PtdIns(3,4,5)P_3 and inositol depletion as a cellular target of mood stabilizers

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Abstract

Lithium (Li^+) is the mood stabilizer most frequently used in the treatment of bipolar mood disorder; however, its therapeutic mechanism is unknown. In the 1980s, Berridge and colleagues proposed that Li^+ treatment acts via inhibition of IMPase (inositol monophosphatase) to deplete the cellular concentration of *myo*-inositol. Inositol depletion is also seen with the alternative mood stabilizers VPA (valproic acid) and CBZ (carbamazepine), suggesting a common therapeutic action. All three drugs cause changes in neuronal cell morphology and cell chemotaxis; however, it is unclear how reduced cellular inositol modulates these changes in cell behaviour. It is often assumed that reduced inositol suppresses Ins(1,4,5)P_3, a major intracellular signal molecule, but there are other important phosphoinostide-based signal molecules in the cell. In the present paper, we discuss evidence that Li^+ has a substantial effect on PtdIns(3,4,5)P_3, an important signal molecule within the nervous system. As seen for Ins(1,4,5)P_3 signalling, suppression of PtdIns(3,4,5)P_3 signalling also occurs via an inositol-depletion mechanism. This has implications for the cellular mechanisms controlling phosphoinositide signalling, and offers insight into the genetics underlying risk of bipolar mood disorder.

Introduction

Bipolar mood disorder is a chronic psychiatric disorder characterized by recurrent episodes of mania and depression. It has a high lifetime prevalence (>1 %) and presents a major socioeconomic burden. Although primarily triggered by life events, there is a high, but complex, genetic component to risk of onset. Despite potential leads offered by current genetic studies, we still lack a clear understanding of the cellular and molecular origins of the disorder.

In many patients, the disorder can be controlled by the use of mood stabilizers, particularly lithium (Li^+). The mood-stabilizing properties of Li^+ were noted in the 19th Century, and, following the pioneering work of John Cade in the late 1940s [1], it has become the most common and cost-effective treatment for bipolar mood disorder. There are, however, other drugs, including VPA (valproic acid), CBZ (carbamazepine) and lamotrigine [2,3], which, although originally selected for their anti-epileptic properties, can also be used as mood stabilizers. Investigating the mechanisms by which these drugs exert their effect will provide insights into the origins of the disorder and suggest improvements to drug therapy.

Key words: bipolar disorder, chemotaxis, *Dictyostelium*, inositol depletion, neuronal cell biology, phosphatidylinositol 3,4,5-trisphosphate, phosphatidylinositol 4,5-bisphosphate.

Abbreviations used: CBZ, carbamazepine; DRG, dorsal root ganglion; F-actin, filamentous actin; G6P, glucose 6-phosphate; GSK3, glycogen synthase kinase 3; IMPase, inositol monophosphatase; IPP, inositol polyphosphate 1-phosphatase; PGM, phosphoglucomutase; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B; PLC, phospholipase C; PO, prolyl oligopeptidase; PTEN, phosphatase and tensin homologue deleted on chromosome 10; VPA, valproic acid.

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Biochemical targets of Li^+

Biochemical studies have identified three protein families that are inhibited directly by Li^+ [4]. These are IMPase (inositol monophosphatase) (EC 3.1.3.25) and related members of the phosphoric monoester hydrolase subfamily, GSK3 (glycogen synthase kinase 3) (EC 2.7.11.26) and PGM (phosphoglucomutase) (EC 5.4.2.2). Each is inhibited by Li^+ with a K_i in the range 0.5–2.0 mM, and hence IC_{50} alone is insufficient to distinguish between the different targets in patients, pre-clinical models or *in vitro* cell systems. We have taken the alternative approach of looking for common effects of the different mood-stabilizer drugs.

We have established that Li^+, VPA and CBZ all have an effect on the morphology of neuronal growth cones in a similar way [5]. Growth cones form at the end of developing axons and dendrites and drive the migration of these projections to their target regions, where they differentiate to form the branches of a synaptic tree. Treatment with Li^+, VPA and CBZ, but not lamotrigine, results in a substantial increase in growth cone spread area [6,7]. In each case, this is accompanied by elevated actin polymerization and an increase in the number of F-actin (filamentous actin)-rich filopodia that project from the growth cone surface [8]. This indicates a common cellular target, which alters actin dynamics and growth cone behaviour. In a link to synaptogenesis, Li^+ and VPA increase the synapse number in mature cultured hippocampal neurons [9].

Whereas the common effect of these drugs causes changes in growth cone morphology and the F-actin cytoskeleton, Li^+ has a second effect on neuronal microtubule dynamics not seen with VPA and CBZ [5]. Microtubules form stable

structural elements within the main part of the axon, but in the growth cone, they exhibit dynamic behaviour [10]. In Li^+-treated neurons, microtubules extend deep into the spread growth cones and take on a distinctive curled morphology [11]. As the growth cone migrates, these altered microtubule dynamics give rise to microtubule-enriched branching along the axon. Similar effects are seen following treatment with the specific GSK3 inhibitors [12] or stimulation with the extracellular ligand Wnt, which leads to inhibition of GSK3 function [13].

A number of further biochemical observations argue against GSK3 as a common mood-stabilizer target. First, despite an initial report to the contrary for VPA, neither it nor CBZ inhibits GSK3 *in vitro* [14]. Secondly, using tau phosphorylation as an *in vivo* monitor of GSK3 activity, neither VPA nor CBZ inhibits GSK3 in rat DRG (dorsal root ganglia) or cultured neocortical cells [14]. Finally, whereas Li^+ inhibition of GSK3 stimulates the Wnt signalling pathway, this does not occur following VPA or CBZ treatment [6,8,14]. These observations argue for a GSK3-mediated effect on microtubule dynamics, but against GSK3 as the common target of other mood stabilizers.

Mood stabilizers and inositol depletion

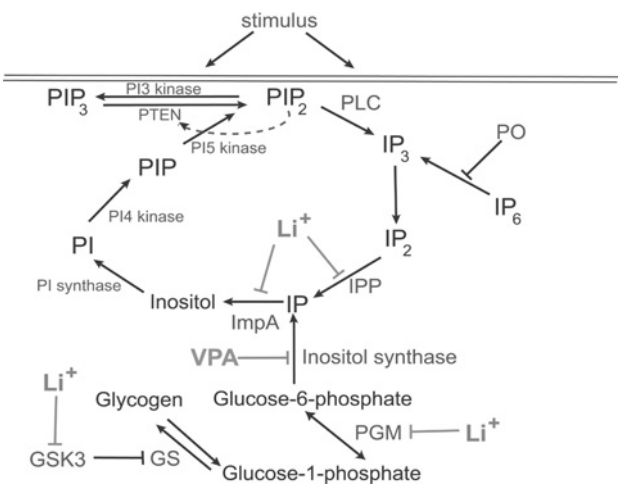
Li^+ inhibition of IMPase and the related IPP (inositol polyphosphate 1-phosphatase) enzyme suppresses the synthesis of *myo*-inositol. In addition, VPA inhibits inositol synthase activity blocking the conversion of G6P (glucose 6-phosphate) to inositol monophosphate, the IMPase substrate [15]. Both drugs lower the cellular concentration of Ins(1,4,5)P_3, indicative of decreased inositol phosphate-based signalling [5,6,16]. As both GSK3 and PGM are involved in the formation of G6P within the cell, it is possible that their inhibition may also affect inositol synthesis, although currently it has not been demonstrated whether inhibition of these enzymes limits the availability of G6P (Figure 1).

Consistent with an inositol-depletion mechanism, the effects of Li^+, VPA and CBZ on DRG neuronal growth cones are reversed by the addition of *myo*-inositol [5,7,16]. Similarly, Li^+ induction of synapse formation is prevented by the addition of inositol [9]. Inhibition of the enzyme PO (prolyl oligopeptidase) elevates the inositol phosphate signalling [17,18]. As seen for addition of inositol, PO inhibitors reverse the effect of Li^+, VPA and CBZ [5,16,19]. These observations indicate that inositol phosphate signalling is a common target of Li^+, VPA and CBZ.

Li^+ and *Dictyostelium* chemotaxis

How does inositol depletion cause changes in cell morphology and behaviour? A powerful means to investigate this question is offered by the social amoeba *Dictyostelium* [20]. Following removal of nutrients, *Dictyostelium* amoebae enter a developmental programme to form a fruiting body, comprising spore and stalk cells. In order to become multicellular, individual cells must aggregate together into a multicellular mass of 10^5 cells. This process requires cell

Figure 1 | Inositol phosphate biosynthesis and signalling
Inositol is recycled from Ins(1,4,5)P_3 (IP_3) by IPP and ImpA. It is also synthesized from G6P via inositol synthase to inositol monophosphate (IP). Glucose is released from glycogen in the form of glucose 1-phosphate, which is then converted into G6P by PGM. Glucose is incorporated into glycogen, via glucose 1-phosphate and UDP-glucose intermediates, by glycogen synthase (GS), which in turn is inhibited by GSK3. Inositol is incorporated into PtdIns(4,5)P_2 (PIP_2) by phosphatidylinositol synthase (PI synthase) and phosphoinositide 4- and 5-kinases (PI4 kinase and PI5 kinase respectively). Upon cell stimulation PtdIns(4,5)P_2 is either converted into PtdIns(3,4,5)P_3 (PIP_3) via activation of PI3K (PI3 kinase) or is hydrolysed to release Ins(1,4,5)P_3. PtdIns(3,4,5)P_3 is dephosphorylated to PtdIns(4,5)P_2 by PTEN, which requires PtdIns(4,5)P_2 to associate with the plasma membrane. PO regulates Ins(1,4,5)P_3 by a mechanism that involves degradation of InsP_6 (IP_6). Li^+ inhibits ImpA, IPP, PGM and GSK3, whereas VPA inhibits inositol synthase. All of these targets have the potential to reduce inositol.

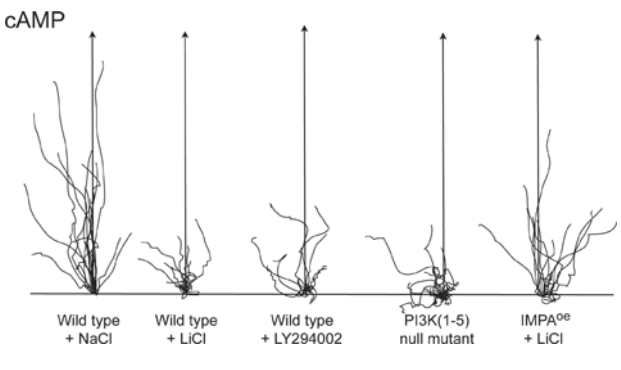


chemotaxis, which is co-ordinated by pulses of extracellular cAMP. In contrast with other cellular systems, the effect of Li^+ on the two major Li^+-sensitive signal pathways, inositol phosphate signalling and GSK3, are separated into different developmental stages [17]. GSK3 inhibition results in mis-patterning of cell differentiation during multicellular stages of development, whereas inositol depletion has significant effects on chemotaxis [17,21].

Li^+ has a specific effect on *Dictyostelium* chemotaxis, causing a decrease in cell speed and an increase in cell turning, but only a slight reduction in the accuracy of cells moving up a chemotactic gradient of cAMP [22]. In contrast with Li^+ treatment, ablation of either the PLC (phospholipase C) gene, which generates Ins(1,4,5)P_3 in response to cAMP, or the Ins(1,4,5)P_3 receptor gene, through which Ins(1,4,5)P_3 causes an increase in intracellular calcium, has no effect on chemotaxis [23,24]. This indicates that Ins(1,4,5)P_3 signalling is not essential for cell chemotaxis and that Li^+ targets a different inositol phosphate. The effects of Li^+, however, closely match the effects of loss of PtdIns(3,4,5)P_3, either using the PI3K (phosphoinositide 3-kinase) inhibitor LY294002 or knockout of the five *Dictyostelium* PI3K genes (Figure 2) [22].

Figure 2 | Analysis of *Dictyostelium* chemotaxis

The tracks of 20 representative cells migrating towards a cAMP source. Wild-type cells treated with Li^+ or the PI3K inhibitor LY294002, or a mutant lacking all five *Dictyostelium* PI3K genes move towards the cAMP source, but move with half the speed and turn more than control (NaCl-treated) cells. Increased expression of *ImpA* reverses this effect. Adapted from [22] with permission.



Indeed, Li^+ treatment reduces the amount of PtdIns(3,4,5)P_3 generated in response to cAMP stimulation to 40% of control cells and suppresses activation of the PtdIns(3,4,5)P_3 effectors, CRAC (cytosolic regulator of adenylate cyclase) and PKB (protein kinase B). Both of these proteins translocate to the plasma membrane via their PtdIns(3,4,5)P_3-binding PH (pleckstrin homology) domains, where PKB is phosphorylated by PDK1 (phosphoinositide-dependent kinase 1) [25]. Li^+ causes a substantial reduction in cAMP-stimulated protein translocation and PKB phosphorylation [22].

It is not possible to directly reverse the effects of Li^+ by addition of inositol to developing *Dictyostelium* cells, as it is sensed as a food source and blocks progress through development. Instead, inositol depletion can be reversed by overexpression of the *Dictyostelium* IMPase gene, *ImpA*. This is possible because Li^+ is an uncompetitive inhibitor of IMPase, and so is alleviated by increasing enzyme not the substrate concentration. Consistent with the biochemistry, increasing expression of ImpA in *Dictyostelium* reduces the effects of Li^+ on chemotaxis and PtdIns(3,4,5)P_3 synthesis [22]. Finally, the effects of Li^+ on PtdIns(3,4,5)P_3 synthesis is not restricted to *Dictyostelium* chemotaxis, and is also observed in the human myeloid cell line HL60 [22], and seen following Li^+ treatment of G93A mice; a model for the study of the neurodegenerative disease ALS (amyotrophic lateral sclerosis) [26].

PtdIns(3,4,5)P_3 signalling plays a variety of roles in the nervous system, including axonal guidance, synaptogenesis and synaptic transmission [27–31]. Mice with elevated PtdIns(3,4,5)P_3 due to loss of the PtdIns(3,4,5)P_3 phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10) suffer seizures [32]. In human patients, PTEN mutations are associated with defects in neural development such as macrocephaly, mental retardation, cerebellar hypertrophy, ataxia and seizures [33], whereas mutations in the TSC2 (tuberous sclerosis 2) gene, which is inhibited by PKB phosphorylation, are often associated with epilepsy and autism [34].

Li^+ targets a limiting pool of PtdIns(4,5)P_2

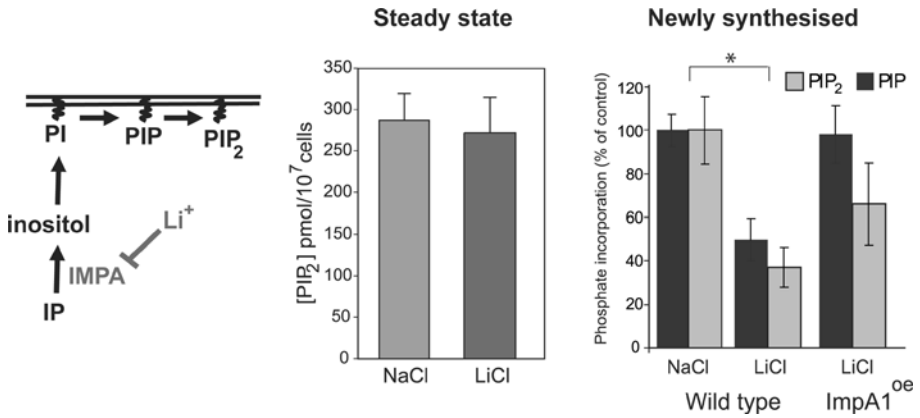
The results described above demonstrate that inositol depletion can affect more than just Ins(1,4,5)P_3 within the cell, and, in *Dictyostelium*, its reduction of PtdIns(3,4,5)P_3 causes a significant change in cell signalling. PtdIns(4,5)P_2 is the common substrate from which both signal molecules are synthesized. This occurs via phosphorylation of phosphatidylinositol by phosphoinositide 4- and 5-kinases. In turn, phosphatidylinositol synthesis requires *myo*-inositol, and so reduced inositol should lead to lower PtdIns(4,5)P_2, reducing the generation of both PtdIns(3,4,5)P_3 and Ins(1,4,5)P_3 following cell stimulation. Implicit in this hypothesis is the notion that the availability of inositol limits the amount of PtdIns(4,5)P_2 substrate available for PI3K and PLC. Consistent with this view, increasing inositol synthesis by overexpressing *ImpA* in *Dictyostelium* cells leads to elevated PtdIns(3,4,5)P_3 synthesis upon cell stimulation in the absence of Li^+ [22] (Figure 1).

Given the evidence in support of Li^+ action via inositol depletion, it is unexpected that the steady-state concentration of PtdIns(4,5)P_2 in *Dictyostelium* is unaltered by Li^+ treatment (Figure 3). Furthermore, in terms of absolute mass, there is 50- and 200-fold excess of PtdIns(4,5)P_2 in the cell over the peak synthesis of Ins(1,4,5)P_3 and PtdIns(3,4,5)P_3. At face value, this argues against inositol depletion having any effect on PtdIns(4,5)P_2 and subsequent signalling processes. This paradox can be resolved if only a small, rapidly turned over and hence Li^+-sensitive pool of PtdIns(4,5)P_2 is utilized for the generation of phosphoinositide-based signal molecules, whereas the majority of PtdIns(4,5)P_2 turns over more slowly and is involved in other processes, such as anchoring the actin cytoskeleton to the plasma membrane [35].

A number of pieces of evidence point to this being the case. First, if the rate of synthesis of PtdIns*P* and PtdIns(4,5)P_2 is measured rather than steady-state concentration, there is a 60% decrease in the amount of PtdIns(4,5)P_2 synthesized over a short time period in Li^+-treated cells. This matches the level of decrease observed for PtdIns(3,4,5)P_3 following Li^+ treatment, and, as is the case for PtdIns(3,4,5)P_3, PtdIns(4,5)P_2 can also be reversed by elevated expression of *ImpA* (Figure 3) [22]. VPA treatment also decreases both PtdIns(3,4,5)P_3 and PtdIns(4,5)P_2 synthesis, although it is not yet clear whether this acts via inositol depletion or another mechanism [31]. Secondly, in *Dictyostelium*, membrane association of PTEN requires PtdIns(4,5)P_2. Again, this is sensitive to Li^+ treatment, indicating loss of PtdIns(4,5)P_2 from the plasma membrane [22]. Finally, electron microscopy studies have shown that there is differential turnover of PtdIns(4,5)P_2 populations on the plasma membrane [36], and diffusion studies in cultured mammalian cells indicate that only a small fraction may be freely available for cell signalling, with the majority of PtdIns(4,5)P_2 bound to cytoskeletal and membrane proteins [37]. These observations may explain why it is difficult to measure altered inositol phosphate signalling

Figure 3 | Li^+ reduces PtdIns*P* synthesis

The total mass of PtdIns(4,5)P_2 (PIP_2) in *Dictyostelium* cells treated with Li^+ is not reduced compared with control (NaCl-treated) cells. However, new synthesis of both PtdIns*P* (PIP) and PtdIns(4,5)P_2 is significantly reduced ($^*P < 0.01$, paired Student's *t* test) following Li^+ treatment. This decrease is reversed by increased expression of *ImpA*. PI, PtdIns. Adapted from [22] with permission.



in patients, as the majority of PtdIns(4,5)P_2, and its precursors may not be relevant to the actual pool targeted by Li^+.

Inositol phosphate signalling and bipolar mood disorder

The observation that elevated inositol synthesis can lead directly to elevated cell signalling offers an attractive hypothesis for a molecular basis for bipolar disorder. At least two genes encoding components of the biosynthetic pathway leading to PtdIns(4,5)P_2 synthesis have been associated with bipolar disorder. Mammals, including humans, possess two IMPase genes [38]. Although *IMPA1* expression is decreased in lymphocytes of bipolar disorder patients, it is not altered in post-mortem brains [39,40]. In contrast, expression of *IMPA2* is elevated in post-mortem brains [41]. This corresponds to the presence of a specific set of single nucleotide polymorphisms in the gene promoter, which appear to lead to elevated expression [41–43]. In addition, a rare polymorphism in the phosphoinositide 5-kinase (*PI5K5A*) gene promoter has been associated with bipolar disorder and schizophrenia [44]. It therefore remains a real possibility that altered regulation of PtdIns(4,5)P_2 synthesis may lead to aberrant signalling that can be reversed by Li^+ and other mood-stabilizer treatment.

Concluding remarks

Recent reports suggest that Li^+, VPA and CBZ have a common effect via inositol depletion. Although the large majority of studies have focused on suppression of Ins(1,4,5)P_3 signalling as primary effect of Li^+ treatment, PtdIns(3,4,5)P_3 is also reduced in both *Dictyostelium* and human HL60 cells. In *Dictyostelium*, Li^+ has a measurable effect on PtdIns(3,4,5)P_3 biochemistry that matches its effect on cell behaviour. Measurement of the total steady-state concentration of PtdIns(4,5)P_2 is not a good measure of Li^+ effect, and this may account for some of difficulty in observing altered PtdIns(4,5)P_2 in clinical studies. However, Li^+ decreases the rate of PtdIns(4,5)P_2 synthesis, effectively changing the flux of phosphoinositides within the cell and leading to suppression of synthesis of both Ins(1,4,5)P_3 and PtdIns(3,4,5)P_3. Finally, altered rates of PtdIns(4,5)P_2 synthesis offer a mechanism to describe the molecular basis of the bipolar disorder in some patients, although further analysis is required to determine whether this occurs in the clinical context.

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