

FEATURE REVIEW

Emerging experimental therapeutics for bipolar disorder: insights from the molecular and cellular actions of current mood stabilizers

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Bipolar disorder afflicts approximately 1–3% of both men and women, and is coincident with major economic, societal, medical, and interpersonal consequences. Current medications used for its treatment are associated with variable rates of efficacy and often intolerable side effects. While preclinical and clinical knowledge in the neurosciences has expanded at a tremendous rate, recent years have seen no major breakthroughs in the development of novel types of treatment for bipolar disorder. We review here approaches to develop novel treatments specifically for bipolar disorder. Deliberate (ie not by serendipity) treatments may come from one of two general mechanisms: (1) Understanding the mechanism of action of current medications and thereafter designing novel drugs that mimics these mechanism(s); (2) Basing medication development upon the hypothetical or proven underlying pathophysiology of bipolar disorder. In this review, we focus upon the first approach. Molecular and cellular targets of current mood stabilizers include lithium inhibitable enzymes where lithium competes for a magnesium binding site (inositol monophosphatase, inositol polyphosphate 1-phosphatase, glycogen synthase kinase-3 (GSK-3), fructose 1,6-bisphosphatase, bisphosphate nucleotidase, phosphoglucomutase), valproate inhibitable enzymes (succinate semialdehyde dehydrogenase, succinate semialdehyde reductase, histone deacetylase), targets of carbamazepine (sodium channels, adenosine receptors, adenylate cyclase), and signaling pathways regulated by multiple drugs of different classes (phosphoinositol/protein kinase C, cyclic AMP, arachidonic acid, neurotrophic pathways). While the task of developing novel medications for bipolar disorder is truly daunting, we are hopeful that understanding the mechanism of action of current mood stabilizers will ultimately lead clinical trials with more specific medications and thus better treatments those who suffer from this devastating illness. *Molecular Psychiatry* advance online publication, 11 May 2004; doi:10.1038/sj.mp.4001518

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Bipolar disorder is a severe, chronic, and often life-threatening (due to medical comorbidities, suicide, and other associated detrimental behaviors) illness, which affects approximately 1–3% of the population.¹ It is equally prevalent in both men and women, and is often characterized by two seemingly opposite mood states: mania and depression. The manic stages of bipolar disorder are characterized by a hyperaroused state (either euphoric or dysphoric), increases in motor activity, racing thoughts, impaired judgment, decreased sleep, and an apparent decreased need for sleep.¹ The depressive phases of the illness present with similar symptomatology as those seen in major depression, including depressed mood, cognitive changes, psychomotoric changes, and a host of neurovegetative symptoms. Functional impairments

during mood episodes have long been recognized; however, there is increasing evidence of significant interepisode impairment as well. This evidence includes major economic, societal, medical, and interpersonal consequences^{2–7} (see also Evans and Charney⁸ and associated issue). The impairment of the disorder is further complicated by the fact that many medications currently used for its treatment are associated with variable, often not much greater than placebo, rates of efficacy in both the acute and maintenance phases of the illness. Furthermore, of the patients who do respond, medication adherence is many times quite poor due to side effects.⁹ An additional concern among those who do respond and continue taking medication is that relapse is common.¹⁰

Considering this evidence it is not surprising that the World Health Organization has listed bipolar disorder as the 6th leading cause of disability worldwide, and has projected a greater impact in the future.¹¹ In spite of these major economic, societal, and comorbid medical findings, in addition to the

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limited efficacy of most of the drugs available, there have been very few major advances in treatments specifically for bipolar disorder in recent years. In fact, recent additions to the bipolar disorder pharmacopeia are primarily the result of testing brain-penetrant drugs developed and approved for other disorders (generally schizophrenia and epilepsy). While many of these medications have some degree of efficacy in bipolar disorder (see Muzina *et al*¹² and Strakowski *et al*¹³ for review), they are not designed specifically for its treatment. In this review, we discuss the prospect of developing new medications for bipolar disorder based upon the mechanism of action of current medications. In a basic sense, approaches to develop novel therapeutics for a complex disorder like bipolar illness can take one of two forms:

- (1) Understanding the precise biochemical targets (both direct and long-term) of antimanic/mood stabilizers currently in use, and using the knowledge gained to design drugs that are more specific to those biochemical target(s).
- (2) Understand the pathophysiology of the illness, and utilizing that knowledge to design therapeutics that control core bipolar symptomatology and either attenuate or prevent the deleterious systemic effects of the illness.

In this review, we focus upon the first approach; in a companion review¹⁴ within this journal issue, we direct our attention toward approach 2. It is noteworthy that the most common medications used to treat bipolar disorder—lithium, anticonvulsants, and antipsychotics—differ greater in their structure and in their presumed initial molecular/cellular targets. Valproate is an eight-carbon branched fatty acid, lithium is a monovalent cation, carbamazepine has a similar structure to the tricyclic antidepressants, and antipsychotics differ widely in structure. With the exception of antipsychotics, these drugs do not appear to specifically target cell surface receptors,^{15,16} but exert their actions on these intracellular targets leading to direct and indirect effects on signaling pathways. We focus in this review on the intracellular molecular targets of lithium, VPA, and carbamazepine, but it requires pointing out that both typical and atypical antipsychotics have proven efficacy in the treatment of different phases of bipolar disorder and any concentrated effort to develop novel medications will require exploration of the mechanisms for the actions of these medications as well.

It may clearly be of use to design drugs based on the initial molecular targets of current mood stabilizers, with modifications designed to enhance potency, specificity and/or side-effect profile. We discuss the evidence supporting some the effects of current mood stabilizers on initial molecular targets. Additionally, since all current medications take weeks to exert their full effects (implicating changes in gene expression, protein function, and—more generally—plastic changes) targets identified after prolonged treatments

in cell- and animal-based models may be a useful approach towards the development of novel therapeutics. This approach may allow greater ‘downstream’ specificity and provide therapeutics with potentially more potent and rapid actions. Such agents may also have efficacy in patients refractory to existing treatments. That is, if these patients have abnormalities in the cascade leading from the initial target to the long-term adaptation, bypassing the ‘defect’ by one or multiple mechanisms may have considerable utility. As we describe, there is no shortage of targets and the task of determining which ones are most therapeutically relevant is difficult. We attempt to focus on those target proteins and pathways with the most evidence, the greatest relevance to current disease models, or where other medications with the same or similar actions have been developed and could be used for ‘proof of concept’ trials.

Developing novel therapeutics by understanding the direct targets of current antimanic/mood stabilizers

It is unknown with any certainty what the therapeutic mechanism of action is of the available mood stabilizing drugs. In spite of this major shortcoming, the following section describes initial molecular targets modulated by the current mood stabilizers lithium, valproate, and carbamazepine. Lithium’s inhibition of selected enzyme(s) probably results in its mood stabilizing effects. In regard to valproic acid (valproate, VPA) and carbamazepine, it is often assumed that their anticonvulsant target is the same as their mood stabilizing target. However, when other drugs with anticonvulsant properties have been tried in double-blind placebo-controlled studies, the result is often one of no beneficial effect. This leads to the conclusion that general anticonvulsant targets *per se* may not be responsible for antimanic effects or mood stabilization. As we discuss, both VPA and carbamazepine additionally have multiple intracellular targets that could be of therapeutic relevance. By determining which target(s) are responsible for mood stabilization, we can begin to design drugs for that target with hopefully a more straightforward pharmacological profile and subsequently less side effects. In this regard, preclinical studies are currently attempting to determine which targets may be most relevant for further study and clinical investigation.^{17–19} However, while preclinical studies are important, true validation (especially considering available animal models of bipolar disorder^{17,20}) will only come from clinical trials with medications that are specific for the targets described.

Direct targets of lithium

Lithium has a hydrated ionic radius, which is very similar to that of magnesium, and inhibits some enzymes through competition for this often required cofactor^{21–23} (Table 1). Lithium has been shown to have some degree of inhibition of a number of

Table 1 Direct targets of lithium

<i>Direct targets of lithium</i>	<i>Description</i>
Inositol monophosphatase (IMPase)	Rate-limiting enzyme in inositol recycling; lithium's inhibition of IMPase led to the inositol depletion hypothesis of lithium's actions
Inositol polyphosphate 1-phosphatase (IPPase)	Enzyme involved in inositol recycling in phosphoinositol signaling; acts prior to IMPase
Bisphosphate nucleotidase (BPNase)	Removes phosphate from 3'-phosphoadenosine 5'-phosphate (PAP) to form adenosine 5'phosphate (AMP); an increase in PAP inhibits sulfotransferases, which transfer sulfur to biological molecules
Fructose 1,6-bisphosphatase (FBPase)	Key enzyme in glyconeogenesis; catalyzes the removal of the 1-phosphate from fructose 1, 6-bisphosphatase to form fructose 6-phosphate
Phosphoglucomutase (PGM)	Key enzyme in glycogenolysis and glycogenesis; catalyzes the formation of glucose 1-phosphate from glucose 6-phosphate during glycogenolysis (and the reverse during glycogenesis)
Glycogen synthase kinase-3 (GSK-3)	Normally active kinase that is inhibited by the activity of many signaling pathways; inhibiting GSK-3 has been linked to neurotrophic support, neuroprotection, and possible modulation of circadian rhythms. Preclinical studies suggest antidepressant-like effects of inhibiting G-SK-3.

enzymes.²⁴ However, only a few are significantly inhibited at therapeutic serum lithium concentrations (0.6–1.2 mM). Defined by John York and colleagues in 1995, lithium inhibits a group of at least four related phosphomonoesterases, which are a group of magnesium-dependent, lithium-sensitive phosphatases that, in mammals, currently includes inositol polyphosphate 1-phosphatase (IPPase), inositol monophosphate phosphatase (IMPase), fructose 1,6-bisphosphatase (FBPase), and bisphosphate nucleotidase (BPNase).²⁵ All members of this small group contain a conserved amino-acid sequence motif, Asp-Pro (Ile or Leu)-Asp-(Gly or Ser)-(Thr or Ser), and have a common core tertiary structure that binds metal ions and participates in catalytic functions of the enzyme.²⁵ Of these enzymes, IPPase, IMPase, and FBPase were originally identified as containing this conserved structure,²⁵ whereas BPNase was identified subsequently based upon its common sequence.²⁶ Newer technology utilizing computer-assisted molecular modeling may allow for more extensive structural characterization of the properties of this binding site, with the potential to discover novel enzymes inhibited by lithium that do not contain this specific motif.

Lithium also inhibits the metabolic enzymes phosphoglucomutase (PGM)^{27–30} and a kinase that functions as an intermediary in numerous intracellular signaling pathways, glycogen synthase kinase-3 (GSK-3)^{31,32} (Table 1). Here, we describe IMPase (secondarily IPPase) and GSK-3 in greater depth (Figure 1), and mention briefly the function of other lithium-inhibitable enzymes. Major research effort has focused upon IMPase and GSK-3 as possible therapeutically relevant targets of lithium inhibition based predominantly upon the roles these enzymes play in neurological functions (for review see Gould *et al.*⁴⁸). Indeed, major pharmaceutical effort has focused on both these targets, and it is quite likely

that an inhibitor of either IMPase or GSK-3 will have lithium-mimetic properties in the treatment of bipolar disorder.

Inositol monophosphatase and inositol polyphosphate 1-phosphatase IMPase and IPPase are enzymes involved in recycling and *de novo* synthesis of inositol, which is a necessary component of a primary intracellular signaling pathway, the phosphoinositol signaling pathway. Many extracellular receptors (such as 5-HT₂, α_1 , M1, 3, 5) are coupled to the G protein, G_{q/11}, which, through activation of phospholipase C (PLC), mediates the hydrolysis of a membrane phospholipid, phospholipase phosphoinositide 4,5-bisphosphate (PIP₂), to form the second messengers diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP₃) (see Gould and Manji¹⁵ and Majerus⁴³ for review). DAG and IP₃ subsequently modulate the activity of a multitude of intracellular events (see greater discussion later in the phosphoinositol signaling section). A number of inositol polyphosphate phosphatase enzymes are involved in the dephosphorylation (recycling) of IP₃ to inositol, which is a precursor of membrane PIP₂ (see Majerus⁴³ for review). This recycling is necessary to maintain phosphoinositol-mediated signaling in cell types where inositol is not freely available. The enzyme IMPase is the final (and rate-limiting) inositol polyphosphate phosphatase prior to conversion to inositol. IPPase removes a phosphate from inositol-1, 4-bisphosphate, at a stage just prior to where IMPase acts. Both appear to be critical steps in the maintenance of inositol levels and continuation of phosphoinositol-mediated signaling. IMPase is also required for the *de novo* synthesis of inositol.⁴⁹ Lithium's direct inhibition of IMPase^{50,51} and secondarily IPPase^{52,53} led to the inositol depletion hypothesis of lithium's action^{44,54} (Figure 1).

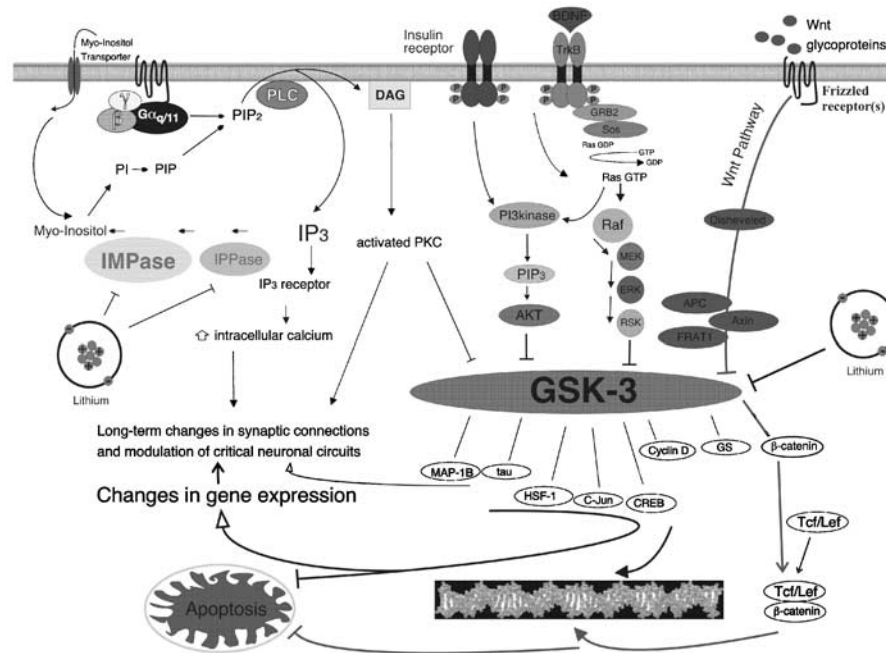


Figure 1 Glycogen synthase kinase-3 and inositol monophosphatase are direct targets of lithium. GSK-3 functions as an intermediary in a number of signaling pathways including neurotrophic signaling pathways, and the insulin/PI3 kinase pathway and the Wnt pathway—activation of these pathways inhibits GSK-3. GSK-3's function while active is generally proapoptotic. Inhibiting GSK-3, for example with lithium, or by phosphorylation via activation of the aforementioned signaling pathways is generally antiapoptotic. Many GSK-3 targets are transcription factors (β -catenin, C-Jun, HSF-1, CREB) and cytoskeletal elements (tau, MAP1B). The influence of neurotrophic factors (such as BDNF) on cell survival is mediated—in part—by activation of the MAP kinase cascade. In this pathway, activation of neurotrophic factor receptors (tyrosine receptor kinases, Trks, TrkB for BDNF), results in activation of the MAP kinase cascade via several intermediate steps. The resultant activation of the small guanosine triphosphate-binding protein Ras (via adapter proteins GRB2 and Sos) leads to activation of a cascade of serine/threonine kinases. These include Raf, MAP kinase kinase (MEK), and MAP kinase (also referred to as extracellular response kinase, or ERK). One target of ERK is Rsk, a kinase that can phosphorylate and deactivate GSK-3. Ras can also activate PI3kinase, a kinase that is activated by insulin signaling as well, and inactivates GSK-3 via phosphorylation. In this pathway, GSK-3 inhibition activates glycogen synthase (GS). GSK-3 is also an important intermediary in the Wnt signaling pathway. Via the frizzled family of receptors, Wnt secreted glycoproteins activate disheveled. Disheveled activation results in inhibition of GSK-3 via interactions within a complex that contains the proteins adenomatous polyposis coli (APC), axin and FRAT1. Under normal conditions, phosphorylation of β -catenin by GSK-3 results in its degradation by ubiquitin. Following GSK-3 inhibition, nondegraded (nonphosphorylated) β -catenin binds to *lef/tcf* transcription factors, targeting transcription of specific genes. In cells, GSK-3 can be inhibited by at least five different mechanisms, which are critically important for the development of novel GSK-3 inhibitors (see Table 2). Lithium appears to be competitive for a magnesium binding site on GSK-3 (#1).^{22,33} In the Wnt pathway, GSK-3 is inhibited by interacting with specific proteins that are part of a larger protein complex (#2). Proteins that inhibit GSK-3 in this manner are axin and FRAT1.³⁴ In the other cellular pathways that inhibit GSK-3 activity, GSK-3 is inactivated by phosphorylation of one of its serine residues (by kinases AKT, P90Rsk, P70 S6, PKC, and PKA)^{35,36} (#3). Most small molecule synthetic inhibitors compete with ATP for a binding site (#4). These include indirubins,³⁷ SmithKline Beecham compounds SB-415286, SB-21676,³⁸ AstraZeneca Compound AR-A014418³⁹ and Chiron compounds.⁴⁰ Recently, synthetic small molecule GSK-3 inhibitors, which are not ATP competitive, have been developed (#5).^{41,42} The upper left portion of the figure depicts lithium's actions on the phosphoinositide signaling pathway. Activation of some G proteins induces phospholipase C hydrolysis of phosphoinositide 4,5-bisphosphate (PIP2) to diacylglycerol (DAG) and inositol-1, 4, 5-triphosphate (IP3). DAG activates protein kinase C (PKC). IP3 binds to the IP3 receptor that also functions as a calcium channel in the cell. This interaction results in the release of intracellular calcium reservoirs from the endoplasmic reticulum; calcium is an activator of many enzymes, and plays a prominent role in many cellular signaling events. IP3 is recycled back to PIP2 by the enzymes inositol monophosphate phosphatase (IMPase) and inositol polyphosphatase phosphatase (IPPase); both of which are inhibited by lithium.⁴³ The inositol depletion hypothesis suggests that lithium exerts its therapeutic actions by depleting free inositol, and thus dampening the activation of downstream signaling pathways in neurons.⁴⁴ Past industry efforts have attempted to develop a brain penetrant IMPase inhibitor by taking the approach of altering the primary substrate of IMPase, inositol monophosphate.⁴⁵ Compounds with sufficient inhibition were developed, but thus far have failed to advance through clinical trials due to being highly charged,⁴⁶ or extremely lipophilic,⁴⁷ both of which limited the bioavailability in the brain (see Atask⁴⁵ for review). Modified, and reproduced, with permission from Gould *et al*¹⁸.

Heuristically, the inositol depletion hypothesis suggests that lithium, via inhibition of IMPase, decreases the availability of inositol, and thus the amount of PIP₂ available for G-protein-mediated signaling that relies upon this pathway.⁴⁴ It is hypothesized that the brain would be especially sensitive to lithium, due to inositol's relatively poor penetration across the blood-brain barrier⁴⁴ or a reduced ability of specific neuronal populations to transport inositol across their cell membrane.⁴⁹ Furthermore, based on the inhibition profile of lithium (ie noncompetitive), more active cells/brain regions may be affected to a greater degree.⁵⁵ Lithium has consistently been shown to decrease free inositol levels in brain sections, and in the brains of rodents treated with lithium (Allison and Stewart⁵⁶; see Attack⁵⁷ for review). Lithium treatment also decreases myoinositol (a form of inositol) in human subjects.⁵⁸ While being proposed more than a decade ago,^{44,54} inositol depletion remains a viable hypothesis for the mechanism of action of lithium. However, no clinically approved inhibitors of either IPPase or IMPase are available; therefore, it remains difficult to test the inositol depletion hypothesis in patients with bipolar disorder. Past industry efforts have attempted to develop a brain penetrant IMPase inhibitor by taking the approach of altering the primary substrate of IMPase, inositol monophosphate.⁴⁵ Compounds with sufficient inhibition were developed, but thus far have failed to advance through clinical trials due to being highly charged,⁴⁶ or extremely lipophilic;⁴⁷ both of which limited the bioavailability in the brain (see Attack⁴⁵ for review). The published crystal structure and modeling studies of IMPase may help to develop novel inhibitors.^{59,60} Downstream molecules (eg protein kinase C; PKC) of IMPase signaling and the phosphoinositol pathway may also be relevant targets.

Glycogen synthase kinase-3

In 1996, Klein and Melton noted that lithium administration to developing *Xenopus* embryos⁶¹ had the same effect, duplication of the dorsal axis, as did downregulation of GSK-3.⁶² This parallelism in a developmental model led them to the study direct effects of lithium on GSK-3, finding that GSK-3 was directly inhibited by lithium.³¹ Lithium was initially found to inhibit GSK-3 with an K_i of 1–2 mM (serum therapeutic range 0.6–1.2 mM).^{31,32} However, evidence showing that lithium competes with magnesium^{22,33} suggests that the original studies utilizing higher than physiological levels of magnesium may have underestimated the degree of inhibition. Indeed, recent studies suggest a significant inhibition of this enzyme in the rodent brain at therapeutic serum lithium levels during long-term treatment. For example, Gould *et al*⁶³ found that 9 days of lithium (mean serum concentration 0.8mM) treatment increased cytosolic protein levels of β -catenin (a transcription factor regulated directly by GSK-3).⁶³ This protein level increase was accompanied by a small but

significant decrease in β -catenin mRNA levels, further suggesting that lithium exerted its actions post-translationally by inhibiting GSK-3 (the mRNA changes reflecting cellular compensation).⁶³ Furthermore, Phiel and colleagues found that 3 weeks of lithium treatment (serum levels 0.8–1.2 mM) decreased brain levels of amyloid- β peptide in AP-Swedish/Tg2576 mice (a model of familial Alzheimer's disease), a finding—given lithium's effect on amyloid- β accumulation in cell culture^{64,65}—that is likely due to inhibition of GSK-3.⁶⁵ These preclinical studies clearly suggest that therapeutic serum concentrations of lithium produce a biologically significant inhibition of GSK-3 in the mammalian brain.

GSK-3 is a serine/threonine kinase that is normally highly active in cells, and is deactivated by signals originating from numerous signaling pathways (for example, the Wnt pathway, PI3 kinase pathway, protein kinase A, protein kinase C, among many others). It is found in two forms, α and β , that have similar—but not always identical—biological functions. Cellular targets of GSK-3 are numerous and often depend upon the signaling pathway that is acting upon it (due to cellular localization and regional sequestration). For example, Wnt pathway inhibition of GSK-3 activates the transcription factor β -catenin, while in the insulin/PI3 kinase signaling pathway inhibition of GSK-3 results in activation of the enzyme glycogen synthase. Targets of GSK-3 include—among others—transcription factors (β -catenin, CREB, c-Jun), proteins bound to microtubules (Tau, MAP1B, kinesin light chain), cell cycle mediators (cyclin D, human ninein), and regulators of metabolism (glycogen synthase, pyruvate dehydrogenase). See Gould *et al*⁴⁸ and Frame and Cohen⁶⁶ for review (Figure 1).

Being a component of many signaling pathways, with multiple cellular targets to choose from, allows GSK-3 to regulate a diverse array of cellular processes such as glycogen synthesis, gene transcription, events related to synaptic plasticity, apoptosis, and the circadian cycle (see Woodget,³⁵ Gould and Manji,⁶⁷ Lenox *et al*,⁶⁸ and Jope and Bijur⁶⁹ for review). While many of these functions are likely critically important to both cellular and organism functioning, at present GSK-3 is receiving foremost interest as a regulator of apoptosis and cellular resilience (Figure 1). Generally, increased activity of GSK-3 is proapoptotic, while inhibiting GSK-3 attenuates or prevents apoptosis (see Gould and Manji,⁶⁷ and Jope and Bijur⁶⁹ for review). At this point, it is critical to note that evidence suggests an association between mood disorders and impairments of neuroplasticity and cellular resilience—with both *in vivo* and postmortem studies suggesting neuron and/or glial cell loss or atrophy in circumscribed brain areas.^{70,14} Importantly, lithium has effects suggestive of neuroprotection clinically, as well as in rodent and cell-based models (see Manji *et al*⁷⁰ and Chuang *et al*⁷¹ for review). Lithium may exert these neuroprotective effects—at least in part—by inhibition of GSK-3.^{67,69}

Table 2 Examples of drug classes that inhibit GSK-3

Compound	Type of inhibition (see Figure 1)	References
Lithium	Magnesium competitive	22,31–33
Zinc	?	82
Indirubines	ATP competitive	37
Maleimides	ATP competitive	83–85
Hymenialdisine	ATP competitive	86
Paullones	ATP competitive	87
Thiadiazolidinones	Non-ATP competitive	41
Synthetic phosphorylated peptide	Substrate competitive	42
Azole derivatives	ATP competitive	39

Recent evidence, utilizing preclinical models, suggests that inhibiting GSK-3 may represent both an antidepressant target and antimanic target of lithium. Two groups have found that administration of GSK-3 inhibitors produce antidepressant-like effects in the forced swim test paradigm following either intracerebral ventricle injections in mice⁷² or peripheral administration to rats.⁷³ As mentioned, GSK-3 has a number of cellular targets; the antidepressant-like behavior observed could putatively be via any one, or a combination of, these targets. However, the acuteness of the forced swim test paradigm (1 or 2 injections over 2 days) argues against sizable plastic changes playing a major role. In this regard, Li *et al*⁷⁴ recently reported that inhibitory phosphorylation of GSK-3 is acutely increased by increased serotonin levels. Specifically, they found that d-fenfluramine (serotonin release stimulator), clorgyline (MAOI), fluoxetine, and imipramine increase serine 9 phosphorylation of GSK-3 β .⁷⁴ These data suggest the possibility that the effects of antidepressant drugs on behavioral measures may be mediated by GSK-3-dependent mechanisms. Hence, GSK-3 inhibition may represent a therapeutically relevant downstream consequence of antidepressant drugs that initially target monoamine levels.

Amphetamine-induced hyperactivity is the most established rodent model for mania; this hyperactivity is attenuated by a number of mood stabilizers including lithium, anticonvulsants, and antipsychotics. Beaulieu *et al*⁷⁵ recently reported that dopamine-dependent activity increases in mice are mediated in large part via a GSK-3-dependent mechanism. They report that both lithium and alternate GSK-3 inhibitors attenuate the hyperactivity in mice lacking the dopamine transporter, that amphetamine administration to wild-type mice results in a decrease in the inhibitory phosphorylation of GSK-3, and that mice heterozygous for GSK-3 β have an attenuated response to amphetamine administration.⁷⁵ We have also found that peripheral administration of a GSK-3 inhibitor decreases amphetamine-induced hyperactivity in rats.⁷³ *In toto*, these data support the possibility that inhibition of GSK-3 may represent lithium's antimanic, as well as its antidepressant target. It will be of

critical future importance to determine which GSK-3 target(s) are responsible for behavior in these models.

In addition to its possible usefulness in the treatment of bipolar disorder,⁴⁸ inactivation of GSK-3 has been suggested as a potential therapy for a number of diseases. Diabetes and Alzheimer's disease have received the most attention. Diabetes has drawn interest because GSK-3 phosphorylates and deactivates glycogen synthase (see Kaidanovich and Eldar-Finkelman⁷⁶ for review). Alzheimer's disease is a target due to the role GSK-3 plays in both the phosphorylation of tau (see Bhat and Budd⁷⁷ and Alvarez *et al*⁷⁸ for review), and the assembly of amyloid- β .^{64,65} Hyperphosphorylation of tau is associated with the formation of neurofibrillary tangles, while accumulation of amyloid- β leads to amyloid plaques. Glycogen synthase kinase-3 inhibitors may also be useful for the treatment of cardiac ischemic injury,⁷⁹ baldness/alopecia (the Wnt pathway is involved in hair growth; see Frame and Cohen⁶⁶ for review), other neurodegenerative disorders^{70,71} and stroke and other neurotraumatic injuries.^{71,80,81}

For these reasons, industry has focused major efforts on the development of selective GSK-3 inhibitors (Table 2). It was reported in 2002 that more than 45 patents for GSK-3 inhibitors had already been filed.⁸⁸ As shown in Table 2, these inhibitors generally act by inhibiting the ability of ATP to bind to its GSK-3 binding site. Thus, ATP competitive inhibitors appear to block GSK-3-mediated phosphorylation of all GSK-3 substrates. A more recent development is the synthesis of compounds that act in a non-ATP competitive manner, including competition for the GSK-3 substrate-binding site.^{41,42} It is suggested that this class of inhibitors holds the promise of possible inhibiting GSK-3-mediated phosphorylation of some substrates but not others.^{89,36}

The element zinc has been identified as an inhibitor of GSK-3 β ⁸² and in a recent placebo-controlled study, adjunctive treatment with zinc has been shown to have antidepressant effects.⁹⁰ Zinc has also been reported to have antidepressant-like properties in the forced swim test and olfactory bulbectomy model in rats,^{91–93} and low (ineffective) doses of zinc combined with ineffective doses of antidepressants

likewise have antidepressant-like effects in preclinical models.^{91,94} These data suggest the possibility that zinc may have these effects through inhibition of GSK-3; however, zinc has other biological effects, including antagonism of NMDA receptors (see Quiroz *et al*¹⁴ for review), that require consideration.

Other preclinical research has identified proteins that naturally inhibit GSK-3 (FRAT1, components of axin), sometimes in a pathway or substrate-specific manner (see Gould *et al*⁴⁸ for review). Understanding the exact mechanism by which these proteins inhibit GSK-3 may lead to another class of compounds. Early phase clinical trials (likely for Alzheimer's disease or diabetes) of GSK-3 inhibitors will likely be initiated in the near future; it is expected that these compounds will also be tested for efficacy in the treatment of bipolar disorder.⁴⁸

Other enzymes: fructose 1,6-bisphosphatase, bisphosphate nucleotidase, phosphoglucomutase Studies have suggested that lithium inhibits other enzymes at therapeutic concentrations. This list includes FBPase, BPNase, and PGM (Table 1).²³ FBPase (a regulator of gluconeogenesis), removes the 1-phosphate from fructose 1, 6-bisphosphate to form fructose 6-phosphate. Lithium's inhibition of FBPase was originally described a number of years ago.^{27,95,96} More recent studies support these findings.^{97,98} However, lithium's effect of FBPase has not received much attention due to dysfunction of glyconeogenesis not being a primary theory of bipolar disorder pathophysiology. Inhibitors of FBPase are under development as possible treatments for diabetes (for example, see Wright *et al*⁹⁹).

Mammalian BPNase acts on bisphosphorylated nucleotides such as 3'-phosphoadenosine 5'-phosphate (PAP) where it removes the 3' phosphate to form adenosine 5'phosphate (AMP).^{26,100,101} Hence, BPNase is also referred to as PAP phosphatase. Sulfotransferases are enzymes, which transfer a sulfur group to various biomolecules, utilizing 3'-phosphoadenosine 5'-phosphosulphate (PAPS) as a sulfur donor. PAP is produced following the removal of the sulfur group from PAPS, and acts as an inhibitor of sulfotransferases. Therefore, inhibition of BPNase (and the subsequent buildup of PAP) will inhibit sulfotransferases. Although work in mammalian systems is lacking, biochemical reactions potentially modulated by BPNase and/or PAP accumulation include RNA processing metabolism, sodium homeostasis, and sulfation assimilation. It has been suggested that the development of nephrogenic diabetes insipidus in patients undergoing lithium therapy may be due to BPNase inhibition.²⁶ BPNase, similar to IPPase, hydrolyzes inositol-1, 4-bisphosphate and lithium prevents BPNase-mediated hydrolysis of both substrates.^{26,100,101} Thus, lithium inhibition of BPNase would be expected to have effects on inositol recycling similar to inhibiting IMPase or IPPase. The recently described crystal structure of BPNase should help develop novel

inhibitors.¹⁰² See Agam and Shaltiel¹⁰³ for a recent review and more extensive discussion regarding some of the possible roles of BPNase in bipolar disorder.

PGM catalyzes the formation of glucose 1-phosphate from glucose 6-phosphate during glycogenolysis (and the reverse during glycogenesis). Lithium was originally identified to inhibit the rabbit and rat enzyme,²⁷⁻²⁹ and more recently has been found to inhibit human and yeast PGM.³⁰ The role of PGM as a therapeutic target in bipolar disorder treatment has been mostly overlooked perhaps due to limited evidence that metabolism of glycogen is involved in the disorder.

Direct targets of valproate

Interest in the potential efficacy of VPA, a simple branched-chained fatty acid, in bipolar disorder initially arose out of the suggestion that facilitating the activity of an inhibitory neurotransmitter like GABA may have antimanic effects. VPA's effect on GABA levels is generally, but not exclusively, believed to be via inhibition of key enzyme(s) within the GABA metabolic pathway (for review see Johannessen¹⁰⁴ and Owens and Nemeroff¹⁰⁵). VPA also inhibits the enzyme histone deacetylase (HDAC),^{106,107} suggesting the possibility that there exist further VPA inhibitable enzymes that may be relevant to the mood stabilizing properties of this fatty acid. Comparison of the sequence homology among the VPA binding site within these enzymes thus may be of great interest.

Valproate also incorporates into the cell membrane in GT1-7 neurons,¹⁰⁸ suggesting that the formation of valproyl-phospholipids (and the resultant effects on cell membrane integrity, function, and/or mediation of signal transduction) is a mechanism that is deserving of more study. Additionally, and similar to carbamazepine (see next section), VPA inhibits sodium channel function at high frequencies (see Macdonald and Kelly¹⁰⁹ for review) and may represent a second site of anticonvulsant action. However, while the mechanism of VPA's efficacy in the treatment of bipolar disorder may be via its anticonvulsant target(s), it may also be via an entirely unrelated mechanism²³ (Table 3).

Enzymes in the GABA metabolic pathways; succinate semialdehyde dehydrogenase, succinate semialdehyde reductase, and GABA transaminase A leading hypothesis regarding how VPA exerts its anticonvulsant effects is by increasing the levels of GABA (see Johannessen¹⁰⁴ and Owens and Nemeroff¹⁰⁵ for review). Numerous studies have documented an increase in GABA concentration in rodent brain after VPA administration (see Johannessen¹⁰⁴ for review). GABA, an inhibitory amino-acid neurotransmitter, inhibits excessive firing of synapses. VPA may exert its effects on GABA through inhibition of critical enzyme(s) in GABA metabolism. A number of studies show

Table 3 Direct targets of valproic acid

<i>Direct targets of valproate</i>	<i>Description</i>
Succinate semialdehyde dehydrogenase (SSA-DH)	Converts succinate semialdehyde to succinate; succinate inhibits GABA-T; inhibiting GABA-T increases GABA levels
GABA transaminase (GABA-T)	Converts GABA to succinate semialdehyde; however, VPA inhibition appears to be above the therapeutic range
Succinate semialdehyde reductase (SSA-R)	Converts succinate semialdehyde to gamma-hydroxybutyrate (GHB)
Histone deacetylases (HDACs)	Enzymes that acetylates histones. Inhibition of this enzyme results in an increase in nonacetylated histones and a resultant increase in transcription of some genes
Sodium channels	Inhibits sodium channel function at times of high-frequency firing

that VPA, at therapeutic concentrations, is an inhibitor of succinate semialdehyde dehydrogenase (SSA-DH).^{104,110–113} This enzyme is critical for the GABA shunt, a pathway that produces both glutamate and GABA by circumventing a portion of the tricarboxylic acid (TCA) cycle. GABA transaminase (GABA-T) converts GABA to succinate semialdehyde (SSA), which is then converted to succinate by SSA-DH. VPA's effect on SSA-DH would be expected to increase levels of SSA, which has a strong inhibitory effect on GABA-T activity. GABA concentration increases as GABA-T is inhibited by an increasing SSA concentration. Although—on average—only 8–10% of the total flux through the TCA cycle enters the GABA shunt,¹¹⁴ it is possible that this percentage changes in different brain regions, or in different cell types.¹¹² Downstream of SSA-DH, the GABA shunt re-enters the TCA cycle; thus, inhibition of the GABA shunt could lead to a lower overall activity of the TCA cycle. A lower TCA activity—or perhaps increased GABA—may explain the decreased glucose metabolism observed during VPA treatment.^{104,115,116} Additionally, VPA inhibits succinate semialdehyde reductase, the enzyme that converts succinate semialdehyde to γ -hydroxybutyrate (GHB) with a reported K_i of 85 μ M.^{104,113} VPA also inhibits GABA-T, but the K_i appears to be well above therapeutic levels.^{104,117,118} However, some evidence suggests that neuronal GABA-T may be more sensitive than glial GABA-T.^{105,119}

VPA's enhancement of GABAergic neurotransmission, and the direct effect of VPA on enzymes involved in GABA metabolism suggests the possible utility of enhancing GABA levels as a therapeutic approach in the treatment of bipolar disorder. A number of newer anticonvulsants that increase GABAergic transmission have been developed, and many have been tried in open labeled studies with somewhat encouraging results; double-blind studies are eagerly awaited (see Ketter and Wang¹²⁰ for a recent review). However, it is worth considering that the relevant effects of inhibition of these enzymes by VPA may not be due to an increase in GABA *per se*, but other cellular processes (such as the TCA cycle as described above). Indeed, there exists concern that

many of the studies on GABA levels have utilized supra therapeutic levels of VPA, which is suggestive of other effects of VPA on the above-mentioned enzymes as being more relevant.¹²¹

Histone deacetylase

Histones are components of nucleosomes, on which DNA is bound to form chromatin (Figure 2). Acetylation of histones reduces their affinity for DNA and is a major epigenetic regulator of gene expression. Histones acetylation is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), and generally activates gene transcription. Two classes of HDACs (I and II) are found in large protein complexes, which suppress gene transcription (of certain genes in specific cell types). VPA is an inhibitor of all class I, and some class II, HDACs *in vitro*, in cell culture, and in intact animals with inhibition within therapeutic serum levels (0.4 – 0.8 mM).^{106,107,124} Recent studies have found that peripheral VPA administration increases histone acetylation in the rodent brain following short^{122,123} and long-term administration.¹²² VPA's inhibition of HDAC promotes acetylation, and thus has the potential to increase transcription of certain genes.

VPA's effect on HDAC could explain many of its effects on cellular signaling pathways (see Gould *et al*²³ and Gurvich and Klein³³ for review). Some HDAC inhibitors are currently being developed and utilized in oncology trials (HDAC inhibition upregulates many tumor suppressor genes; see Marks *et al*¹²⁵ for an excellent review). Recently, the HDAC inhibitors, suberoylanilide hydroamic acid (SAHA) and sodium butyrate were found to readily penetrate the blood-brain barrier and ameliorate motor deficits in a mouse model of Huntington's disease.^{126,127} These studies suggest the possibility that brain-penetrant HDAC inhibitors may be available for bipolar disorder trials.

Direct targets of carbamazepine

Similar to valproate, it is not clear if carbamazepine has efficacy in treating mania due to its target in epilepsy (generally thought to be inhibition of the high-frequency firing of high-frequency sodium

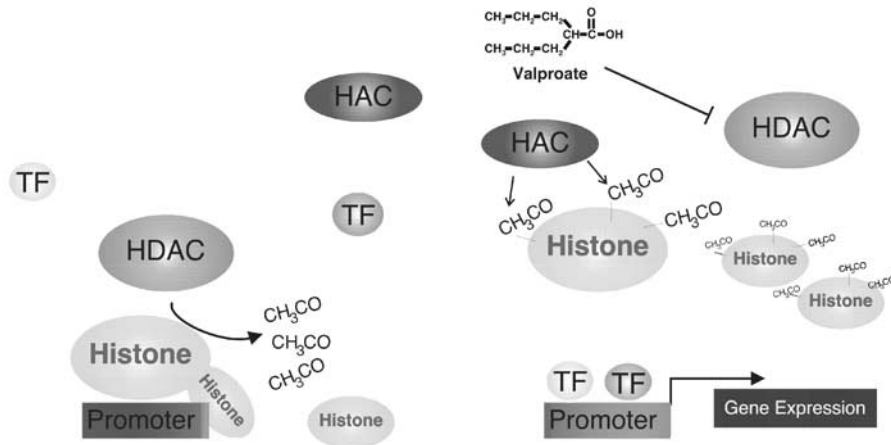


Figure 2 Histone deacetylase is a direct target of valproic acid. Histones are components of nucleosomes, on which DNA is wrapped to form chromatin. Acetylation of histones reduces their affinity for DNA and by this mechanism represents a major epigenetic regulator of gene expression. Two classes of HDACs (I and II) are found in large protein complexes, which suppress gene transcription (of certain genes in specific cell types). Histone acetylation is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACS), and acetylation generally activates gene transcription (for example reelin¹²²) by allowing for increased interaction of transcription factors (TF) and the promoter. VPA is an inhibitor of HDACs *in vitro*, in cell culture, and in animals with inhibition within therapeutic serum levels (0.4–0.8 mM).^{106,107,122,123} VPA's effect on HDAC could explain many of its effects on cellular signaling pathways (see Gould *et al*²³ and Gurvich and Klein³³ for review).

Table 4 Direct targets of carbamazepine

Direct targets of carbamazepine	Description
Sodium channels	Inhibits sodium channel function at times of high-frequency firing
Adenosine receptor	Antagonist at A1 subtype; increases adenosine receptor protein levels in rats. Adenosine has modulatory functions on neurotransmitter release and numerous behavioral and cognitive functions
Adenylate cyclase (AC)	AC forms cyclic AMP from ATP, and as such is a principle regulator of the adenylate cyclase signaling pathway; carbamazepine attenuates cyclic AMP signaling and appears to have an inhibitory effect on AC or an AC-associated protein

channels), or because of an unrelated target.²³We selectively review some of carbamazepine's targets here (Table 4). However, additional findings with carbamazepine including potentiating effects on GABA_B agonists, blockade of calcium influx, effects on peripheral benzodiazepine receptors, among others, are not described in this review, but could also be of critical importance in understanding carbamazepine's mechanism of action.

Sodium channels

It is widely accepted that carbamazepine exerts its antiepileptic effects by inhibiting the high-frequency firing of sodium channels. Carbamazepine, in cultured neurons¹²⁸ and in voltage-clamp experiments,¹²⁹ blocks voltage-dependent sodium channels; thus, inhibiting repetitive neuronal firing—a process that is thought to contribute to aggregation of neural firing (epileptogenesis). Under physiological conditions,

sodium channels are thought to be in one of three conformations: a resting state, an open state, or an inactive state. Carbamazepine's specific interaction appears to be via modulating the sodium channel only during the inactive state, thus prolonging the time of inactivation.¹³⁰ Carbamazepine does not appear to affect the amplitude or duration of individual action potentials, but does reduce the ability of a neuron to produce trains of action potentials at a high frequency. While the antiepileptic function of this is intuitive, it is not clear if these physiological effects are relevant to the treatment of mania. Additional drugs that act as antagonist of sodium channel firing have been developed as anticonvulsants. Similar to carbamazepine, VPA produces a functional blockade of voltage-gated sodium channels that could be related to its mood stabilizing properties. Lamotrigine has similar effects on sodium channels, but recent studies suggest a primary antidepressant effect and in

preventing mood episode relapses.^{131,132} Phenytoin is a classic anticonvulsant that blocks voltage-gated sodium channels. Mood stabilizing effects of phenytoin have been addressed in double-blind adjunct studies^{133,134} and, if replicated in monotherapy trials, will lend substantial support to the hypothesis that some mood stabilizers may act—in part—through inhibition of sodium channels. Future anticonvulsants with this mechanism of action may likewise be suitable for testing in patients with mood disorders.

Adenosine receptors Adenosine receptors are generally G-protein coupled receptors that can modulate neurotransmitter release, neuronal damage, and neurovegetative functions such as sleep, arousal, and cognition. Carbamazepine has both acute and long-term effects on the levels, and signaling events involving adenosine receptors when administered at therapeutic concentrations to rodents. In addition to reports suggesting that carbamazepine effects adenosine-mediated second messenger signaling,^{135–137} the drug also appears to bind to adenosine receptors.^{138–142} Carbamazepine appears to act as an antagonist, with general specificity for the A₁ subtype of adenosine receptors.¹⁴² Carbamazepine also appears to affect protein levels of adenosine receptors. After 11 days of treatment with carbamazepine, levels of adenosine receptors in rats were significantly increased.¹⁴³ The changes persisted long after (up to 8 weeks) discontinuation of treatment.¹⁴⁴ Signaling events downstream of adenosine receptors also appears to be altered by carbamazepine. Specifically carbamazepine, via blockade of adenosine A₁ receptors, appears to modulate adenosine's potentiating effect on activation by neurotransmitters of the phosphoinositol second messenger pathway (an effect similar as has been proposed for lithium—the inositol depletion hypothesis).^{135,142,145} Other A₁ antagonists (eg Maemoto *et al*¹⁴⁶) are available for preclinical use, suggesting the possibility of future clinical studies.

Adenylate cyclase

As discussed in the next section of this review, evidence suggests that carbamazepine attenuates stimulated cyclic AMP signaling.^{15,136,142,147–152} These findings eventually led to studies suggesting that carbamazepine may directly inhibit adenylate cyclase (AC; see description in the next section).¹⁵¹ Chen *et al*¹⁵¹ reported that at therapeutic concentrations carbamazepine inhibited both basal and stimulated cyclic AMP production. Carbamazepine exerted this effect regardless of whether AC-coupled receptors, or AC itself was stimulated, suggesting the possibility of a direct inhibitory effect. These effects were also found in AC extracts; suggesting that carbamazepine inhibits AC directly, or acts via a closely associated factor that purifies with the enzyme.¹⁵¹ Most of the evidence discussed has been accumulated during acute treatment, and it remains to be seen if this

action can be temporally associated with carbamazepine's therapeutic effects in bipolar disorder. Interestingly, in addition to its putative role in the pathophysiology of mania, AC and the cyclic AMP signaling pathway has been postulated to play a role in epilepsy.^{153,154} Thus, it is worth considering that carbamazepine may exert both its antimanic and antiepileptic effects by inhibiting this enzyme and attenuating cyclic AMP-mediated signaling.

Developing novel therapeutics by understanding downstream targets of current mood stabilizers

There is considerable excitement in the field that the development of novel, improved and potentially more rapidly acting medications may be based upon more downstream actions of mood stabilizers. This approach may be especially promising considering that mood stabilizers generally take week(s) to exert their initial effects and longer to exert full effects. The possibility thus exists to develop medications that may act more proximal to the relevant function of medications, and thus have a more rapid action. It has been noted that individual mood stabilizers regulate a number of signaling pathways in preclinical cellular and rodent models. To tackle this dilemma, many investigators have taken the approach that if multiple, structurally dissimilar, mood stabilizers act in a similar manner on a pathway, then it may be relevant for treatment. While a number of pathways exist that multiple mood stabilizers regulate (see Gould *et al*²³ for discussion), we selectively review those where there is among the greatest evidence and a likelihood of medication trials, *vide licet* the adenylate cyclase, phosphoinositol, neurotrophic, and arachidonic acid signaling pathways.

Cyclic AMP-mediated signal transduction

In addition to the direct effects of carbamazepine on AC as discussed in the preceding section, significant modulation of cyclic AMP-mediated signaling has been noted with lithium (especially) and also VPA. G proteins modulate intracellular cyclic adenosine monophosphate (cAMP) levels by mediating the effect of neurotransmitters (via extracellular receptors) on AC, an integral membrane protein of which there exist numerous subtypes. AC catalyzes the conversion of adenosine triphosphate (ATP) to cyclic AMP. Stimulation of G proteins G_{α_s} and G_{α_o} increase AC activity, while stimulation of G_{α_i} results in a decrease in AC activity. The physiologic effects of cyclic AMP appear to be mediated primarily by activation of protein kinase A (PKA), an enzyme that phosphorylates and regulates many proteins including ion channels, cytoskeletal elements, transcription factors, and other enzymes. One major CNS target for the actions of PKA is the transcription factor CREB (cyclic AMP responsive element binding protein), which plays a major role in long-term neuroplasticity, and is a downstream target of antidepressants (see Gould and Manji¹⁵ and Duman¹⁵⁵ for review). One of

the genes activated by CREB is brain-derived neurotrophic factor (BDNF); a protein implicated in neuronal survival and synaptic plasticity. There is a growing body of data suggesting that agents, which directly modulate the cAMP/PKA/CREB/BDNF cascade, may have utility for the treatment of depression (see Manji and Duman¹⁵⁶ for review of antidepressant effects). In addition to antidepressant effects on cyclic AMP-mediated signaling, mood stabilizers also appear to regulate this pathway. Interestingly, both lithium and VPA increase BDNF levels in the brains of rats treated chronically with these drugs^{157–159} and, as we discuss, appear to have inhibitory effects on stimulated AC-mediated signaling. Thus, it is useful to keep in mind the multiple interactions between signaling pathways (for example, CREB activity and BDNF expression are regulated by multiple signaling pathways including neurotrophic signaling pathways as discussed later in this review), and that cyclic AMP signaling pathway does much more than simply regulate CREB activity.

As mentioned in the preceding section, multiple lines of evidence indicate that carbamazepine is a modulator of cyclic AMP-mediated signaling. Specifically, in mouse cerebral cortex and cerebellar tissue, carbamazepine decreases the basal concentration of cyclic AMP.¹⁴⁷ It also lowers cyclic AMP following stimulation by norepinephrine,^{147,148} adenosine,^{142,147,150} and the epileptogenic compounds ouabain^{136,147,149} and veratridine.¹⁴⁹ Additionally, carbamazepine appears to attenuate β -adrenoceptor and muscarinic cholinergic coupling to G proteins in the rat cortex,¹⁶⁰ decreases the levels of G_s and G_i and attenuates cyclic AMP-mediated phosphorylation of CREB in C6 glioma cells.¹⁵¹ Similarly, in pheochromocytoma (PC12) cells carbamazepine inhibits cyclic AMP-mediated increases in c-fos gene expression.¹⁵² This evidence eventually led to studies discussed in the preceding section suggesting that carbamazepine may directly inhibit adenylate cyclase.¹⁵¹

Lithium appears to have complex effects on cyclic AMP-mediated signaling, with the preponderance of the data demonstrating an elevation of basal AC activity, but an attenuation of receptor-stimulated responses in both preclinical and clinical studies (see Jope¹⁶¹ for an excellent review). Thus, a number of independent research laboratories have found in preclinical models that the ability of the receptor-mediated signal to be propagated via adenylate cyclase to decrease after lithium treatment (see Gould and Manji¹⁵ and Jope¹⁶¹ for review). These extensive cellular findings are consistent with an animal model where cholera toxin (a stimulator of the G proteins, G_s and G_{olf}) induces hyperactivity when injected into the nucleus accumbens of rats. Cholera toxin induced hyperactivity was decreased by lithium administration,¹⁶² consistent with decreased G_s and/or G_{olf} activity during lithium treatment. But while stimulated levels are decreased, there is evidence to suggest an increase in basal cyclic AMP activity (see Jope¹⁶¹ for an excellent review of this literature). Thus, the

literature describing the effect of lithium on G proteins suggests that lithium both increases basal activity and inhibits stimulated AC (see Jope¹⁶¹ for review).

In contrast to the multiple studies that describe the effects of lithium and carbamazepine on G-protein coupled and/or cyclic AMP-mediated signaling, only one published report exists for VPA.¹⁶³ This study found that at therapeutically relevant concentrations in a cell line model, VPA decreased the density of β ARs and attenuated both receptor- and postreceptor-stimulated cyclic AMP production. These authors additionally reported that levels of G_{α_s} 45, but none of the other G protein subunits examined were decreased.¹⁶³ Thus, VPA may exert an effect on cyclic AMP signaling at multiple levels.

Postmortem and peripheral cell studies are also consistent with a role of cyclic AMP in mood disorders. Postmortem brain studies of patients who suffered from bipolar disorder have reported increased levels of G_{α_s} and postreceptor stimulated adenylate cyclase activity.^{164,165} Generally, the experiments measuring adenylate cyclase activity in unipolar depression find both reduced immediate and long-term effects (see Wang *et al*¹⁶⁶ for a comprehensive review). Thus, though an oversimplification, the majority of the evidence reports increased activity of the adenylate cyclase system in bipolar disorder and a decrease in activity in unipolar depression.

The finding that lithium, carbamazepine, and VPA exert roughly similar effects on cyclic AMP signaling suggests a common target of these two mood stabilizers.^{15,161,167} While caution is clearly warranted when attempting to correlate these preclinical and postmortem studies with human disease, the available evidence is substantial. There are numerous compounds that inhibit AC activity. A good deal of specificity has been observed particularly with analogs of the nucleoside adenosine, namely P-site inhibitors.^{168,169} Ideally, novel compounds would be isoform-selective in order to avoid peripheral side effects due to the widespread distribution of multiple AC isoforms in different organs in the body. The development of these compounds suggests the eventual possibility of trials with these medications in the treatment of bipolar disorder. Stimulators of adenylate cyclase (eg forskolin) may be useful for challenge studies.

Phosphoinositol-mediated signaling

Inositol phospholipids play a major role in receptor-mediated signal-transduction pathways, involved in a diverse range of responses such as cell division, secretion, neuronal excitability, and responsiveness. The phosphatidylinositol (PI) pathway is initiated by the activation of G proteins coupled receptors. Muscarinic (m1, m3, m5), noradrenergic (α_1) and serotonergic (5HT2) receptors coupled to $G_{\alpha_q/11}$ induce phospholipase C (PLC) hydrolysis of the membrane component phosphoinositide 4,5-bisphosphate (PIP2).

Hydrolysis of PIP₂ by PLC results in the formation of the intracellular second messengers inositol-1,4,5-triphosphate (IP₃) and 1,2-diacylglycerol (DAG; an endogenous activator of protein kinase C (PKC)). IP₃ binds to the IP₃ receptor facilitating the release of calcium from intracellular stores, in particular the endoplasmic reticulum (see Gouldi and Manji¹⁵ for review). Among other proteins, the Ca²⁺-receptor protein calmodulin (CaM) is activated stimulating calmodulin-dependent protein kinases (CaM-Ks) that regulate the activity of diverse proteins, including ion channels, signaling molecules, proteins which regulate apoptosis, scaffolding proteins, and transcription factors.¹⁷⁰ As described in an earlier section IPPase and IMPase (enzymes that are involved in recycling of inositol-1, 4, 5-triphosphate (IP₃) back to PIP₂) are directly inhibited by lithium 34 (Figure 1). Lithium's inhibition of these enzymes led to the inositol depletion hypothesis of lithium's action, which suggests that lithium, via inhibition of IMPase, decreases the availability of myo-inositol, and thus the amount of PIP₂ available for G-protein-mediated signaling events that rely upon this pathway.⁴⁴

The inositol depletion hypothesis led to a number of studies, both in cultured cells and animal models, to determine if the PI pathway may be involved in the pathophysiology or treatment of bipolar disorder (see Manji and Lenox¹⁷¹ for review). Interestingly, a number of studies have suggested the possibility that multiple mood stabilizers may regulate the PI signaling pathway. These include studies of the sodium/myo-inositol cotransport (SMIT), a high-affinity myo-inositol transport system that has been characterized in various cell types, including those of neural origin (see van Calker and Belmaker¹⁷² for review). The activity and expression of SMIT mRNA in cultured astrocytes is downregulated after chronic treatment with therapeutic concentrations of lithium^{172,173} Downregulation of SMIT was also observed after VPA and carbamazepine treatment.^{172,173} If replicated *in vivo*, these findings suggest that SMIT may represent a novel target for the development of new drugs.

Another finding implicating phosphoinositol signaling in the actions of mood stabilizers comes from Williams *et al*¹⁷⁴ who used a tissue-culture assay that measures sensory neuron growth-cone stability to conclude that the depletion of neuronal inositol (1,4,5) trisphosphate (IP₃) may be a common mechanism of action. These investigators demonstrated that lithium, VPA and carbamazepine all inhibit the collapse of sensory neuron growth cones and increase growth cone area; effects which were reversed by inositol. The authors then used *Dictyostelium*, which relies on IP₃ for its development, to identify mutants that confer resistance to the drugs. Null mutations of prolyl oligopeptidase confer lithium resistance and elevate intracellular levels of IP₃. The authors established a link by showing that prolyl oligopeptidase inhibitors abolished the effects of lithium, carbama-

zepine and VPA on growth-cone collapse and area in their tissue-culture assay.¹⁷⁴

Both lithium and valproic acid regulate protein kinase C isozymes and MARCKS

Protein kinase C (PKC) is a primary target of DAG, and as such has been an object of research in regard to the action of mood stabilizers on the PI pathway. It is highly enriched in brain, where it plays a major role in regulating both pre- and postsynaptic aspects of neurotransmission.¹⁷⁵ Recent studies have suggested that PKC activation may facilitate neurotransmitter release via a variety of mechanisms, including modulation of several ionic conductances regulating Ca²⁺ influx, upstream steps regulating release of Ca²⁺ from intracellular stores, recruitment of vesicles to at least two distinct vesicle pools, and the Ca²⁺ sensitivity of the release process itself. PKC has been demonstrated to be active in many other cellular processes including stimulation of transmembrane glucose transport, secretion, exocytosis, smooth muscle contraction, gene expression, modulation of ion conductance, cell proliferation, and desensitization of extracellular receptors.¹⁷⁵

PKC and PKC signaling appear to be a target of lithium and VPA.¹⁷¹ Chronic lithium treatment decreases the level of PKC isozymes α , and ϵ ¹⁷⁶⁻¹⁷⁸ in cells and treated rodents. The precise mechanisms by which lithium exerts these isozymes-selective actions is unknown, but there is evidence that it is due to lithium's inhibition of IMPase.^{171,176} Further supporting the effect of lithium on PKC, lithium decreases the levels and phosphorylation of a major PKC substrate, myristoylated alanine-rich C kinase substrate (MARCKS), following chronic treatment in rats.¹⁷⁹ In cultured cells, it was found that this effect appears to be dependent on low media inositol concentrations, thus implicating lithium's inhibition of IMPase and/or IPPase as a causative factor.^{171,180}

Evidence also suggests that PKC is a target of VPA. In cultured cells, VPA reduces PKC activity in both membrane and cytoplasmic fractions.¹⁸¹ VPA also selectively reduces the protein levels of the same PKC isozymes reduced by lithium, α and ϵ , further suggesting possible importance in the treatment of bipolar disorder.¹⁸¹ The reduction in the α isozyme has also been confirmed in the brains of rats treated chronically with VPA at therapeutically relevant concentrations.¹⁷¹ Also, similar to the effects of lithium, VPA decreases the levels of MARCKS.¹⁸² The mechanism by which VPA exerts these effects is unknown; however, they appear to be independent of myoinositol.¹⁸²

PKC signaling in animal models of mood disorders

Current models of mania that have been used in the study of mood disorders include kindling, behavioral/amphetamine sensitization, and glucocorticoid administration.^{20,68,183} Kindling is an animal model for epilepsy that has been proposed to have similarities with pathophysiological aspects of

bipolar disorder, in which repeated administration of electrical stimulus (that are subthreshold to produce seizures *per se*) results in an epileptic focus and a permanent state of hyperexcitability to the stimulus. These studies on rats have consistently shown the upregulatory effect of hippocampal kindling on PKC activity and protein concentration,^{184–189} findings that also were demonstrated to be valid in other brain structures such as the amygdala^{190,191} and neocortex.^{192,193}

Studies have also implicated alterations in PKC activity as mediators of long-term alterations in neuronal excitability in the brain following chronic stimulant use. Thus, several independent laboratories have now demonstrated that both acute and chronic amphetamine produce an alteration in PKC activity, its relative cytosol to membrane distribution, as well as the phosphorylation of a major PKC substrate, GAP-43, which has been implicated in long-term alterations of neurotransmitter release.^{194–198} Furthermore, PKC inhibitors have been shown to block the acute responses (as assessed by both behavioral and *in vivo* microdialysis studies) to both amphetamine¹⁹⁹ and cocaine as well as cocaine-induced sensitization.^{200,201}

Abnormalities of circulating glucocorticoids are well known to be associated with affective symptomatology, and interestingly, elevated glucocorticoids have been associated with both depressive and manic symptomatology.^{202,203} Repeated administration of dexamethasone for 10 days results in a significant increase in Bmax of [³H]PDBu binding to PKC, increased PKC activity, and increased the levels of PKC α and ϵ in the rat hippocampus.²⁰⁴ It is striking that behavioral sensitization and kindling (models which have been postulated to represent models of BD and mania) as well as dexamethasone administration all produce alterations in the PKC signaling pathway in critical limbic structures, since lithium and VPA also target the very same biochemical targets. Thus, although considerable caution obviously needs to be employed when extrapolating from rodent brain and animal behavioral models, the fact that these two models and glucocorticoid administration are associated with opposite effects on PKC signaling to those observed with chronic lithium or VPA is compelling indeed.

Interestingly, there is also evidence suggesting that chronic antidepressants may also modulate PKC activity in limbic and limbic-associated areas of rat brain.^{205,206} and PKC has been demonstrated to regulate the activity of norepinephrine, dopamine, and serotonin transporters.^{207–210} Whether these complex effects of antidepressants on PKC activity underlies their apparent ability to trigger manic episodes, and perhaps promote rapid cycling in susceptible individuals,¹ remains to be determined.

Protein kinase C as potential therapeutic target

In view of the pivotal role of the PKC signaling pathway in the regulation of neuronal excitability,

neurotransmitter release, and long-term synaptic events, its modulation by both lithium and valproate, and supporting evidence from animal models of mania, it was postulated that the attenuation of PKC activity might have efficacy in treating mania. These findings led to a single-blind clinical trial investigating possible antimanic properties of the PKC inhibitor tamoxifen.²¹¹ While best known for its antiestrogenic properties, tamoxifen is also a potent PKC inhibitor at high concentrations.^{212,213} Initial results are encouraging, finding that tamoxifen treatment resulted in a significant decrease in manic symptoms rated by the Young Mania Rating Scale, with a greater than 50% decrease in the Young Mania Rating Scale score occurred in five of seven patients enrolled in the initial trial.²¹¹ Larger double-blind placebo-controlled studies of tamoxifen are in progress.

Inhibition of PKC activity has been advanced as a method to treat diabetic complications, and selective PKC inhibitors are presently in late-stage clinical trials (see Frank²¹⁴ and Wheeler²¹⁵ for review) for this indication. One of the compounds furthest in development is LY333531, ruboxistaurin, a relatively selective PKC β inhibitor.^{215,216} Several other groups of PKC inhibitors are currently under investigation, including rottlerin, indolocarbazoles, PKC412, bisindolylmaleimides and balanol (reviewed in Aiello²¹⁶ and Parker²¹⁷). Compounds with properties similar to these may be utilized as potential medications for the treatment of mania.

Neurotrophic signaling cascades

Neurotrophins are a family of regulatory factors that mediate the differentiation and survival of neurons, as well as the modulation of synaptic transmission and synaptic plasticity. The neurotrophin family now includes—among others—nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin (NT) 3, NT4/5, and NT6. BDNF and other neurotrophic factors are necessary for the survival and function of neurons, implying that a sustained reduction of these factors could affect neuronal viability. However, what is sometimes less well appreciated is the fact that BDNF also has a more acute effects on synaptic plasticity and neurotransmitter release, and facilitates the release of glutamate, GABA, dopamine, and serotonin (see Du *et al*²¹⁸ for review).

Thus, BDNF is best known for its long-term neurotrophic and neuroprotective effects, which may be very important for its putative role in the pathophysiology and treatment of mood disorders. In this context, it is noteworthy that although endogenous neurotrophic factors have traditionally been viewed as increasing cell survival by providing necessary trophic support, it is now clear that their survival-promoting effects are mediated in large part by an inhibition of cell death cascades (see Du *et al*²¹⁸ for review). Increasing evidence suggests that neurotrophic factors inhibit cell death cascades by activating the ERK kinase signaling pathway, the

phospholipase C- γ cascade, and the phosphatidylinositol-3 kinase (PI-3K)/Akt pathway. It has been demonstrated that chronic stress (21 days foot-shock; an animal model of depression) induced a pronounced and persistent extracellular response kinase 1/2 (ERK1/2) hyperphosphorylation in dendrites of the higher prefronto-cortical layers, while phospho-CREB was reduced in several cortical regions including frontal cortex.²¹⁹ Since CREB is phosphorylated and activated by phospho-ERK1/2 directly, this reduction indicates that chronic stress could downregulate CREB phosphorylation indirectly, and subsequently downregulate the transcription of some genes such as Bcl-2 and BDNF. In this context, it is noteworthy that severe stress exacerbates stroke outcome by suppressing Bcl-2 expression.²²⁰ In this study, mice exposed to aggressive social stress expressed ~70% less Bcl-2 mRNA than unstressed mice following ischemia. Furthermore, stress greatly exacerbated infarct in control mice but not in transgenic mice that constitutively express increased neuronal Bcl-2. Finally, high corticosterone concentrations were significantly correlated with larger infarcts in wild-type mice but not in Bcl-2 overexpressing transgenic mice. Thus, enhanced Bcl-2 expression appears to be capable of offsetting the potentially deleterious consequences of stress-induced neuronal endangerment, and suggests that pharmacologically induced upregulation of Bcl-2 may have considerable utility in the treatment of a variety of disorders associated with endogenous or acquired impairments of cellular resilience. Overall, it is clear that the neurotrophic factor/ERK MAP kinase/Bcl-2 signaling cascade plays a critical role in cell survival in the CNS, and that there is a fine balance maintained between the levels and activities of cell survival and cell death factors. Dysregulation of the BDNF-ERK-CREB coordination may be a key mechanism by which prolonged stress induces atrophy of selective subpopulations of vulnerable neurons and/or distal dendrites. Conceivably, the precise kinetics of ERK and CREB activation will ultimately dictate whether the activated kinases participate in a cell survival or death-promoting pathway.

In view of the important role of ERK MAP kinases in mediating long-term neuroplastic events, it is noteworthy that lithium and valproate, at therapeutically relevant concentrations, have been demonstrated to activate the ERK MAP kinase cascade in human neuroblastoma SH-SY5Y cells²²¹ and in critical limbic and limbic-related areas of rodent brain.¹⁵⁷ Interestingly, as noted, neurotrophic factors are now known to promote cell survival by activating MAP kinases to suppress intrinsic, cellular apoptotic machinery, not by inducing cell survival pathways (see Du *et al*²¹⁸ for review). Thus, a downstream target of the MAP kinase cascade, ribosomal S-6 kinase (Rsk) phosphorylates the cAMP response element binding protein (CREB) and this leads to induction of bcl-2 gene expression. Consistent with an activation

of neurotrophic signaling cascades, chronic treatment of rats with 'therapeutic' doses of lithium or valproate produces an increase in the activation of Rsk, and CREB, and a doubling of bcl-2 levels in frontal cortex, effects which are primarily due to a marked increase in the number of bcl-2 immunoreactive cells in layers II and III of frontal cortex.^{222–224} Interestingly, the importance of neurons in layers II–IV of the frontal cortex in mood disorders has recently been emphasized, since primate studies indicate that these areas are important for providing connections with other cortical regions, and that they are targets for subcortical input.²²⁵ Further suggestive evidence that lithium and VPA activate the ERK MAP kinase pathway and/or targets of this pathway comes from the finding that both lithium and VPA increase the expression of BDNF in rodent brain following chronic treatment¹⁵⁸ (in addition to activating the ERK MAP kinase pathway, the ERK MAP kinase pathway also initiates—via CREB—the transcription of BDNF).

Consistent with its effects on neurotrophic signaling cascades, lithium has been demonstrated to be neuroprotective in animal models of ischemia, Huntington's disease, promotes neurogenesis in the hippocampus of rats, increases the regeneration of CNS axons,²²⁶ and is neuroprotective in many cell culture models (see Manji *et al*⁷⁰ and Chuang *et al*⁷¹ for review). Recent evidence suggests that the neuroprotective effect of lithium in cortical neurons requires BDNF expression.²²⁷ Valproic acid also exerts neuroprotective actions in a number of cellular models including glutamate toxicity, β -amyloid toxicity, and following exposure to other toxins.^{228–231}

Human evidence for the neurotrophic effects of lithium While the body of preclinical data demonstrating neurotrophic and neuroprotective effects of mood stabilizers is striking, considerable caution must clearly be exercised in extrapolating to the clinical situation with humans. In view of lithium robust effects on the levels of the cytoprotective protein bcl-2 in the frontal cortex, Drevets *et al* reanalyzed older data demonstrating ~40% reductions in subgenual PFC volumes in familial mood disorder subjects.²³² Consistent with neurotrophic/neuroprotective effects of lithium, they found that the patients treated with chronic lithium or valproate exhibited subgenual PFC volumes, which were significantly higher than the volumes in nonlithium or VPA-treated patients, and not significantly different from control (WC Drevets, personal communications). In a more recent study, Drevets and colleagues have investigated glial cell densities in mood disorder patients. Although the sample sizes are small, they made the intriguing observation that unipolar patients exhibited reduced glial cell densities, whereas only the bipolar patients off chronic lithium or VPA exhibited similar reductions.²³³

Although the results of the afore-mentioned studies suggests that mood stabilizers may have exerted

neuroprotective effects during naturalistic use, considerable caution is warranted in view of the small sample size and cross-sectional nature of the study. To investigate the potential neurotrophic effects of lithium in humans more definitively, a longitudinal clinical study was recently undertaken using proton magnetic resonance spectroscopy (MRS) to quantitate *N*-acetyl-aspartate (NAA, a putative marker of neuronal viability) levels.²³⁴ Four weeks of lithium treatment produced a significant increase in NAA levels, effects which were localized almost exclusively to gray matter.²³⁵ These findings provide intriguing indirect support for the contention that chronic lithium increases neuronal viability/function in the human brain. Furthermore, a ~ 0.97 correlation between lithium-induced NAA increases and regional voxel gray matter content was observed, thereby providing evidence for colocalization with the regional specific bcl-2 increases observed (eg gray vs white matter) in the rodent brain cortices. These results suggest that chronic lithium may not only exert robust neuroprotective effects (as has been demonstrated in a variety of preclinical paradigms),^{70,71} but also exerts neurotrophic effects in humans.

A follow-up volumetric MRI study has demonstrated that 4 weeks of lithium treatment also significantly increased total gray matter content in the human brain,²³⁶ suggesting an increase in the volume of the neurophil (the moss-like layer comprised of axonal and dendritic fibers which occupies much of the cortex gray matter volume). A finer grained subregional analysis of this brain imaging data is ongoing, and clearly shows that lithium produces a regionally selective increase in gray matter, with prominent effects being observed in hippocampus, caudate and prefrontal cortex (unpublished observations; GJ Moore and HK Manji). Furthermore, no changes in overall gray matter volume are observed in healthy volunteers treated chronically with lithium, suggesting that lithium is truly producing a reversal of illness-related atrophy, rather than nonspecific gray matter increases. Recently, cross-sectional studies have corroborated the gray matter findings,²³⁷ and NAA findings.²³⁸

Ongoing studies are attempting to determine the precise relationship between the lithium-induced increases in regional NAA levels and gray matter volumes and treatment response. In this context, a preliminary electroconvulsive therapy (ECT) study of severely depressed patients is noteworthy.²³⁹ Michael *et al*²³⁹ investigated the effects of a course of ECT on NAA levels in left amygdalar region in 28 severely depressed patients. They found that significant increase in NAA was observed only in ECT responders ($n = 14$). Moreover, five of 14 nonresponders to ECT monotherapy were then treated with adjunctive antidepressants (while ECT continued), and re-scanned; these investigators found that this group showed both clinical improvement and a significant increase in NAA. While preliminary, these clinical results suggest that the neurotrophic effects of anti-

depressant treatments (and likely lithium) are indeed associated with treatment response (although a causal relationship has yet to be established).

An increasing number of strategies are being investigated to develop strategies to enhance neurotrophic signaling as treatment of neurogenerative disorders (see Deigner *et al*²⁴⁰ for review). Human phase I/II trials of recombinant methionyl human brain-derived neurotrophic factor have already been undertaken, wherein the BDNF was administered by intrathecal infusion to patients with amyotrophic lateral sclerosis.²⁴¹ Unfortunately, side effects such as sensory symptoms, including paraesthesias or a sense of warmth, sleep disturbance, dry mouth, agitation, and other behavioral effects were encountered at higher doses, precluding further study. Strategies are also being investigated to develop small molecule switches for protein-protein interactions, which have the potential to regulate the activity of growth factors, MAP kinases cascades, and interactions between homo- and heterodimers of the bcl-2 family of proteins. In view of the robust effects of bcl-2 on neurite sprouting, neurite outgrowth, and axonal regeneration (see Du *et al*²¹⁸ for review), and protection against the deleterious CNS effects of severe stress,²²⁰ it is possible that bcl-2 enhancers will have utility in the treatment of bipolar disorder. Indeed, lithium's ability to robustly upregulate bcl-2 may play a role in its antidepressant potentiating effects. It is also noteworthy that the dopamine agonist pramipexole upregulates bcl-2 in several brain areas,²⁴² and has been shown to exert antidepressant effects in preliminary studies.²⁴³ While the dopamine agonistic effects of pramipexole may clearly also contribute to its purported antidepressant effects, its neurotrophic effects suggest that it may have broader utility as an antidepressant potentiator. In this context, recent studies at the NIMH²⁴⁴ and elsewhere²⁴⁵ have found pramipexole to be more effective than placebo in treating bipolar depression; ongoing longitudinal studies at the NIMH are further exploring putative neurotrophic effects with the use of serial MRS measurements of NAA, and volumetric MRIs.

Arachidonic acid metabolism

Arachidonic acid (AA) functions as an important mediator of second messenger pathways within the brain.^{246,247} It is released from membrane phospholipids via receptor/G protein-initiated activation of phospholipase A2.²⁴⁸ This action results in release of AA from the cellular membrane and cyclooxygenase-mediated production of eicosanoid metabolites such as prostaglandins and thromboxanes. These metabolites mediate numerous subsequent intracellular responses and, due to their lipid permeable nature, transynaptic responses.

Arachidonic acid metabolism as a target of mood stabilizers was originally suggested by studies done by Rapoport, Chang, and colleagues in 1996 and 2001 showing that chronic lithium and VPA treatment of rats results in selective reductions in the turnover rate

in the brain phospholipids of AA.^{249–251} In the case of lithium the reduction was 80%, and it was subsequently demonstrated that lithium downregulated the gene expression and protein levels of an AA-specific phospholipase A2 (cPLA2)^{252,253} and the protein levels of cyclooxygenase-2 (COX-2).²⁵⁴ VPA also decreased the turnover of AA by 33%,²⁴⁹ has no apparent effect on cPLA2 protein levels,²⁴⁹ but decreases protein levels of COX-1 and COX-2.²⁵⁵ These findings suggest that effects of mood stabilizers on cell membranes—and specifically AA turnover—might be relevant to the pharmacological action of lithium and VPA.^{247,251} Further general support for the involvement of the arachidonic acid signaling pathway in bipolar disorder comes from other preclinical studies. For example, recent studies in rats found that administration of nonselective cyclooxygenase inhibitors indomethacin and piroxicam prevented amphetamine-stimulated locomotor activity²⁵⁶ and blocks cocaine sensitization²⁵⁷ (both are rodent models of mania²⁰). Furthermore, inhibition of COX-2 with NS-398 attenuates restraint stress (a model of depression) induced oxidative changes.²⁵⁸ The inflammatory hypothesis (distinct from the AA hypothesis) of bipolar disorder has led to a clinical trial addressing the effect of a specific COX-2 inhibitor as an adjunct treatment in bipolar patients.²⁵⁹

Conclusions

Bipolar disorder affects approximately 1–3% of the world's population. However, there has been little progress in developing truly novel drugs specifically for the treatment of bipolar disorder, and most recent additions to the pharmacopeia are brain penetrant drugs developed for the treatment of epilepsy or schizophrenia. Thus, there exists a critical need to develop novel approaches for the treatment of bipolar disorder. While the task of developing novel medications is very difficult, new medications will be developed, and we predict that they will likely derive from both understanding mechanisms of current drug actions and by directing medications at a renewed understanding of bipolar disorder pathophysiology. In this review we discussed the first approach.

Studies of targets of mood stabilizers lithium, valproate, and carbamazepine focus on both initial molecular targets and downstream effects on cellular signaling pathways that are shared between multiple drugs. As we discussed, molecular and cellular targets of current mood stabilizers include lithium inhibitable enzymes inositol monophosphatase (IMPase), inositol polyphosphate 1-phosphatase, glycogen synthase kinase-3 (GSK-3), fructose 1,6-bisphosphatase, bisphosphate nucleotidase, and phosphoglucomutase. Of these targets, IMPase and GSK-3, have received the most attention. The pharmaceutical industry has yet to develop potent brain-penetrant IMPase inhibitors (see Atack⁴⁵ for review), but GSK-3 inhibitors are rapidly being developed, and we believe will be useful to discern the role of

inhibition of GSK-3 in the treatment of bipolar disorder.⁴⁸ In addition to inhibiting high-frequency opening of sodium channels, valproate inhibits some enzymes including succinate semialdehyde dehydrogenase, succinate semialdehyde reductase, and histone deacetylase (HDAC). Valproate's effects on the first two likely relate to its inhibitor effect on GABA levels, while inhibition of HDAC could lead to changes in gene expression, protein levels, and functional alterations which have widely been hypothesized to be the desired outcome of psychotropic medications. HDAC-specific inhibitors are being developed for the treatment of various cancers, and it is thus likely that they may be available for treatment trials of bipolar disorder. Carbamazepine targets include sodium channels, adenosine receptors and adenylate cyclase. The fact that both carbamazepine and VPA inhibit sodium channel activity suggests the possibility that sodium channel inhibition may have therapeutic relevance. More studies with alternate sodium channel inhibitors, such as the studies of phenytoin^{133,134,260} are necessary to discern this possibility. In addition to these direct targets, we also discussed signaling pathways regulated by multiple drugs including phosphoinositol/protein kinase C, cyclic AMP, arachidonic acid signaling, and neurotrophic signaling pathways. The protein kinase C inhibitor tamoxifen is currently being investigated as an antimanic agent; initial results from a single-blind study are encouraging,²¹¹ and these are being followed up by large double-blind studies. Mechanisms to enhance neurotrophic pathways is a major focus for the treatment of neurodegenerative disorders, and it is likely that novel medications for this intent may be soon available.

Critical to remember is that while theories abound, ultimate validation (especially considering available animal models of bipolar disorder^{17,20}) will rest in the results of clinical trials. We are hopeful that understanding the mechanism of action of current mood stabilizers will lead to clinical trials with drugs of more specific actions, and ultimately, improved medications for the treatment of those who suffer from bipolar disorder.

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