International Journal of Neuropsychopharmacology (2003), **6**, 181 189. Copyright © 2003 CINP
DOI: 10.1017/S1461145703003419

SPECIAL SECTION

Cellular mechanisms and second messengers: relevance to the psychopharmacology of bipolar disorders

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Abstract

The discovery of lithium's e cacy as a mood-stabilizing agent revolutionized the treatment of patients with bipolar disorder and after five decades, lithium continues to be the mainstay of treatment for bipolar disorder. Recent research on the molecular mechanism underlying the therapeutic effect of lithium has focused on how it changes the activities of cellular signal transduction systems, especially the cyclic AMP and phosphoinositide second-messenger systems. Considerable data suggest that carbamazepine and valproate (VPA) are an alternative or adjunctive treatment to lithium. VPA, despite being dissimilar structurally to lithium, shares most of the effects of lithium at the level of protein kinase C (PKC). Like lithium, VPA reduces the activity of PKC and reduces the protein levels of different PKC isoforms, however the effects of VPA appear to be largely independent of myoinositol. The long-term e cacy of VPA and lithium in bipolar disorder suggested that modulation of gene expression might be an important target for these drugs. Both VPA and lithium altered the expression of the early inducible genes for c-*fos* and c-*jun* thus promoting the expression of specific proteins. The genes known to be regulated by the AP-1 family of transcription factors include genes for various neuropeptides, neurotrophins, receptors, transcription factors, enzymes, proteins that bind to cytoskeletal elements, and cytoprotective proteins such as bcl-2. In conclusion chronic treatment with lithium and other mood stabilizers, by regulating transcriptional factors, may modulate the expression of a variety of genes that compensate for aberrant signalling associated with the pathophysiology of bipolar disorder.

Received 7 October 2002; Accepted 13 November 2002

Key words: Gene expression, lithium, mood stabilizers, second messengers.

Introduction

Manic-depressive illness is a common, severe, chronic and life-threatening illness, and represents one of the leading causes of disability worldwide (Müller-Oerlinghausen et al., 2002). Several drugs have shown e cacy in controlling acute manic symptoms (Thase and Sachs, 2000). These include anticonvulsants, benzodiazepines, antipsychotics, calcium channel blockers, as well as lithium. Some of these agents, particularly the mood-stabilizing anticonvulsants and lithium are also effective in the prophylactic management of bipolar disorder.

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The three agents with mood-stabilizing properties supported by the strongest data are lithium, carbamazepine (CBZ) and valproic acid. CBZ and valproate (VPA) are now the first-line therapy for lithium-resistant or -intolerant patients. Most recently, newer anticonvulsants such as lamotrigine, gabapentin, topiramate, and oxcarbazepine have been examined in preliminary studies as possible alternatives in the treatment of this disorder (Chengappa et al., 1999; McElroy et al., 1997; Sporn and Sachs, 1997). Other newer anticonvulsants that could also have a role in the treatment of bipolar disorder are tiagabine, vigabatrin, felbamate, but there are still no systematic reports on their effects in mood disorder patients.

Understanding the molecular and cellular mechanisms by which these drugs achieve their therapeutic action, especially their shared effects, would represent a valuable step in clarifying the pathophysiology and

possibly the pathogenic process that lead to mania and bipolar depression.

Mechanism of action of mood stabilizers: from ion transport to second messengers

Early biological theories regarding mechanisms of action of mood stabilizers and antidepressants focused on modulation of neurotransmitter synthesis, release, turnover and uptake (Lenox et al., 1998). However, although a number of acute, in-vitro effects of these agents are well established, their therapeutic effects are only seen after chronic administration, thereby precluding any simple mechanistic interpretations based on acute biochemical effects. In the past decade the focus has shifted to post-synaptic events, such as the regulation of signal transduction mechanisms. More recent studies with lithium and mood stabilizers have focused on transcription factors coded by immediate early genes and regulation of cytoskeletal protein function (Lenox and Hahn, 2000). The search for the mechanisms of action of mood stabilizers has been facilitated by a growing appreciation that rather than any single neurotransmitter system being responsible for depression or mania, multiple interacting and overlapping systems are probably involved in regulating mood and that most effective drugs probably do not work on any particular neurotransmitter system in isolation, but rather affect the functional balance between interacting systems (Chen et al., 1999a).

In search for a link between the mechanism of action of lithium and neurotransmission, the effect of lithium has been extensively studied on virtually every neurotransmitter system (Lenox and Hahn, 2000). Chronic lithium has been reported to prevent behavioural and biochemical manifestations of haloperidol-induced dopamine receptor supersensitivity. The effect of lithium on norepinephrine receptor binding has been generally inconclusive, however it has been shown that lithium treatment enhances receptor subsensitivity following antidepressant treatment and prevents β-adrenergic receptor supersensitivity induced by neurotransmitter depletion. One of the most consistent observations, in vivo as well as in vitro, is that β-adrenergic receptor-mediated cAMP accumulation is decreased by acute lithium treatment. Preclinical studies have shown that lithium affects serotonergic transmission at multiple levels resulting in an enhancement of serotonergic neurotransmission. Chronic treatment of lithium increases basal and stimulation-induced serotonin (5-HT) release, moreover it produces a subsensitivity of presynaptic inhibitory 5-HT-1A receptors, which can result in a net increase of 5-HT released per impulse. These findings provide the neurochemical basis for the clinical observation of lithium e cacy as an adjunct to antidepressants in the treatment of resistant depression.

In plasma and CSF of human subjects as well as various regions of the rat brain, lithium treatment has been shown to increase the level of GABA; GABA-B receptors were found to be increased in the hippocampus following chronic treatment with lithium as well as with anticonvulsant mood stabilizers.

It has been recently reported that lithium acutely increases synaptic concentrations of glutamate, leading then, upon chronic administration, to an increase and stabilization in glutamate uptake transporter capacity (Jope, 1999). This effect could mediate a reduction and stabilization in excitatory neurotransmission after lithium treatment and may contribute to a relatively large and diverse group of findings in the literature indicating that lithium has neuroprotective effects. This neuroprotective action may be mediated by increased glutamate uptake, attenuated increases in intracellular calcium levels associated with excitatory neurotransmission, and the recent finding that lithium up-regulated the anti-apoptotic protein bcl-2 (Chen and Chuang, 1999; Chen et al., 1999b).

Clinical evidence clearly indicates that the therapeutic effects of lithium and mood stabilizers are only seen after chronic administration; therefore the search for their mechanism of action should not be based on acute biochemical effects. In recent years, research aimed at elucidation of the cellular mechanisms underlying the therapeutic effects of mood stabilizers have focused on their chronic action on second-messenger generating systems (Manji et al., 1995, 1996).

Numerous studies have demonstrated that the cAMP second-messenger system is modulated by lithium (Jope and Williams, 1994). In general, these show that lithium increases basal levels of cAMP but impairs receptor-coupled stimulation of cAMP production. These dual effects of lithium might be due to inhibition by lithium of the G proteins that mediate cAMP production. Receptor-mediated production of cAMP is controlled by a stimulatory G protein (Gs), and a counter-balancing inhibitory G protein (Gi). Under basal conditions, cAMP production is tonically inhibited by the prevailing Gi in uence. Increased basal cAMP levels caused by lithium may occur at least in part because lithium reduces the activity of Gi (Masana et al., 1992), apparently by shifting its equilibrium between a free active conformation and an inactive heterotrimeric conformation towards the

inactive form. Therefore, inhibitory effects of lithium on Gi can elevate the basal level of cAMP. On the other hand, attenuation of stimulus-induced increases in cAMP production, such as by norepinephrine, may be caused by an inhibitory effect of lithium on the activation of Gs, through an as yet unspecificied mechanism. Overall, it appears that these actions of lithium reduce the magnitude of uctuations in cAMP levels by increasing the lowest basal levels and decreasing maximal stimulated increases, thus stabilizing the activity of this signalling system.

In the last 10 yr the main focus of the research on the molecular mechanisms underlying the therapeutic effects of lithium has been the receptor-coupled hydrolysis of phosphatidyl inositol 4,5-biphosphate (PIP2). Activation of receptors coupled to phosphatidyl inositol hydrolysis results in the breakdown of phosphoinositide 4,5-biphosphate (PIP2) into two second messengers: IP3 and diacylglycerol (DAG), which is an endogenous activator of protein kinase C (PKC). Lithium, at therapeutically relevant concentrations, is an uncompetitive inhibitor of inositol monophosphatase ($K_i = 0.8$ mM) and results in an accumulation of inositol 1-monophosphate as well as a reduction in free inositol specifically in those cells undergoing the highest rate of PIP2 hydrolysis (for a review see Shaldubina et al., 2001). It was proposed that the physiological consequence of lithium's action is derived through a depletion of free inositol and that its selectivity could be attributed to its preferential action on the most overactive receptor-mediated neuronal pathways. Following these observations it was hypothesized that lithium's action derived through a depletion of free myoinositol in the brain, the so-called inositol depletion hypothesis'. It was proposed that this action might result in the depletion of inositol-containing lipids thereby attenuating receptor-coupled phosphoinositide signalling. Despite the di culty in demonstrating that lithium treatment reduces resynthesis of PIP2 in all cell systems, a body of preclinical data, however, suggests that some of the initial actions of lithium may occur with a relative depletion of myoinositol initiating then a cascade of secondary changes at different levels of the signal transduction process and gene expression in the CNS, effects that are ultimately responsible for lithium's therapeutic e cacy (Jope, 1999; Manji et al., 1995).

Since lithium has been shown to interact with the two major receptor G protein-coupled signalling pathways, studies have examined the direct effects of lithium on G protein activity. Although some studies have reported modest changes in the levels of G protein subunit, several independent laboratories have reported that chronic lithium does not modify G-protein levels per se, but clearly modifies G protein function. Recent studies have also examined the effects of long-term lithium administration on G protein function in humans and have generally observed reduced receptor/G protein coupling (Li et al., 2000).

The final target of second-messenger transduction systems is the activity protein kinases (PKA, PKC) (Casebolt and Jope, 1991). A primary action of cAMP is to stimulate the activity of cAMP-dependent PKA. Several studies found that lithium can modulate stimulated PKA-mediated protein phosphorylation. Using a purified system, Mori et al. (1996) have demonstrated that the phosphotransferase activity of PKA can be directly inhibited by therapeutic concentrations of lithium salts. Moreover chronic lithium treatment resulted in a significant increase in cAMP binding, as well as in the levels of the PKA subunits in the soluble, but not in the particulate fractions of different brain areas (Mori et al., 1998).

Lithium's effects on signalling processes downstream from phosphoinositide hydrolysis have also been studied extensively, and many reports describe the modulation of PKC by lithium. PKC exists as a family of closely related subspecies, has a heterogeneous distribution in the brain (with particularly high levels in presynaptic nerve terminals), and plays a major role in the regulation of neuronal excitability, neurotransmitter release, and long-term alterations in gene expression and plasticity (Stabel and Parker, 1991). Short-term lithium treatment may activate PKC, whereas long-term lithium exposure results in an attenuation of phorbol ester-mediated responses, which may be accompanied by down-regulation of PKC isozymes in the brain (Manji et al., 1996). One of the major substrates of PKC, myristoylated alanine-rich C kinase substrate (MARCKS), has been studied extensively and lithium was found to reduce the levels of MARCKS, an effect that is also caused by direct activation of PKC, suggesting a modulatory effect of lithium on PKC activity which modulates MARCKS. MARCKS is involved in cytoskeletal architecture, indicating that this function may be affected by lithium (Lenox et al., 1992, see Table 1).

Second-messenger mediated pathways represent the target of lithium, it has therefore been of interest to test whether other putative mood-stabilizing agents exert similar effects on the same signalling pathways. Considerable data suggest that CBZ and VPA are an alternative or adjunctive treatment to lithium, both for acute manic episodes as well as for long-term prophylaxis in bipolar affective disorder (Brambilla et al., 2001). However the cellular mechanisms underlying

Table 1. Main effects of lithium on signal transduction mechanisms

Adenylyl cyclase
Increased basal levels of cAMP
Decreased receptor stimulated levels of cAMP
Phosphatidylinositol cycle
Uncompetitive inhibition of inositol monophosphatase
Accumulation of inositol 1-monophosphate
Reduction in free inositol
G proteins
Attenuation of receptor-stimulated adenylyl cyclase activity
Attenuation of receptor-mediated and GTPγ S-mediated phosphoinositide turnover
Attenuation of agonist-induced [^{3}H]GTP binding
Reversal of the effect of lithium by increasing GTP
Increase in pertussis toxin-catalysed [^{32}P]ADP ribosylation in platelets and in rat brain
Reduction in Gαs, Gαi1, Gαi2 mRNA in rat cortex
Protein kinases
In-vitro inhibition of PKA catalytic subunit
Increased cAMP binding to PKA regulatory subunit and increased levels of R and C subunit after chronic treatment in the rat
Short-term activation and long-term attenuation of phorbol ester-mediated neurotransmitter release
Reduced in-vitro PKC-mediated phosphorylation of major PKC substrates
Reduced [^{3}H]PDBU binding in hippocampal structures, CA_1 and subiculum
Reduced immunolabelling of PKCα, PKCε, and MARCKS in rat hippocampus and culture cells

both the anticonvulsant and mood-stabilizing effects of CBZ and VPA have not yet been completely identified.

CBZ is chemically related to the tricyclic antidepressants. Widely used in the treatment of partial and generalized tonic-clonic seizures, CBZ has been reported to stabilize the inactive form of the Na^+ channel in a voltage-, frequency-, and time-dependent fashion. Inhibition of glutamatergic neurotransmission has also been implicated in the mechanism of CBZ action. Recent evidence suggests that it inhibits the rise in intracellular-free Ca^{2+} induced by NMDA and glycine in rat cerebellar granule cells and blocks veratridine-induced release of endogenous glutamate (Kwan et al., 2001). CBZ does not affect the PKC-signalling pathway or G proteins, however, it produces many effects on the cAMP-signalling pathway. CBZ decreases the basal concentrations of cAMP in the mouse cerebral cortex and cerebellum and reduces cAMP production induced by norepinephrine and adenosine. Moreover it has been recently reported that CBZ inhibits both basal and forskolin-stimulated activity of purified adenylate cyclase (AC). These data suggest that CBZ inhibits cAMP production by acting directly on AC and/or through factors tightly associated with or co-purified with AC (Chen et al., 1996). Consistent with these results, it has been demonstrated that CBZ attenuates forskolin-induced c-*fos* (an immediate-early gene) expression and inhibits forskolin-induced phosphorylation of CREB. Because c-*fos* and CREB are known to be involved in mediating a number of long-term neuronal responses, these effects might be postulated to play a role in the delayed therapeutic effect of CBZ.

Sodium valproate has proved to be an extremely useful anti-epileptic drug, with a broad spectrum of activity and particular e cacy in the generalized epilepsies, however the precise mechanisms by which it exerts its anti-epileptic as well as mood-stabilizing effects remain to be determined. VPA has been reported to block voltage-dependent Na^+ channels and to reduce sustained repetitive firing of neurons in culture. There is evidence to suggest that VPA elevates whole brain GABA levels and potentiates GABA responses, possibly by enhancing GAD activity and inhibiting GABA degradation (Löscher, 1999). Anecdotal reports suggest that the drug also augments GABA release and blocks GABA uptake. Some studies have suggested that VPA causes decreased excitatory transmission in the brain by reducing the number of action potentials elicited by application of NMDA. VPA would then exert its mood-stabilizing effect through preventing hyperexcitability in membranes via inhibiting voltage-activated sodium channels, its action on excitatory neurotransmitters, or its various GABAergic actions (Johannessen, 2000).

Following the idea that drugs belonging to the same therapeutic class (e.g. anti-manic agents) even if possessing distinct chemical structures, may share common biochemical targets when administered in a therapeutically relevant' paradigm, the effects of VPA on second-messenger pathways have been investigated. In view of the significant effects of lithium on PKC, it has been found that VPA even if structurally unrelated, produces effects on the PKC signalling pathway that are strikingly similar to those of lithium (Chen et al., 1994; Watson et al., 1998). Long-term administration of lithium and VPA seems to regulate PKC isozymes by distinct mechanisms and VPA's effects seem to be largely independent of myoinositol. This biochemical observation is consistent with clinical observations that some patients have a preferential

response to one or the other agent and that additive therapeutic effects often occur when the two agents are co-administered.

Neurobiology of mood disorders

Early biological theories regarding the pathophysiology of bipolar disorders have focused on various neurotransmitters, in particular, the biogenic amines. The behavioural and physiological manifestations of bipolar disorders are complex and undoubtedly mediated by a network of interconnected neuronal circuits and it is thus not surprising that the brain systems which have received the greatest attention in neurobiological studies of mood disorders have been the monoamines since they are extensively distributed throughout the network of limbic, striatal and prefrontal cortical neuronal circuits thought to support the behavioural and visceral manifestations of mood disorders. Assessments of CSF chemistry, neuroendocrine responses to pharmacological challenge and neuroreceptor binding have demonstrated a number of abnormalities of the serotonergic, noradrenergic and other neurotransmitter and neuropeptide systems in mood disorder (Garlow et al., 1999). The observations that clinical effects of mood stabilizers are only observed after chronic (days to weeks) administration and that regulation of cellular signalling transduction pathways play a major role in the therapeutic effects of lithium and mood stabilizers, have led to the agreement that while dysfunction within the monoaminergic neurotransmitter systems is likely to play important roles in mediating some aspects of the pathophysiology of mood disorders, it probably represents the epiphenomena of more primary abnormalities (Manji and Lenox, 2000; Manji et al., 2001). Thus the most recent research into the pathophysiology and treatment of bipolar disorders has focused on intracellular signalling pathways (Vawter et al., 2000).

Studies of receptor and post-receptor function in mood disorders have been limited to indirect strategies, such as characterization of receptor function in readily accessible blood elements.

Among these pathways, the upstream components of cAMP signalling have been extensively investigated. In particular, a large number of studies has demonstrated alterations in the levels and function of Gαs and Gαi, as well as in the activity of AC and PKA-mediated phosphorylation in peripheral cells and the post-mortem brain of patients with affective disorders (Perez et al., 1999, 2000; Warsh and Li, 1996; Warsh et al., 2000; Zanardi et al., 1997). In view of the indirect evidence for abnormalities at post-receptor sites, it is not surprising that several independent laboratories have examined G proteins in patients with mood disorders. Increased levels of Gαs as well as increases in forskolin-stimulated AC activity have been described in post-mortem brains of bipolar patients (Young et al., 1993).

Despite the clear role of modulation of PKC activity in the mechanism of action of mood stabilizers, there have only been a limited number of studies directly examining PKC in bipolar patients (Jope et al., 1996; Soares and Mallinger, 1997). When measuring PKC isozyme levels, activity, and translocation in post-mortem brain tissue from bipolar patients, Wang and Friedman (1996) reported increased PKC activity and translocation in bipolar disorder brains, compared to control subjects, effects that were accompanied by elevated levels of selected PKC isozymes in cortices of bipolar disorder subjects.

Beyond second messengers

Any relevant biochemical models proposed for the effects of mood stabilizers must account for their special temporal clinical profile; patterns of effects requiring prolonged administration of the drug suggest alterations at the genomic level. The putative effects of mood stabilizers on gene expression have been recently investigated by examining their effects on the DNA-binding activity of transcription factors, especially the AP-1 family of transcription factors (Chen et al., 1997; Chen et al., 1999c; Ozaki and Chuang, 1997; Yuan et al., 1998). AP-1 is a collection of homodimeric and heterodimeric complexes composed of products from two transcription factor families, Fos and Jun. These products bind to a common DNA site (known as the TPA response element) in the regulatory domain of the gene and activate gene transcription in response to PKC activators, growth factors, cytokines, and other agents (including neurotransmitters). The genes known to be regulated by the AP-1 family of transcription factors in the brain include genes for various neuropeptides, neurotrophins, receptors, transcription factors, enzymes involved in neurotransmitter biosynthesis, and proteins that bind to cytoskeletal elements. It has recently been demonstrated that both lithium and VPA, at therapeutically relevant concentrations, produce a time- and concentration-dependent increase in the AP-1 DNA-binding activity that translates into changes at the gene expression level (Asghari et al., 1998).

The precise mechanism by which lithium and VPA regulate AP-1 DNA binding activity remains

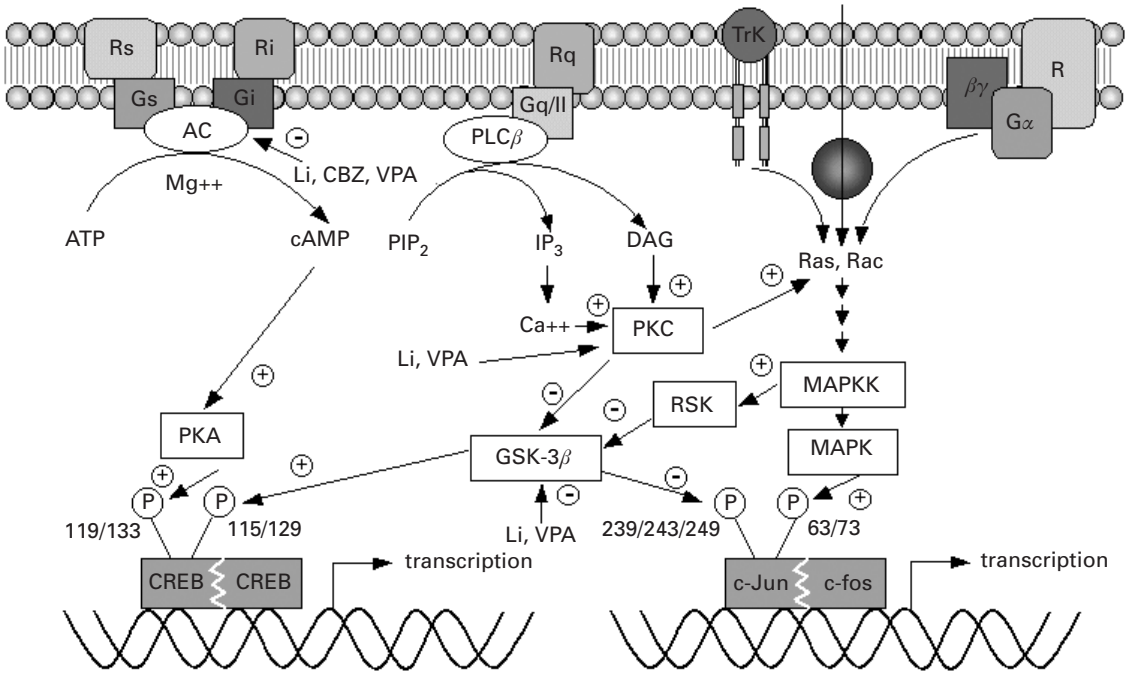

Figure 1. Effect of mood stabilizers on signalling pathways and gene transcription mediated by AP-1. In addition to regulating the activity of β-catenin, GSK-3β also regulates other transcription factors. Two important factors are Jun and cAMP response-element-binding protein (CREB). GSK-3 phosphorylation of Jun inhibits its binding to DNA, and because Jun is a subunit of activator protein-1 (AP-1), this can explain why lithium promotes AP-1 activity in human neuronal cell lines. By contrast, GSK-3β phosphorylation of CREB activates gene expression. CREB also requires phosphorylation by the cAMP-dependent protein kinase (PKA), an enzyme regulated through adenylate cyclase coupled receptors. These receptors are a major target of antidepressants and provide a possible mechanism by which lithium could enhance the effects of these drugs. (Adapted from Chen et al., 1999a.)

to be fully delineated, but may involve effects on mitogen-activated protein (MAP) kinases. PKC activates certain MAP kinases, which phosphorylate c-Jun thus enhancing the formation of the active AP-1 complex (Figure 1).

Interestingly it has recently been demonstrated that lithium as well as VPA inhibit the activity of a specific kinase, glycogen synthase kinase 3β (GSK-3β) (Chen et al., 1999d; Stambolic et al., 1996; Williams and Harwood, 2000). GSK-3β plays an important role in the CNS by regulating various cytoskeletal processes through its effects on Tau and Synapsin I and by inducing long-term nuclear events through phosphorylation of c-Jun and nuclear translocation of β-catenin. GSK-3β is known to phosphorylate c-Jun at sites adjacent to the DNA binding domain, thereby reducing AP-1 binding. Thus, the acute inhibition of GSK-3β by lithium and VPA has the potential to bring about long-term changes in the CNS through the transcriptional activity of both β-catenin and AP-1 (Figure 1).

Unlike many protein kinases, GSK-3β is highly active in resting cells and is primarily regulated by inactivation. Several recent studies have found that inhibition of GSK-3β by lithium reduces the phosphorylation of microtubule associated protein (MAPs) such as tau and MAP-1B (Hong et al., 1997). The intracellular neurofibrillary tangles found in Alzheimer's disease are composed of straight and paired helical filaments that contain an aberrantly hyperphosphorylated form of the MAP, tau. Even if GSK-3β is only one of the kinases involved in phosphorylating tau, inhibition of GSK-3β by lithium may reduce levels of hyperphosphorylated tau.

By acting on transcription factors, mood-stabilizing drugs have the potential to regulate the expression of a number of critical genes in the CNS (Manji et al., 1999a). One of the genes whose expression is markedly increased by lithium and VPA treatments encodes another transcription factor, the β-subunit of the polyomavirus enhancer-binding protein 2 (PEBP2β), involved in the regulation of the major neuroprotective protein Bcl-2 (B-cell lymphoma/leukaemia 2). Chronic treatment of rodents with therapeutic doses of lithium and VPA was found to produce

a doubling of Bcl-2 levels in the frontal cortex, an effect accompanied by a marked increase in the number of Bcl-2 immunoreactive cells in frontal cortex layers II and III (Manji et al., 2000a). Moreover, lithium has very recently been demonstrated to reduce the levels of the pro-apoptotic protein p53 (Chen and Chuang, 1999). Thus, overall the data clearly shows that chronic lithium robustly increases the levels of the neuroprotective protein Bcl-2 and at least in cultured cell systems, reduces the levels of the pro-apoptotic protein p53. These data suggest that mood-stabilizer treatment should result in neuroprotective effect (Manji et al., 1999b, 2000b; Chuang et al., 2002).

Consistent with these neuroprotective effects, lithium has been shown to increase grey-matter volume in bipolar patients (Moore et al., 2000a), to increase the levels of *N*-acetyl-aspartate, a putative marker of neuronal viability, in bipolar patients and healthy volunteers (Moore et al., 2000b), and to enhance neurogenesis in the rat hippocampus (Chen et al., 2000). Lithium has also been demonstrated to exert robust effects against diverse insults both in vivo and in vitro, including the robust inhibition of brain infarct volume and neuronal death by lithium pretreatment in rodent models of CNS disease (Nonaka and Chuang, 1998).

In this context it is of note that recent neuroimaging studies have demonstrated significant reductions in regional CNS volume and cell numbers (both neurons and glia), findings that are complemented by postmortem observations of cell atrophy and loss (Drevets et al., 1999; Drevets, 2000). It is still unclear whether these findings represent neurodevelopmental abnormalities, disease progression that fundamentally involves loss of glia and neurons, or the sequelae of the biochemical changes accompanying repeated affective episodes per se.

Several studies have been undertaken to unravel the molecular mechanisms underlying the neurotrophic and neuroprotective effects of lithium and VPA. It is widely accepted that several endogenous growth factors exert many of their neurotrophic effects via the MAP kinase signalling cascade, known to play an important role in mediating long-term neuroplastic events. Recent studies have demonstrated that both lithium and VPA activate the ERK MAP kinase cascade, which may contribute to the long-term changes in synaptic plasticity and morphology that follow chronic treatment (Yuan et al., 2001). One target of the MAP kinase cascade is the phosphorylation of CREB, which leads to increased expression of the anti-apoptotic factor Bcl-2. Together, the regulation of different signalling pathways brings about an enhancement of synaptic connectivity potentially necessary for long-term stabilization of mood.

In conclusion it is becoming more and more evident that rather than looking for a single site of action, many different actions should be integrated to obtain a cohesive picture of how neuronal function is modulated by long-term exposure to mood stabilizers. Lithium and mood stabilizers modulate excitatory and inhibitory synapses and adjust signalling activities regulating second messengers, transcription factors, and gene expression. Multiple actions may be necessary because lithium has anti-manic, antidepressant, and prophylactic stabilizing actions, and because bipolar disorder is a complex illness involving multiple systems.

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