Cellular consequences of inositol depletion

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Abstract
The inositol-depletion hypothesis was suggested to explain the therapeutic mechanism of mood-stabilizing drugs. Focus was previously on the phosphatidylinositol signalling pathway and on the regulatory roles of \(\text{Ins}(3,4,5)P_3\) and DAG (diacylglycerol). Recent findings indicate that inositol and inositol-containing molecules, including phosphoinositides and inositol phosphates, have signalling and regulatory roles in many cellular processes. This suggests that depleting inositol may lead to perturbation of a wide range of cellular functions, at least some of which may be associated with bipolar disorder.

Introduction
Over 20 years ago, the inositol-depletion hypothesis was put forward by Berridge et al. [1,2] in an attempt to explain the therapeutic mechanism of lithium (Li\(^+\)), a drug that has been used for the treatment of bipolar disorder for over 60 years despite the lack of understanding of the mechanism underlying its therapeutic effects. Interestingly, other drugs that have been, and continue to be, used effectively for the treatment of this disorder, such as VPA (valproic acid) and carbamazepine, also lead to inositol depletion, albeit by different mechanisms. Although some have doubted its validity, the inositol-depletion hypothesis has continued to generate much enthusiasm, primarily focused on the phosphatidylinositol signalling pathway and the regulatory roles of \(\text{Ins}(3,4,5)P_3\) and DAG (diacylglycerol). Recent research has identified numerous inositol-containing compounds involved in signalling pathways that are linked to neuronal function. Thus decreased inositol levels caused by anti-bipolar drugs may have far more consequences than altering \(\text{Ins}(3,4,5)P_3\) and DAG, and may explain the therapeutic effects by altering other signalling pathways affected by phosphoinositides. The present review discusses the consequences of inositol depletion beyond the effects of \(\text{Ins}(3,4,5)P_3\) and DAG.

The connection between inositol depletion and bipolar disorder
The involvement of myo-inositol in bipolar disorder has been well documented. Several studies have implicated abnormalities in nerve cell myo-inositol levels and/or phosphoinositide-cycle regulation in the pathophysiology and/or treatment of several psychiatric disorders, including bipolar disorder [3]. Perturbation of intracellular inositol regulation has also been linked to Alzheimer’s disease [4], diabetes mellitus [5] and multiple sclerosis [6]. Shaldubina et al. [7] observed that an inositol-deficient diet reduced the severity of affective disorder in ten out of 15 rapid-cycling or drug-resistant bipolar patients. Decreased inositol levels in the frontal cortex of post-mortem brains of bipolar patients have been reported [8]. This emphasizes the correlation between altered inositol levels and this disorder.

Sources and levels of inositol in bipolar disorder
Cells normally derive inositol from three sources: (i) \textit{de novo} biosynthesis from glucose 6-phosphate by MIPS (1-D-myoinositol-phosphate synthase) [9]; (ii) dephosphorylation of inositol phosphates derived from the breakdown of inositol-containing membrane phospholipids [10]; or (iii) uptake from the extracellular fluid via specialized \textit{myo}-inositol transporters [11]. The concentration of \textit{myo}-inositol in the cerebrospinal fluid is in the range 100–500 \(\mu\)M, but it reaches 10 mM or more in brain cells [12]. This is largely dependent on the activity of the two \textit{myo}-inositol transporters, SMIT (\(\text{Na}^+-\text{myo}-\text{inositol transporter}\)) and HMIT (\(\text{H}^+-\text{myo}-\text{inositol transporter}\)), responsible for the uptake of inositol from the extracellular fluid in the brain. The elevated level of inositol in neurons probably reflects the increased demand for the continuous synthesis and turnover of membrane phospholipids needed for neuronal plasticity and increased synapse formation in these cells.

Regulation of inositol biosynthesis
Both Li\(^+\) and VPA affect \textit{de novo} biosynthesis of inositol. Regulation of inositol biosynthesis in human cells is not well understood. In contrast, regulation of inositol biosynthesis has been extensively studied in the yeast \textit{Saccharomyces cerevisiae} [13,14]. Two enzymes are required for \textit{de novo} inositol biosynthesis, MIPS and IMPase (inositol monophosphatase). MIPS, coded for by the structural gene \textit{INO1}, catalyses the conversion of glucose 6-phosphate into MIP (\textit{myo}-inositol 3-phosphate) [9]. The second enzyme, IMPase, coded for by the genes \textit{INM1} and \textit{INM2} (IMPA1 and IMPA2 in humans), dephosphorylates MIP to \textit{myo}-inositol [15]. \textit{INO1} is the most highly regulated of the genes required for
phospholipid biosynthesis [13,14]. In the presence of inositol, expression of INO1 is repressed by the negative regulator Op1p [14]. Inositol also regulates expression of INM1 [16]. Expression of INO1 is derepressed by a heterodimer formed by the transcriptional activators Ino2p and Ino4p, which bind to UASINO (inositol upstream activator sequence) in the absence of inositol. Conservation of function from yeast to humans has been demonstrated for the INO1-encoded enzyme MIPS [17]. As discussed below, both enzymes of de novo synthesis are affected by anti-bipolar drugs.

**Inositol depletion and anti-bipolar drugs**

Three major drugs used for the treatment of bipolar disorder cause inositol depletion, although by different mechanisms. Li+ leads to inositol depletion and the dampening of the phosphoinositide cycle by inhibiting inositol monophosphatase and inositol bisphosphate phosphatase [18,19]. VPA, a branched-chain carboxylic acid, also causes inositol depletion. Shaltiel et al. [20] showed that VPA, like Li+, acutely decreases inositol concentrations in the mouse brain. VPA indirectly inhibits MIPS, both in yeast [21] and in human brain crude homogenates [17,20]. Repression of transcription of INO1 is not the mechanism of inhibition of MIPS, as increased levels of INO1 mRNA were observed in the presence of VPA [22]. In a recent study involving Caenorhabditis elegans, Tokuoka et al. [23] showed that PLC (phospholipase C)-mediated hydrolysis of PtdIns(4,5)P2 to form DAG and Ins(3,4,5)P3 is defective in the presence of VPA. Consistent with this, Ins(3,4,5)P3- and DAG-regulated behaviours are inhibited in the presence of VPA, most likely by inhibiting neuro-regulators such as UNC-13. Carbamazepine causes inositol depletion possibly by inhibiting uptake of inositol. A knockout of the mouse Smul1 gene resulted in a 92% reduction in intracellular inositol in the fetal brain [24]. However, depletion of phosphoinositides was not observed [25]. This raises the question of whether the therapeutic effects of inositol depletion are due to dampening of the phosphoinositide cycle by altering the levels of phosphoinositides and inositol phosphates or to alternative mechanisms involving the inositol-containing molecules.

**Inositol depletion and GSK3 (glycogen synthase kinase 3)**

An alternative to the inositol-depletion hypothesis was suggested by the observation of Klein and Melton [26] that Li+ inhibits GSK3β at a nearly therapeutic concentration (K_i=2 mM). Li+ inhibits GSK3β directly by uncompetitive inhibition and indirectly by increasing the phosphorylation of GSK3β [27]. The effects of VPA, on the other hand, are controversial. Although several groups have reported that VPA inhibits GSK3β in mammalian cells [28], the study of DiDaniel et al. [29] suggested that GSK3 is not involved in the inositol-reversible effect of mood stabilizers including VPA. Interestingly, using the yeast model, Azab et al. [30] showed that GSK3 is required for optimal MIPS activity and de novo inositol biosynthesis, and that loss of GSK3 activity causes inositol depletion [30]. This finding links two targets of anti-bipolar drugs and highlights a new possible mechanism for the regulation of inositol biosynthesis, i.e. that inositol depletion by anti-bipolar drugs may derive from inhibition of GSK3.

**Consequences of inositol depletion**

Since the inositol-depletion hypothesis was first proposed, numerous studies have addressed the role of inositol in the aetiology of bipolar disorder and the therapeutic mechanisms of anti-bipolar drugs. To date, most studies have dealt with inositol depletion and its effects in the context of the phosphoinositide cycle. However, inositol is a key component of important intracellular signalling molecules that affect phospholipid synthesis, the UPR (unfolded protein response) and protein secretion [17,31–33]. Indeed, Jesch et al. [34] reported altered expression of more than 712 genes in response to inositol. Furthermore, inhibition of inositol synthesis leads to changes in expression of hundreds of genes in essential pathways. Thus inositol depletion results in a plethora of consequences possibly due to alteration of signalling pathways.

**Regulatory roles of phosphoinositides and inositol phosphates may be altered by inositol depletion**

Whereas great progress has been made in elucidating the functions of proteins in signalling cascades, less is known about the regulatory roles of phosphoinositides and inositol phosphates. Phosphoinositides are derived from PtdIns by the reversible phosphorylation of the inositol ring at positions 3, 4 and 5. Synthesized in the ER (endoplasmic reticulum), PtdIns is delivered to other membranes within the cell, either by vesicular transport or via cytosolic phosphoinositide-transfer proteins [35]. Seven phosphoinositide species have been identified, each of which has a unique subcellular distribution. Metabolically, the phosphoinositides are strikingly dynamic and undergo acute and reversible phosphorylation by kinases and phosphatases. They also exhibit differential intracellular distribution. In spite of their low abundance in cells, they are indispensable mediators of signalling events in cellular compartments, motility and intracellular membrane trafficking [36,37]. PtdIns(3,4)P2 and PtdIns(3,4,5)P3, for example, activate a wide range of effector proteins by targeting them to specific membrane locations where they activate signal transduction pathways [38,39]. PtdIns3P, PtdIns4P and PtdIns(3,5)P2 are associated predominantly with intracellular membranes, where they are involved in membrane trafficking and vesicular transport.

Inositol phosphates are derived from Ins(3,4,5)P3, the second messenger produced along with DAG by the hydrolysis of the membrane phospholipid PtdIns(4,5)P2 by PLC. Ins(3,4,5)P3 signals the mobilization of calcium from intracellular stores [40]. DAG, along with calcium, activates protein kinase C, which translocates to the plasma membrane, where it activates a variety of proteins, or to the nucleus, where it regulates gene expression. In addition to its role in the phosphatidylinositol signalling pathway, Ins(3,4,5)P3 may be phosphorylated to produce a variety of
metabolism of PtdIns(3,5)P_{6} and Lowe’s syndrome [46]. Mutations affecting diseases such as cancer, myotubular myopathy, neurodegenerative enzymes have been implicated in human disorders and Lowe’s syndrome [46].

Inositol polyphosphates are distinguished by their high energy bonds. In addition, they are able to directly phosphorylate prephosphorylated proteins in an ATP-independent manner, thereby identifying a new post-translational protein modification [45].

Dysfunctions in the regulation of phosphoinositide biosynthesis due to perturbation of phosphoinositide-metabolizing enzymes have been implicated in human diseases such as cancer, myotubular myopathy, neurodegeneration disorders and Lowe’s syndrome [46]. Mutations affecting metabolism of PtdIns(3,5)P_{2} have been associated with neurodegeneration in mice and are responsible for a variant of Charcot–Marie–Tooth disorder (CMT4J) in humans [47]. Pathogenicity of some bacteria, e.g. Shigella, has been attributed to their ability to interfere with phosphatidylinositol metabolism in the host cell, thereby increasing their virulence [48]. Shigella injects a protein with domains similar to the actin-binding protein monomeric actin binding protein (mAbp) into the host cell cytoplasm, which disrupts cytoskeletal attachment of the membrane and facilitates entry to the host cell.

Phosphoinositides may be the most versatile regulatory molecules conserved from yeast to humans [49]. The versatility arises from the inositol headgroups and the pattern of distribution of phosphoinositides among subcellular membranes. Clearly, the variety of roles played by the phosphoinositides and inositol phosphates suggests that depleting inositol may lead to perturbation of a wide range of cellular processes.

**Inositol depletion and mitochondrial dysfunction**

Mitochondrial dysfunction has been implicated in bipolar disorder on the basis of the findings of impaired oxidative phosphorylation, a decrease in intracellular pH and an increased level of lactate in the brains of bipolar patients [50–53]. Neural mitochondria play an important role in apoptosis and in the regulation of intracellular calcium, which is ultimately needed for the release of neurotransmitters. Stork and Renshaw [50] suggested that impaired phospholipid metabolism in bipolar disorder, as is evident from alteration in levels of choline, myo-inositol, inositol monophosphates and phosphomonoesters, is the direct result of energy shortages caused by mitochondrial dysfunction. Consistent with this, a study carried out by Ju and Greenberg [22] indicated that inositol depletion in yeast caused by VPA led to an increase in the level of cardiolipin, a phospholipid synthesized in the mitochondria and needed for optimal mitochondrial function. It is tempting to speculate that the therapeutic role of VPA may derive from improved mitochondrial function as a result of increasing cardiolipin levels. Further evidence for mitochondrial dysfunction in bipolar disorder comes from gene array analysis of 12558 nuclear genes expressed in the hippocampus of healthy individuals, patients with bipolar disorder and patients with schizophrenia [54]. This study identified 43 genes that exhibited decreased expression in bipolar disorder, but not in schizophrenia or in healthy controls. Of these, 18 genes coded for mitochondrial proteins.

**Inositol depletion and the UPR**

The UPR is a highly conserved ER-associated stress response pathway that functions to protect cells from the deleterious accumulation of misfolded proteins [55,56]. Jesch et al. [57] showed that cells grown in inositol-limiting conditions induce the UPR pathway. Several lines of evidence support the involvement of UPR dysfunction in the pathophysiology of bipolar disorder. Hayashi et al. [58] showed that the ER-stress response is impaired in lymphoblastoid cell lines derived from bipolar patients. The study by Konradi et al. [54] indicated that expression of genes coding for proteins of the ubiquitin–proteasome system is greatly decreased in the hippocampus of bipolar patients. One interpretation of these findings is that the therapeutic benefits of inositol depletion induced by anti-bipolar drugs may derive from induction of the UPR and/or proteasome degradation.

**Concluding remarks**

Although it is not yet clear whether the therapeutic benefits of anti-bipolar drugs can be attributed to inositol depletion, decreased inositol is a common outcome of at least three structurally dissimilar drugs: Li^{+}, VPA and carbamazepine. A significant body of research has focused on the effects of inositol depletion on the phosphoinositide cycle and the regulatory functions of Ins(3,4,5)P_{3} and DAG, both of which play important roles in cell signalling. However, as discussed above, inositol is involved in many processes in addition to those pertaining to DAG and Ins(3,4,5)P_{3}. Thus, as can be seen from studies of the myriad inositol-containing molecules that have been discovered, and no doubt will be seen from future studies of inositol compounds awaiting discovery, the consequences of inositol depletion are far greater than initially expected.

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**References**


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