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Pharmacogenetics in model systems: Defining a common mechanism of action for mood stabilisers

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Abstract

Defining the underlying causes of psychiatric disorders has provided an ongoing and intractable problem. The analysis of the genetic basis of manic depression, in particular, has been impeded by the absence of a suitable model system and by the lack of candidate causative genes. One recent approach to overcome these problems has involved identifying those genes which control the sensitivity to anti-manic drugs in a model organism. Characterisation of the role of these genes and their encoded proteins in this model has allowed the analysis of their mammalian homologues to elucidate the therapeutic role of these drugs and the possible aetiology of manic depression. This approach has been used successfully with the cellular slime mould, *Dictyostelium discoideum*. This article introduces the use of model systems for pharmacogenetics research. It describes the identification of prolyl oligopeptidase in *D. discoideum* as a modulator of inositol phosphate signalling, and the subsequent identification of a common mechanism of action of three anti-manic drugs in mammalian neurons. The use of pharmacogenetics in model systems will provide a powerful tool for the ongoing analysis of both the treatment and cause of psychiatric disorders.

Keywords

Dictyostelium discoideum; Lithium; Manic depression; Model systems; Pharmacogenetics; Valproic acid

Abbreviations

cAMP, cyclic adenosine 3',5'-phosphate; CBZ, carbamazepine; DAG, diacylglycerol; DpoA, *Dictyostelium* prolyl oligopeptidase; Gsk3/A, glycogen synthase kinase 3/A; IMPase, inositol monophosphatase; InsP₃, inositol (1,4,5) trisphosphate; PIP₂, phosphatidyl-inositol (4,5) bisphosphate; PLC, phospholipase C; PO, prolyl oligopeptidase; REMI, Restriction Enzyme Mediated Integration; VPA, valproic acid

1. Introduction

'Manic depression' or bipolar disorder occurs with a lifetime prevalence of 1.9% (ten Have et al., 2002). It severely impairs quality of life in most sufferers and carries a 30- to 50-fold increased risk of suicide (Muller-Oerlinghausen, 2001). The substantial socio-economic burden from the disorder, most of which is due to indirect societal costs, has been estimated at £2 billion annually in the UK (Das Gupta and Guest, 2002). Advances in the understanding of bipolar disorder have so far been serendipitous, with current

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psychopharmacology treatments being based on empirical approaches. However, in contrast with other fields of biomedical research, it has proved nearly impossible to test the *in vivo* efficacy of new drugs for bipolar disorder because no suitable animal models exist.

Pharmacogenetics offers a novel approach to aiding research into this condition. The isolation of genes that control the effect of drugs used to treat bipolar disorder can help to elucidate the molecular pathophysiology of the disorder and to identify the mechanisms by which existing drugs function. In order to identify these genes, and in some cases to define how they operate, researchers are starting to use model systems. This article outlines the use of the social amoeba *Dictyostelium discoideum* as a model system for pharmacogenetic analysis and describes the success of this model in elucidating a common mechanism of action for drugs used to treat bipolar disorder.

2. Pharmacogenetics of model systems

How can we identify human genes whose products may either cause a particular inherited disease or are targeted by drugs that effectively treat such diseases? This would be possible if we could change the activity of every human gene and thereby identify which genes cause the disease or alters the effect of therapeutic drugs. The systematic modulation or ablation of every gene in an animal model is currently not feasible (Brown and Nolan, 1998). However, this is possible in lower-order organisms, providing some basic principles are followed.

Pharmacogenetics can be successfully employed in model systems provided the following criteria are fulfilled:

1. The ability to knock out every gene in the organism and to isolate clonal lines of each mutant. Thus, providing loci are non-lethal, every gene in the organism can be analysed for the ability to cause drug resistance or sensitivity.
2. The ability to screen each mutant, enabling the identification of loci causing drug resistance or sensitivity. The drug must therefore cause some phenotypic change in the model system and the screen must be sufficiently large to ensure every gene is examined.
3. The presence of putative signalling cascades or orthologous target genes within the genome of the model system.
4. The ability to analyse the biochemical processes giving rise to resistance, requiring the availability of sufficient quantities of cellular material from each resistant clone for analysis.

Of course, this approach will isolate all genes whose products are affected by a drug and not just those involved in the treatment of a disorder. The additional non-therapeutic targets, which may be involved in side effects of therapy, are unlikely to be shared by other structurally diverse treatments for the same disorder. Hence, it is possible to distinguish between gene targets involved in the therapeutic effects and side effects of a drug by defining genes causing resistance to multiple treatments for the same disorder.

3. *D. discoideum*: a pharmacogenetics model system

D. discoideum has been studied extensively over the last 60 years, predominantly as a model system for cell signalling, differentiation and motility (Maeda et al., 1997). It is now increasingly being used in other research areas. It is a single-celled (Fig. 1A), haploid eukaryote amoeba, containing close to 11,000 genes (around one third that found in humans). At a genetic level it is more closely related to vertebrates than to fungi and plants

(Glockner et al., 2001). Furthermore, it has a large number of genes that are not present in other lower eukaryotes such as fungi and yeast.

D. discoideum is found worldwide in soil, where it survives by consuming micro-organisms (Raper, 1935). The developmental aspect of its lifecycle begins when a single cell runs out of nutrients and releases a signal (cyclic-AMP), causing migration and differentiation of around 10,000 surrounding cells to form a multi-cellular fruiting body (Fig. 1B). This fruiting body, which is approximately 1 mm high and composed of three basic cell types (stalk, spore and basal disk), develops over a 24-h period (Fig. 1B).

There are numerous advantages in using *D. discoideum* as a model system for pharmacogenetics. The single-cell stage of the *D. discoideum* life-cycle allows cell transformation and the subsequent isolation of isogenic mutant lines. Mutagenic screening of the whole genome is readily possible using Restriction Enzyme Mediated Integration (REMI, Kuspa and Loomis, 1992), whereby an antibiotic selection cassette is integrated at frequently occurring restriction enzyme sites around the genome in intact cells (Fig. 2). This cassette can then facilitate the isolation of the flanking DNA, allowing rapid isolation of the ablated gene (Keim et al., 2004). The single-cell stage of growth also allows rapid production of high quantities of cells, in excess of a gram in weight.

In addition to *D. discoideum* providing a genetically malleable model, the multi-cellular stage of its life-cycle also provides an enormous advantage to pharmacogenetic research. The development of a homogeneous population of cells into a differentiated multi-cellular organism requires a variety of cell signalling events common to those found in vertebrates. It is therefore easy to identify changes in these common signalling pathway, caused by drug effects or gene knockouts, by identifying alterations in the morphology of the fruiting body (Figs. 1C–G and 2). Thus, *D. discoideum*, the most primitive multi-cellular eukaryote, provides an excellent system for identifying the functions and targets of therapeutic drugs.

4. Can the effects of mood stabilising drugs be analysed in *D. discoideum*?

In order that *D. discoideum* can be used as a model system for studying the pharmacogenetics of mood disorder drugs, there must be clear phenotypic effects of these drugs on *D. discoideum* growth or development. In addition, signalling pathways affected by these drugs must also be present in this organism, and some knowledge of how these putative pathways function would be advantageous.

The phenotypic effects of lithium on *D. discoideum* development have been well documented (Maeda, 1970; Harwood et al., 1995; Williams et al., 1999). Cells developed in the presence of 7 mM lithium produce a small fruiting body with an increased basal disk and a severely reduced spore head (Fig. 1C,D). At concentrations of 10 mM, fruiting body formation is lost entirely (Fig. 1E). This two-tiered effect suggests that lithium acts on at least two targets within the cell. There is also evidence that these effects are reversible, as cells exposed to lithium can resume normal development if removed from the drug early in development.

The two primary targets of lithium in mammalian cells, glycogen synthase kinase 3 (Gsk3) and inositol phosphate (InsP) signalling, have also been well examined in *D. discoideum* (Fig. 3). The first of these is the serine threonine kinase, glycogen synthase kinase A (GskA), which is orthologous to the mammalian Gsk3 β (Klein and Melton, 1996; Stambolic et al., 1996). Gsk3 β was originally identified through its phosphorylation of glycogen synthase in sugar metabolism (Embi et al., 1980). It has since been recognised as the central component of the wnt signalling pathway, controlling various cytoskeletal proteins and transcription factors, and cell patterning, during development (Dominguez et al., 1995; He et

al., 1995; Klein and Melton, 1996; Stambolic et al., 1996). Harwood et al. (1995) have defined the role of the *D. discoideum* GskA enzyme in a pathway analogous to the wnt signalling pathway. Its activity is controlled by seven trans-membrane cAMP-receptors, analogous to the wnt receptor, frizzled (Louis et al., 1994; Ginsburg and Kimmel, 1997; Plyte et al., 1999; Kim et al., 1999, 2002), and it has been shown to modulate a downstream β -catenin homologue, aardvark (Grimson et al., 2000; Coates et al., 2002). The phenotype of cells treated with 7 mM lithium closely resembles that of the GskA null mutant, owing to an increase in the pre-spore to pre-stalk cell ratio controlled by this enzyme (Fig. 1C,E; Harwood et al., 1995). This signalling pathway is considered a likely target of mood disorders drugs (Hall et al., 2000; Phiel et al., 2001).

The remaining primary target for lithium in the cell is InsP signalling. This complex signalling system (Fig. 3) has been extensively analysed in *D. discoideum* as a model for the mammalian cell signalling (Stephens and Irvine, 1990; Menniti et al., 1993; Van Dijken et al., 1995, 1996; Van Haastert and Van Dijken, 1997). Lithium exerts its effect on InsP signalling as an uncompetitive inhibitor of a family of phosphatases that includes inositol monophosphatase (Leech et al., 1993) and polyphosphatase (York et al., 1995), two enzymes involved in the breakdown and recycling of $\text{Ins}(145)\text{P}_3$ (Fig. 3). This signalling pathway starts with the binding of a signal molecule to a membrane-bound receptor, leading to the transient activation of phospholipase C (PLC) and the generation of both diacylglycerol (DAG) and InsP_3 from phosphatidylinositol (4,5) bisphosphate (PIP_2). DAG functions to bind and activate PKC or is used to produce phosphatidic acid via DAG kinases (for review see Payraastre et al., 2001). The rapid, transient peak of InsP_3 release following activation of PLC triggers calcium release from the endoplasmic reticulum. Termination of this InsP_3 signal occurs by the hydrolysis of this compound to InsP_2 , followed by the multi-step recycling via inositol to PIP_2 .

The first theory describing the action of mood disorder drugs was proposed by Berridge and Irvine (1984). This theory, called the 'inositol depletion theory', proposed that lithium works by 'dampening down' an over-active InsP_3 signalling cascade. This over-active cascade could be caused by a variety of signalling molecules that lead to elevated PLC activity (Fig. 3). The uncompetitive nature of lithium inhibition of both inositol phosphatases is of particular relevance, as the nature of this inhibition would result in increased enzyme inhibition at elevated substrate concentrations (Leech et al., 1993; York et al., 1995). Thus, an over-active signalling system would be inhibited to a greater degree than a normally functioning system. This is, in fact, what is seen with respect to mood stabilisation with lithium, where healthy volunteers do not experience any effects on normally occurring mood fluctuations whilst taking lithium.

Research into the function of InsP signalling has proved extremely difficult owing to the highly complex nature of the family of InsP compounds, the multitude of effector molecules within this family, and the problems involved in separating and quantifying these compounds. It is also clear that the analysis of this signalling system is dependent upon cell type. Some authors have shown that lithium increases InsP_3 levels by reducing its breakdown (Ishima et al., 1993; Hokin and Dixon, 1993; Dixon and Hokin, 1994; Tritsaridis et al., 2001), while others have shown it to reduce InsP_3 levels through inositol depletion (Jenkinson et al., 1993; Batty and Downes, 1994; Lubrich et al., 1997). In either case, the outcome may be to dampen down overactive InsP_3 signalling.

5. Isolation of the *D. discoideum* prolyl oligopeptidase gene

The first step in identifying a common mechanism of action for mood stabilisers began with a screen for lithium-resistant *D. discoideum* mutants. A bank of 30,000 REMI mutants was

screened for resistance to 10 mM lithium during growth and development (Williams et al., 1999). Thirteen mutants showed increased aggregation and development in comparison to wild-type cells. The first of these mutants produced a phenotypically wild-type fruiting body in the presence of 10 mM sodium chloride, but overcame the inhibitory effect of lithium on aggregation. This mutant contained an ablated *D. discoideum* prolyl oligopeptidase (PO) gene, *DpoA* (Williams et al., 1999), and showed no PO activity. To test if lithium may directly inhibit this enzyme, *DpoA* was partially purified and assayed in the presence of lithium. Lithium had no effect on prolyl oligopeptidase activity at up to 20 times the plasma concentrations used in therapeutic treatment of mood disorders (Williams et al., 1999), suggesting this enzyme may confer lithium resistance via an indirect mechanism. The clinical importance of this gene product was recognised when PO levels were found to be elevated in patients with bipolar depression and reduced in patients with unipolar depression (Maes et al., 1994, 1995).

6. Prolyl oligopeptidase

PO (EC 3.4.21.26), previously called prolyl endopeptidase, is a serine endopeptidase and is conserved from bacteria to man (for review see Polgar, 2002). The distinctive seven-bladed β -propeller structure of the enzyme functions to limit access of potential substrates to the active site (Fulop et al., 2000), thereby ensuring only oligopeptides of up to 30 amino acids may be cleaved at the C-terminal bond of a proline residue (Moriyama et al., 1988). Proline is an imino acid and hence its bond to adjacent amino acids is relatively resistant to hydrolysis by peptidases. This often leads to the protection of the prolyl-containing peptide from degradation (Vanhoof et al., 1995). Although the in vivo physiological targets of PO are unclear, in vitro studies have shown these peptides substrates may include a variety of peptide signals and neural hormones (e.g. angiotensin, vasopressin, substance P), all containing proline residues (Cunningham and O'Connor, 1997). The cleavage of target peptides may either degrade an active oligopeptide (e.g. angiotensin II, bradykinin, luteinising hormone releasing hormone) or activate peptides by cleavage of an inactive precursor (e.g. gonadotropin-releasing hormone) (Yamanaka et al., 1999). In view of its potential role in neural hormone degradation or regulation, considerable efforts have been made in defining the substrate specificity and novel inhibitors of PO. There appears to be no sequence homology within the known in vitro targets of the enzyme. Its activity can be controlled by either the phosphorylation of the target peptide to reduce product cleavage (Kaspari et al., 1996) or by the regulation of enzyme activity by an unknown mechanism (Tsukahara et al., 1990; Amin et al., 1999; Kimura et al., 2002).

A single copy of PO has been found in all mammals, although other proline-cleaving enzymes, namely dipeptidyl peptidase IV and prolylcarboxypeptidase, cleave related substrates (Vanhoof et al., 1995). The enzyme is highly conserved at the amino acid level and has similar biochemical activities in different organisms (Williams et al., 1999). The enzyme is found in the cytoplasm of the cell and has no localisation or secretion signals, although it may be associated with the outer cell membrane (Lew et al., 1994; Williams and Harwood, unpublished data). The location of PO provides one of the major problems concerning its role, since its potential neural hormone targets are extra-cellular and thus would have no access to the cytoplasmically located enzyme.

PO levels have been reported to be elevated in patients with bipolar depression and schizophrenia, and decreased in patient with unipolar depression (Maes et al., 1994, 1995). These studies suggested that this enzyme may function in the immune response, often found in these conditions. Maes et al. (1995) then Breen et al. (2004) also showed that the enzyme activity reduced following treatment with mood disorder drugs, raising the possibility that the enzyme may cause the disorder. PO activity has since been shown to vary in patients

with a variety of medical conditions, often showing increased activity with stress and decreased activity with depression (Maes et al., 1998a,b, 1999, 2001). PO levels have also been found to effect learning and memory (Morain et al., 2002), and the enzyme is currently being examined for a role in Alzheimer's disease (Toide et al., 1998). However, it is worthwhile to note that many of these studies have used non-specific substrates, hence some care needs to be taken in defining enzymatic activity. In addition, previously reported results are based upon PO cleaving activity found in blood plasma and it is not clear whether altered blood plasma levels reflect similarly altered neuronal levels.

7. Prolyl oligopeptidase: a modulator of inositol phosphate signalling and mood stabilising drug sensitivity

The recognition that PO is not directly affected by lithium suggested that this enzyme may modulate the targets of lithium in the cell. To define how this enzyme controlled lithium sensitivity, Williams et al. (1999) examined the activity of the two primary targets of lithium in the *DpoA* mutant: the GskA signalling pathway and InP signalling. This mutant showed no change in the GskA signalling pathway and, as such, showed normal distribution of spore and stalk cells in the mature fruiting body (Harwood et al., 1995). However, it was found to have 3- fold higher InsP₃ levels during growth and early development, thus overcoming the reduction in InsP₃ levels caused by lithium-catalysed inositol depletion. This increase did not occur through altered PLC activity, but through elevated multiple inositol polyphosphate phosphatase (MIPP) activity, which controls the breakdown of higher order InsPs (Williams et al., 1999). This suggests, therefore, that PO may regulate the entry of InsP₃ into this signalling cascade from the large stored pools of higher order InP species, InsP₅₋₈ (Fig. 3). The role of these higher order InP species is still undetermined, but they exist at high concentration in the cell, with InsP₆ at concentrations of up to 100 μ M (French et al., 1991; Bunce et al., 1993). Hence the mobilisation of these stores can have a profound effect on signalling. The elevation of InsP₃ levels was specifically dependent upon reduced *DpoA* activity, as this could be reproduced using an inhibitor to PO during the first 5 h of development (Williams et al., 1999).

Thus, in *D. discoideum*, the action of lithium in the reduction of InsP₃ levels is reversed by the removal of PO activity. This is consistent with the 'inositol depletion' theory of manic depression drug action (Berridge and Irvine, 1984; Berridge et al., 1989), but adds a new regulatory element to this process. These results then pose the question, "Do all mood disorder drugs function through a common mechanism of inositol depletion?" To test this, Williams et al. (2002) examined the effect of valproic acid (VPA), on *D. discoideum* development. VPA, a short chain fatty acid (2 propyl pentanoic acid), was originally used as an anti-epileptic treatment and is now a first-line treatment for mood disorders (Kerwin, 1999). VPA-like lithium-inhibited cell aggregation in *D. discoideum*, but caused dissimilar morphological changes in the mature fruiting body (Fig. 1F). Thus, the common effect of lithium and VPA was to retard development, not to cause cell fate changes in the fruiting body. Since the *DpoA* mutant showed resistance to the effect of lithium over this period through elevated InsP₃, Williams et al. (2002) examined the effect of VPA on the *DpoA* mutant. They found that the ablation of the *DpoA* gene conferred resistance to both lithium and VPA during aggregation, implicating the inhibition of InsP signalling as a common mechanism of action of both mood stabilising drugs (Williams et al., 2002).

The success of a model system in pharmacogenomic studies is dependent upon the transfer of information from the model to a suitable mammalian system. In pursuit of this, the information gained using *D. discoideum* was applied to primary rat dorsal root ganglia (DRG) neurons. In this study, neurons were treated with the three most commonly used mood stabilising agents—lithium, VPA and carbamazepine—with the intention of defining

common effects of all three drugs (Williams et al., 2002). In a similar manner to that seen in *D. discoideum*, these structurally diverse drugs were found to have both unique and common effects. Lithium increased axonal branching and caused microtubule extension into the developing front of a primary neuron, the growth cone, as seen previously (Fig. 4; Goold et al., 1999). VPA caused a decrease in branching, but had no effect on microtubules within the growth cone. Carbamazepine had no effect on either of these processes. The drug specificity of these effects suggested they were not involved in the common therapeutic action of these drugs. However, all three mood stabilising agents were found to increase the area or size of the developing growth cone (Williams et al., 2002; Fig. 4). This suggests that the non-common effects of these drugs on neurons may be related to the side effects of each drug, and that the common effect on neuronal growth cone area may reflect their common mood stabilising properties.

The resistance of the *D. discoideum* *DpoA* mutant to both lithium and VPA suggested that changes in the activity of this enzyme may control the efficacy of mood disorder drugs. To test this, DRG neuronal cultures were treated with all three mood stabilisers, in combination with two structurally distinct PO inhibitors (Demuth et al., 1993; Augustyns et al., 1995). The PO inhibitors alone had little effect on growth cone size, but both reversed size increases caused by all three mood stabilisers (Williams et al., 2002). These inhibitors had no effect on the unique actions of each mood stabiliser, e.g. on axonal branching. This indicated that PO activity in primary mammalian neurons controls the effect of mood stabilising drugs and suggests this effect may occur through altered InsP_3 signalling.

It is currently very difficult to measure InsP_3 levels within a cell and it is not possible to examine these levels in a developing growth cone. Many researchers overcome this by using 'inositol rescue', whereby the addition of inositol to a test system should allow cells to replenish inositol depletion by the uptake of exogenous inositol. Thus, if the addition of inositol overcomes an effect, that effect is likely to result from inositol depletion. To test whether the common effect of mood stabilisers occurred through inositol depletion, the experiments described above were repeated in the presence of different levels of *myo*-inositol. The common increase in growth cone area was reversed by the addition of 1 mM *myo*-inositol, suggesting that—as in *Dictyostelium*—these drugs work through the reduction in phosphoinositide signalling. This effect was not visible at higher *myo*-inositol concentrations (Williams, Mudge and Harwood, unpublished data), perhaps because high exogenous inositol concentrations have been shown to reduce *myo*-inositol uptake (van Calker and Belmaker, 2000; Lubrich et al., 2000; Wolfson et al., 2000). These results provide the most compelling support of the inositol depletion theory for the action of mood disorder drugs.

8. Summary

Pharmacogenetics is a growing field of research that can help elucidate the aetiologies of medical disorders and the pharmacological effects of drugs. The use of model systems to examine drug function represents a new area in this field. This approach takes advantage of the tractable nature of model systems, which enables the isolation and characterisation of novel genes involved in drug response. An example of this process is seen in the discovery of the role of PO in mood disorders. Characterisation of this gene, of previously unknown importance, suggests that it functions by modulating inositol signalling. Information gained in this study enabled targeted experiments in primary mammalian neurons and led to the identification of a common mode of action of the three most commonly used mood stabilisers. This work has clearly indicated the useful role of model systems, in particular *D. discoideum*, in pharmacogenetics. Further research in this model is likely to lead to the

discovery of new pharmacological targets and signalling cascades involved in medical disorders.

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Fig. 1.

Dictyostelium discoideum is a single-cell amoeba that develops into a multi-cellular fruiting body. A: In the single-cell stage, *D. discoideum* cells survive by consuming micro-organisms, or in culture conditions by fluid uptake. Here, a field of cells are shown containing the cytoplasmically located prolyl oligopeptidase protein linked to green fluorescent protein (black bar=10 μ m). B: Upon starvation, cells aggregate and develop to form multi-cellular fruiting bodies composed of distinct cell types: stalk, spore and basal disk cells. Lithium functions, C, D: at 7 mM to reduce fruiting body size, and to alter developmental patterning by reducing spore cell and increasing stalk cell production, and E: at 10 mM to block aggregation. F: Elimination of the *D. discoideum* Gsk3 orthologue (GskA) partially phenocopies 7 mM lithium. G: These morphological effects on fruiting body development are dissimilar to that observed with valproate (VPA), whereby fruiting bodies developed on 1 mM VPA are small and show enlarged spore head and reduced stalk (white bar=0.5 mm).

Fig. 2. Restriction Enzyme Mediated Integration (REMI) bank can be used to isolate mutants resistant to therapeutic drugs. A, B: *Dictyostelium discoideum* cells (hatched) are electroporated in the presence of an antibiotic resistance gene and a restriction enzyme. C: Transformants containing the integrated resistance gene are selected in the presence of the antibiotic and individual mutants are grown and differentiate in the presence of the drug. D: Drug-resistant colonies are selected by their ability to overcome phenotypic changes caused by the drug. The integrating cassette is used to identify the ablated gene.

Fig. 3.

The primary targets of lithium in the cell are the glycogen synthase kinase 3/A (Gsk3/A) and inositol trisphosphate (InsP₃) signalling pathways. Lithium inhibits mammalian Gsk3 or the *Dictyostelium discoideum* homologue, GskA, causing changes in the cytoskeleton and in gene transcription. Lithium also inhibits the recycling of inositol phosphates, by inhibiting inositol monophosphatase (IMPase) and inositol polyphosphatase (IPPase). The 'inositol depletion' theory of bipolar disorder treatment proposes that the therapeutic effect of lithium is to reduce inositol levels in the cell, resulting in the attenuation of an over-stimulated InsP₃ signalling pathway. Prolyl oligopeptidase presumably regulates the cleavage of an oligopeptide signal controlling the activity of multiple inositol polyphosphate phosphatase (MIPP), which functions to breakdown higher-order inositol phosphates to InsP₃.

Abbreviations: DAG: diacylglycerol; GS: glycogen synthase; Gsk3/A, glycogen synthase kinase A/3; IMPase; inositol monophosphatase; InsP₅₋₆: inositol pentakisphosphate and hexakisphosphate; InsP₃: inositol (1,3,5) trisphosphate; IPPase: inositol polyphosphatase; PIP₂: phosphatidylinositol bisphosphate; PLC: phospholipase C.

Fig. 4.

Mammalian neuronal growth cones increase in size when treated with mood stabilizers. Rat dorsal root ganglia cells stained for actin (red) and acetylated tubulin (green) after treatment with mood stabilizers. A: In comparison to untreated cells treatment with B: 10 mM lithium and C: 3 mM valproate increases growth cone size. Only lithium treatment caused microtubule extension into the growth cone. Bar=10 μ M.