]. Wnt7A is expressed in the mouse cerebellum during post-natal development [61]. Wnt7A, dvl, and lithium all induce large-scale changes in the axonal cytoskeleton (Fig. 2) and redistribution of the presynaptic protein synapsin I when applied to cells isolated from this region [61–63]. Changes are particularly apparent in the microtubule cytoskeleton [64]. In growth cones of developing neurons, Wnt and lithium treatment causes changes in the distribution of stable microtubles. In addition, branching increases along the axon length [64]. Therefore, inhibiting GSK-3 could potentially lead to an increased synapse number and size, although this has yet to be demonstrated. These effects do not appear to require β-catenin, but are seen in a double-knockout mouse, which lacks both of the microtubule binding proteins MAP1B and tau, both substrates of GSK-3 [65].

Although pointing to important roles for GSK-3 in the development of both fine and gross structure within the brain, none points to a direct target for the therapeutic action of lithium. The discovery of neurogenesis and apoptosis within the adult brain presents a new potential role for GSK-3. In mammals, adult neurogenesis (the production of new neurons) occurs in certain layers of the hippocampus, where about 3% of the cells undergo division [66,67]. In rodents and primates, both proliferation and survival of cells in this region are increased by exercise, environmental enrichment, and learning, whereas stress reduces neurogenesis [68–72]. Neuroimaging and examination of postmortem brains show localized changes in volume and cell number within the

hippocampus and frontal cortex of patients with bipolar disorder [41]. Lithium treatment of mice increased the number of dividing cells and those expressing the anti-apoptotic gene *Bcl-2* within the dentate gyrus of the hippocampus [73].

Cell number is the net balance between cell division and cell death, and both could potentially be regulated by GSK-3. Deletion of the GSK-3 sites of β-catenin, inactivating mutations of Axin, and failure to degrade β-catenin protein have been associated with a wide range of cancers [74]. The molecular basis of these cancers is found in the up-regulation of gene expression. Although not all experiments agree, common genes up-regulated include c-*myc* and cyclin D1 [75–77]. Regulation of β-catenin also involves the presinilin protein PS-1 [78,79]. Mutations of PS-1 are associated with familial Alzheimer's disease (AD), indicating a role in the brain. Loss of PS-1 in mice leads to increased β-catenin protein and cell division [80]. GSK-3 has also been shown to be a component of the Hh signaling pathway, which is active during brain development (Fig. 2). In *Drosophila*, GSK-3 acts on the transcription factor Cubitus interruptus (Ci) [81,82]. Additionally, Hh signaling lead to changes in expression of the cyclin D and cyclin E genes (Fig. 2) [83,84]. Finally, GSK-3 phosphorylation also regulates the export of cyclin D1 from the nucleus during S-phase [85]. As a consequence of these interactions, there are many potential routes by which GSK-3 could regulate cell division in the adult brain; however, none of these has actually been demonstrated.

4.3. GSK-3 and cell death

GSK-3 may act as a modulator of apoptosis. Treatment of rat hippocampal neurons with the β-amyloid (Aβ) peptide, which builds up in patients with AD, both increases GSK-3β expression and induces apoptosis [86,87]. The apoptotic effect is blocked by antisense oligonucleotides directed at GSK-3. Wnt stimulation also protects against apoptosis, although this may be due to indirect induction of IGF proteins [88]. Insulin, IGFs, NGF, and BDNF can all inhibit GSK-3 through activation of PI3 kinase [53] (Fig. 2). Inhibition of PI3 kinase, by use of chemical inhibitors or serum withdrawal, leads to increased GSK-3 activity, and this correlates with apoptosis [89–91]. In addition, full GSK-3 activity requires phosphorylation at an internal tyrosine (Tyr^{216}) [54]. Interestingly, it has been shown that several apoptotic stimuli induce an increase in Tyr^{216} phosphorylation and increase GSK-3 activity [92]. Consistent with these observations, overexpression of GSK-3 also correlates with neuronal degeneration [93]. Moderate increases in GSK-3 activity in human neuroblastoma SH-SY5Y cells did not increase the basal rate of apoptosis or caspase-3, which sits within the apoptotic signal transduction pathway, but they are associated with increased sensitivity to apoptotic stimuli [94,95]. These effects are blocked by expression of the anti-apoptotic protein Bcl-2 and expression of a dominant-negative p53 mutant [96,97]. Consistent with a role of GSK-3, many of these effects can be reduced by lithium treatment [98].

5. VPA

We are therefore presented with two plausible lithium targets active in neural systems; both can modulate neuronal structure, in addition to their roles during animal development. It is therefore possible that inhibition of either target could explain the therapeutic properties of lithium on bipolar mood disorder. A number of researchers are beginning to examine the molecular actions of other mood-stabilizing drugs in an attempt to resolve this issue.

VPA is now the most widely prescribed antiepileptic drug and is being used increasingly in the treatment of bipolar disorder, especially in the United States. As with other anticonvulsants, VPA inhibits sodium, potassium, and calcium channel function, although its direct *in vivo* target has not been identified definitively [99]. In common with lithium, it has been shown to reduce expression of the protein kinase C isoforms PKCα and PKCε, activate MAP kinase and its downstream targets, increase expression of Bcl-2, and activate AP-1-dependent transcription [100–103]. Unlike lithium, VPA appears to be a potent human teratogen, increasing the risk of a number of congential anomalies, including 1–3% increased risk for spina bifida, congential heart defects, and anomalies of limb and digit development. VPA administration to mice leads to embryonic neural tube defects [104].

Initially, VPA was reported to directly inhibit GSK-3 activity; however, this has not been substantiated in a number of further experiments [105–107]. One report suggests that VPA treatment could lead to an indirect change in GSK-3 activity, but this is not seen in all cases [43,107]. In *Dictyostelium*, VPA treatment does not phenocopy the *gskA* loss of function mutant, indicating that it does not target GSK-3 [43]. VPA, however, has been demonstrated to mimic the effect of lithium on the Wnt signaling pathway by increasing the expression of β-catenin [106] This occurs through direct inhibition of the transcriptional repressor histone deacetylase (HDAC), and increased β-catenin expression is also seen after treatment with the chemically unrelated HDAC inhibitor trichostatin A (TSA). This mechanism may explain some of the effects of VPA on gene expression and animal development. The effects of VPA inhibition of HDAC appear to be cell context specific and vary between specific cases. For example, VPA or TSA treatment of DRG cells for 48 hr failed to increase β-catenin expression and had the opposite effect to lithium on axonal branching [43]. The non-teratogenic VPA analogue valpromide (VPM) does not inhibit HDAC, but has been reported to possess mood-stabilizing properties [106–108]. It has a "synchro-

nizing'' effect on the reduced heart rate circadian rhythm seen in patients with unipolar and bipolar disorders [109]. This argues against HDAC as a therapeutic target in the treatment of mood disorders.

The effect of VPA on inositol signaling has been investigated in mammals and a number of microorganisms. VPA decreases *myo*-inositol concentrations in yeast, in *Dictyostelium*, and in the human and rat brain [43,110–112]. The intracellular inositol concentration feeds back to regulate expression of the inositol-1-phosphate synthase enzyme and expression of the gene encoding this enzyme, *ino-1*, can be used to indirectly monitor the intracellular inositol concentration. This has been done for both the yeast *S. cerevisiae* and *Dictyostelium* [43,110]. In both cases, lithium and VPA increase *ino-1* expression, and this effect is reversed by addition of exogenous *myo*-inositol. In *Dictyostelium*, both lithium and VPA reduce total Ins(1,4,5)P_3 concentrations and impair cell aggregation. Here, combining lithium and VPA at subthreshold concentrations leads to an enhanced effect, suggesting that each drug works within the same signal transduction pathway, but on different components. In rat DRG cells, lithium and VPA both have a common effect on growth cone spreading. This effect is again reversed by the addition of *myo*-inositol, indicative of an inositol-depletion mechanism [43]. These observations are consistent with a common effect of VPA on inositol signaling.

6. Carbamazepine

Carbamazepine is a second anticonvulsant found to be beneficial in the treatment of bipolar disorder and, as for VPA, may be advantageous over lithium in non-classical bipolar conditions such as mixed mood states and rapid cycling conditions [113]. Again, it appears to have teratogenic effects and, like VPA, has been associated with an increased risk of neural tube defects; however, many of its targets appear to be distinct from lithium and VPA [104]. Few studies have been made on its biochemical and cell biological effects. Carbamazepine has no direct or indirect effect on GSK-3 activity[1] and, in contrast to lithium and VPA, has no effect on β-catenin protein stability or expression [43]. Its effects have been examined on caspase-3 activity in a GSK-3 transfected neuroblastoma SH-SY5Y cell line [114]. Here, induction of caspase-3 activity by staurosporine and heat shock is reduced in cells treated with lithium and VPA but not carbamazepine. It is also interesting to note a report suggesting that the recently discovered anticonvulsant mood stabilizer lamotrigine behaves like lithium and VPA in these cells [114]. Although apparently this may suggest a common pathway for three out of four drugs, it is important to consider the multiple pathways, including feedback loops, which regulate caspase activity. The common outcome of decreased caspase-3 activity may be sensitive to inhibition of these multiple, but distinct pathways.

Two reports favor a common interaction of lithium, VPA, and carbamazepine in the regulation of InsP signaling. First, it has been seen that carbamazepine can reduce the elevated free intracellular calcium ion concentrations found in blood platelets and lymphocytes of patients with bipolar disorder [115]. Second, the effect of carbamazepine has been examined on growth cone spreading of rat DRG cells [43]. Here, as seen with lithium and VPA, carbamazepine increases the spreading and reduces the contraction of growth cones. As seen with lithium and VPA, addition of *myo*-inositol to the medium reverses the effect and returns growth cone spread areas to that seen in untreated controls.

7. PO and lithium resistance

Dictyostelium is a haploid organism and can be manipulated using the full range of molecular genetic tools [116]. Insertional mutagenesis has been used to raise a collection of mutants with reduced sensitivity to lithium [117]. These mutants appear to fall into two classes: one affecting the GSK-3-mediated signal transduction pathway, the other reversing the effect of lithium on InsP signaling. The mutant *lisA* can aggregate in the presence of high concentrations of lithium (>10 mM) and has an elevated Ins(1,4,5)P_3 concentration [117]. The increase in Ins(1,4,5)P_3 arises through up-regulation of the activity of the enzyme multiple inositol polyphosphate phosphatase (MInsPP). This enzyme is present in *Dictyostelium* and mammals and generates Ins(1,4,5)P_3 from InsP_6 [118]. InsP_6 is very abundant in the cell and may act as a cellular store of inositol and phosphate. Increasing MInsPP activity could therefore lead to lithium resistance by increasing the cellular pool of inositol (Fig. 1).

The gene disrupted in the *lisA* mutant has been cloned and encodes a PO. This enzyme has been characterized by its ability to cleave peptides of less than 3 kDa at a proline residue; such peptides include peptide hormones and neuropeptides [119]. It is unclear, however, whether these biopeptides are physiologically relevant substrates as PO activity appears to be cytosolic, with only small extracellular amounts. However, PO activity is important for brain function as its inhibitors enhance memory [120–123]; lithium has the opposite effect on memory [124]. Both lithium resistance and elevated Ins(1,4,5)P_3 can be induced in wild-type *Dictyostelium* cells by treatment with PO inhibitors [117]. The inverse relationship between PO activity and Ins(1,4,5)P_3 concentration is also present in the astroglioma cell line U343 [125]. Changes of PO activity have been associated previously with mood disorders as well as a small number of other mental illnesses. Maes *et al.* [126,127] showed that patients with unipolar

[1] Ryves WJ and Harwood AJ, unpublished data.

mood disorder have lowered PO activity and those with bipolar mood disorder have higher PO activity.

Although isolated for lithium resistance, *lisA* has been tested for its effect on sensitivity to VPA. Consistent with an effect on InsP signaling, the *lisA* mutant is cross-resistant to VPA treatment. An effect on GSK-3 can be eliminated, and a double mutant combining *gskA* and PO mutations shows no genetic interaction. To test whether these genetic and biochemical observations hold true for the mammalian neurons, rat DRG cells treated with lithium, VPA, or carbamazepine were also treated with PO inhibitors [43]. In all three cases, PO inhibition reversed the effect of drug treatment. These observations again argue for a common effect of these three mood-stabilizing drugs on InsP signaling. The interaction between the InsP signaling pathway and PO raises the intriguing possibility that changes in InsP metabolism may, in part, underlie the cellular basis for mood disorders.

Consistent with a hypothesis that high PO activity associated with bipolar disorder leads to decreased InsP metabolism—the opposite effect to PO inhibition—inositol has indeed been found to be lower in bipolar patients. Shimon *et al*. [128] and Davanzo *et al*. [129] report reduced postmortem frontal cortex inositol levels in bipolar patients [130]. Inositol levels in lymphocytes derived from cell lines from bipolar patients were also significantly lower than those of cell lines from controls [131]. Banks *et al*. [132] reported a reduced inositol incorporation into membrane phosphoinositides of lymphoblastoid cell lines derived from bipolar patients, and reduced levels of phosphatidylinositol bisphosphate are seen in platelet membranes from bipolar patients [133]. The decreased inositol concentration is accompanied by an approximately 50% reduction of IMPase activity in lymphocytes from cell lines derived from bipolar patients [134,135] and a 66% decrease in mRNA of the IMPase gene, *IMPA1*, in fresh lymphocytes derived from drug-free bipolar patients [136]. These results, however, present a paradox as lithium and the other mood stabilizers would be expected to further lower inositol levels and hence be detrimental to treatment. In contrast, lithium, VPA, and carbamazepine would be expected to be beneficial in situations where PO activity is reduced, such as in patients with unipolar depression.

8. Inositol uptake and mood stabilizers

A further twist to the inositol-depletion hypothesis is that lithium, VPA, and carbamazepine inhibit inositol uptake. Some cells such as isolated oocytes during embryonic development or developing *Dictyostelium* may experience low or no environmental inositol. However, for most cells, inositol is readily available—we ingest 1 g of inositol per day in our diet. The cerebrospinal fluid inositol concentration is between 400 and 600 μM, 10- to 20-fold higher than the K_m of the sodium *myo*-inositol transporter (SMIT) [137] (Fig. 1). Given these figures, cells within the brain ought to be protected from inositol depletion. Among brain cells, the astrocytes express particularly high levels of SMIT. Lithium, VPA, and carbamazepine treatment of astrocyte cultures reduces both SMIT activity and mRNA concentrations [138]. This effect accumulates slowly with a time course remarkably close to the therapeutic time course. It is also dependent upon *myo*-inositol concentration, and curiously at low inositol concentrations (25 μM) mood stabilizers increase inositol uptake [139]. The mechanism by which these drugs affect SMIT is unclear. Effects on SMIT activity could also explain the unexpected result that inositol itself may act as a mood stabilizer as high external concentrations of inositol may down-regulate inositol uptake [140,141].

9. Conclusions

Lithium is an efficacious and effective drug used to treat bipolar disorder, and is also beneficial against other forms of mood disorders. It is recognized that it has a number of limitations. At the cell and molecular level, it is clear from this discussion that it has a wide range of protein targets and impinges on a broad spectrum of cell processes, including metabolism, gene expression, cell division, and death. It seems inconceivable that all of these processes are therapeutic targets of lithium in the treatment of mood disorders. Rather, it is more likely that many contribute to side-effects and, in fact, limit its use. There is, therefore, a strong case for the development of new mood-stabilizing drugs. The rational for the development of new drugs relies on a good knowledge of potential molecular targets and, hence, a clear understanding of cell and molecular mechanisms involved in the origins and progression of the illness. This is currently lacking for all mood disorders. It is hoped, however, that investigating the mechanisms of action of the current treatments will illuminate the underlying molecular mechanisms.

The logic in seeking common effects of lithium and the other mood-stabilizing drugs is that they represent the relevant pathway targeted in mood disorders, whereas unrelated effects would be specific for a single drug or a subset of drugs. Considering the conservation of signal transduction pathways between different cellular processes and throughout evolution, this approach has the advantage that initial studies can be carried out in cell culture and even with genetically tractable microorganisms such as yeast and *Dictyostelium*, before progressing to animal behavior and clinical studies. A disadvantage is that given the complexity of bipolar disorder there may not actually be a single molecular target. Treatment may, in fact, result from the balance of inhibition of a number of pathways. Indeed, while some of the patients benefit from the combination of lithium and VPA or carbamazepine, other respond well to either lithium or VPA alone.

Considering all of the evidence, it is clear that at present no definitive conclusion can be drawn as to the identity of a

single common target in the treatment of mood disorders. Recent observations on the pharmacology of VPA and carbamazepine, the properties of PO mutants, and effects on SMIT activity give new perspectives and strengthen the long-standing proposition that lithium treatment acts through an inositol-depletion mechanism. There remain difficulties, however, in relating changes in inositol metabolism to clinical observations of bipolar patients. On the other hand, recent observations on changes in brain structure could relate to inappropriate GSK-3 activity, although at present there is no definitive evidence that this is the case for lithium or the other common mood stabilizers. What is certain is that the search for a common target will continue, and further studies examining other drugs with mood-stabilizing properties will either strengthen the case for InsP signaling, swing the argument back in favor of GSK-3, or reveal a new, unexpected alternative common target.

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