Clinical and Preclinical Evidence for the Neurotrophic Effects of Mood Stabilizers: Implications for the Pathophysiology and Treatment of Manic–Depressive Illness

Husseini K. Manji, Gregory J. Moore, and Guang Chen

Recent neuroimaging studies have demonstrated regional central nervous system volume reductions in mood disorders, findings that are complemented by postmortem observations of cell atrophy and loss. It is thus noteworthy that lithium and valproate have recently been demonstrated to robustly increase the expression of the cytoprotective protein bcl-2 in the central nervous system. Chronic lithium not only exerts neuroprotective effects in several preclinical paradigms but also enhances hippocampal neurogenesis. Valproate robustly promotes neurite outgrowth and activates the ERK mitogen-activated protein kinase pathway, a signaling pathway utilized by many endogenous neurotrophic factors. Consistent with its preclinical neurotrophic/neuroprotective effects, chronic lithium treatment of patients with manic–depressive illness increases brain N-acetylaspartate (a putative marker of neuronal viability and function) levels, an effect that is localized almost exclusively to gray matter. To determine if lithium was producing neuropil increases, quantitative three-dimensional magnetic resonance imaging studies were undertaken, which revealed that chronic lithium significantly increases total gray matter volume in the human brain of patients with manic–depressive illness. Together, these results suggest that a reconceptualization about the optimal long-term treatment of recurrent mood disorders is warranted. Optimal long-term treatment for these severe illnesses may only be achieved by the early use of agents with neurotrophic/neuroprotective effects, irrespective of the primary, symptomatic treatment. Biol Psychiatry 2000;48:740–754 © 2000 Society of Biological Psychiatry

Key Words: Mood disorders, lithium, valproate, manic–depressive illness, bcl-2, MAP kinase, neurite, neurotrophic, N-acetylaspartate, gray matter

Introduction

Manic–depressive illness (MDI) is a common, severe, chronic, often life-threatening illness (Goodwin and Jamison 1990). Suicide is the cause of death in 10–20% of individuals with MDI, and the risks of suicide in MDI may be higher than those in unipolar depression. In addition to suicide, major mood disorders are also associated with many other deleterious health-related effects, and the costs associated with disability and premature death represent an economic burden of tens of billions of dollars annually in the United States alone (for a review, see Goodwin and Jamison 1990). Despite extensive research, the biochemical abnormalities underlying the predisposition to and the pathophysiology of MDI remain to be fully elucidated (Joffe and Young 1997). Although mood disorders have traditionally been regarded as “good prognosis diseases,” a growing body of data suggests that the long-term outcome for many patients is often much less favorable than previously thought. Indeed, according to the Global Burden of Disease Study, MDI is one of the leading causes of disability worldwide (Murray and Lopez 1997a, 1997b). In this context, although mood disorders were classically viewed as recurring conditions with essentially well periods between episodes, it has been increasingly recognized that interepisode recovery is incomplete in many patients, with a progressive decline in overall functioning (Goldberg and Harrow 1999).

Are Mood Disorders Associated with Structural Brain Changes?

In view of the deteriorating long-term clinical course observed in many patients (vide supra), it is not surprising that recent studies have been investigating potential structural brain changes in mood disorders. It is noteworthy that although mood disorders have traditionally been conceptualized as neurochemical disorders, there is now considerable literature support from a variety of sources demon-
and laminar thickness in MDI subjects completing suicide. Philosophical techniques to demonstrate decreased cortical volume and cell numbers in mood disorders. Rajkowska unipolar depression or MDI. Several recent postmortem postmortem brain samples obtained from patients with the right putamen, and bilateral pallidum externum in reported reduced volumes of the left nucleus accumbens, (Baumann and Bogerts, in press; Baumann et al 1999) reduction in interneurons in the anterior cingulate cortex in BD (Vincent et al 1997) and reductions in nonpyramidal neurons in the DLPFC and orbitofrontal cortices in mood disorders (Rajkowska 2000) glial cell number and density in the DLPFC in mood disorders (Rajkowska 2000) glia (but not neurons) in the subgenual PFC in familial MDD (24% reductions) and BD (41% reductions; Drevets 2000)

Table 1. Postmortem Morphometric Studies Suggesting Cell Death and/or Atrophy in Mood Disorders

<table>
<thead>
<tr>
<th>Study</th>
<th>Findings</th>
</tr>
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<tbody>
<tr>
<td>- Layer-specific reduction in interneurons in the anterior cingulate cortex in BD (Vincent et al 1997)</td>
<td>- nonpyramidal neurons in the CA2 region in BD (and schizophrenia; Benes et al 1998)</td>
</tr>
<tr>
<td>- cortical/laminar thickness in BD (suicide; Rajkowska 1997)</td>
<td>- volumes of (L) nucleus accumbens, bilateral basal ganglia (Baumann and Bogerts, in press; Baumann et al 1999)</td>
</tr>
<tr>
<td>- density and size of cortical neurons in the DLPFC and orbitofrontal cortices in mood disorders (Rajkowska 2000)</td>
<td>- glial cell number and density in the DLPFC in mood disorders (Rajkowska 2000)</td>
</tr>
<tr>
<td>- glial cell number and density in the DLPFC in mood disorders (Rajkowska 2000)</td>
<td>- glia (but not neurons) in the subgenual PFC in familial MDD (24% reductions) and BD (41% reductions; Drevets 2000)</td>
</tr>
</tbody>
</table>

BD, bipolar disorder; DLPFC, dorsolateral prefrontal cortex; PFC, prefrontal cortex; MDD, major depressive disorder.

Stratifying that mood disorders are also associated with significant reductions in regional central nervous system (CNS) volume and cell numbers (both neurons and glia). One line of evidence comes from structural imaging studies, which have recently begun to provide important clues about the neuroanatomic basis of mood disorders. In toto, the majority of the volumetric neuroimaging studies have demonstrated an enlargement of third and lateral ventricles, as well as reduced basal ganglia and frontal cortical volumes (for reviews, see Drevets et al 1999; Sheline 2000). Within the frontal lobe, volumetric neuroimaging studies have fairly consistently shown reduced volumes; in particular, recent volumetric magnetic resonance imaging (MRI) studies in familial bipolar depressives and familial unipolar depressives have demonstrated reductions in the mean gray matter volume of approximately 40% in the prefrontal cortex (PFC) ventral to the genu of the corpus callosum (Drevets 2000). Lending support to the structural neuroimaging literature are multiple functional brain imaging studies that have shown abnormalities in metabolic rate and blood flow in the striatal, frontal, and temporal regions in mood disorders (for reviews, see Drevets et al 1999; Mayberg et al 2000). In addition to the accumulating neuroimaging evidence, several postmortem brain studies are now providing direct evidence for reductions in regional CNS volume and cell numbers (Table 1). Thus, studies have reported a layerspecific reduction in interneurons in the anterior cingulate cortex (Vincent et al 1997) and reductions in nonpyramidal neurons (~40% lower) in CA2 of the hippocampal formation in MDI subjects, as compared with control subjects (Benes et al 1998). Baumann and associates (Baumann and Bogerts, in press; Baumann et al 1999) reported reduced volumes of the left nucleus accumbens, the right putamen, and bilateral pallidum externum in postmortem brain samples obtained from patients with unipolar depression or MDI. Several recent postmortem studies of the PFC have also demonstrated reduced CNS volume and cell numbers in mood disorders. Rajkowska (1997) has used three-dimensional cell counting and morphological techniques to demonstrate decreased cortical and laminar thickness in MDI subjects completing suicide. In thorough follow-up series from the same group, morphometric analysis of the density and size of cortical neurons in the dorsolateral PFC and orbitofrontal cortex revealed significant reductions in mood disorders. Additionally, unexpected reductions in the glial cell number and density were also observed (Rajkowska 2000). Also in the PFC, a histologic study of area sg24 located in the subgenual PFC found striking reductions in glial cell numbers in patients with familial major depression (24% reductions) and MDI (41% reductions), as compared with control subjects (Drevets 2000). This striking finding is consistent with this group’s neuroimaging findings showing cortical volume loss in this same region on volumetric MRI in a similar diagnostic group. Most recently, Drevets and associates have investigated the effects of medications on the volumetric changes observed in the subgenual PFC; in view of lithium and valproate’s robust effects on the levels of the cytoprotective protein bcl-2 in the frontal cortex (discussed in detail later), Drevets and associates reanalyzed their data to determine if there was an effect of medication use. Entirely consistent with a neurotrophic/neuroprotective role for bcl-2, they found that the patients treated with lithium or valproate exhibited subgenual PFC volumes that were significantly higher than the volumes in non–lithium- or valproate-treated patients and not significantly different from those of control subjects.

Together, the majority of the data from the neuroimaging studies and the growing body of postmortem evidence present a convincing case that there is indeed a reduction in regional CNS volume, accompanied by a reduction in cell numbers in at least a subset of patients with mood disorders. It remains to be elucidated if these findings represent neurodevelopmental abnormalities, disease progression that fundamentally involves loss/atrophy of glia and neurons, or the sequelae of the biochemical changes (e.g., in glucocorticoid levels) accompanying repeated affective episodes per se. In support of the latter, chronic stress or glucocorticoid administration has been demonstrated to produce atrophy or even death of vulnerable hippocampal neurons in rodents and primates, and MRI studies have also revealed reduced hippocampal volumes in patients with Cushing disease and posttraumatic stress...
disorder. The distinction between repeated episodes and disease progression is not a trivial one, since it may be possible to utilize therapeutic strategies to reduce the cell loss/atrophy associated with disease progression, even if these agents do not have a major impact on the intensity/frequency of the affective episodes per se.

**Can Disease-Related CNS Cell Death or Atrophy Be Attenuated or Reversed?**

As discussed above, it is now clear that mood disorders are often associated with cell loss and atrophy; these structural brain changes may contribute to the poor long-term outcome observed in many patients. Thus, the development of strategies to attenuate or even reverse regional brain cell loss/atrophy may be critical for improved long-term outcome. Are such therapeutic strategies even plausible? The dependence of neuronal survival on specific “survival factors” and genetic programs represents an intricate and elegant scheme by which much of the establishment, molding, and refining of neuronal circuitry occurs physiologically. A growing body of data has shown that many of the same pathways may also be involved in the cell death and atrophy that occurs pathologically in certain disorders. With the realization that disease-related cell death may arise from aberrantly activated gene-directed processes and/or the absence of critical trophic signals, the loss or atrophy of large numbers of cells in the CNS no longer has to be accepted as an unavoidable fate.

In addition to the identification of specific cellular pathways that may be involved in regulating cell survival, it has recently been demonstrated that neurogenesis occurs in the adult human brain (Eriksson et al 1998; Kempermann and Gage 1999). The localization of pluripotent progenitor cells and neurogenesis occurs in restricted brain regions. The greatest density of new cell birth is observed in the subventricular zone and the subgranular layer of the hippocampus. Cells born in the subventricular zone migrate largely to the olfactory bulb, and those in the subgranular zone into the granule cell layer. A recent study has demonstrated that new neurons, originating from the subventricular zone, are found also in areas of the association cortex of nonhuman primates (Gould et al 1999b). The newly generated neurons send out axons and appear to make connections with surrounding neurons, indicating that they are capable of integrating into the appropriate neuronal circuitry in the hippocampus and cerebral cortex. Neurogenesis in the hippocampus is increased by enriched environment, exercise, and hippocampal-dependent learning (Gould et al 1999a, 2000; Kempermann et al 1997; van Praag et al 1999). Upregulation of neurogenesis in response to these behavioral stimuli and the localization of this process to the hippocampus and association cortex have led to the proposal that new cell birth is involved in learning and memory (Gould et al 1999a, 2000).

Recent studies have shown that decreased neurogenesis occurs in response to both acute and chronic stress (Gould et al 2000). Removal of adrenal steroids (i.e., adrenalectomy) increases neurogenesis, and treatment with high levels of glucocorticoids reproduces the downregulation of neurogenesis that occurs in response to stress. Aging also influences the rate of neurogenesis. Although neurogenesis continues into late life, the rate is significantly reduced. The decreased rate of cell birth could result from upregulation of the hippocampal–pituitary–adrenal axis and higher levels of adrenal steroids that occur in later life. Lowering glucocorticoid levels in aged animals restores neurogenesis to levels observed in younger animals, indicating that the population of progenitor cells remains stable but is inhibited by glucocorticoids (Cameron and McKay 1999). These observations raise the interesting possibility that corticotropin-releasing factor antagonists, currently being developed for the treatment of mood and anxiety disorders, may have particular utility in the treatment of elderly depressed patients. Also of potential relevance to our understanding of the neurobiology and treatment of mood disorders, ovariectomy decreases the proliferation of new cells in the hippocampus, effects that are reversed by estrogen replacement (Gould et al 2000). The rate of neurogenesis fluctuates over the course of the estrus cycle in rodents, and the total rate of cell birth is higher in female rodents. In addition to potentially playing a role in the beneficial cognitive effects of estrogen, the regulation of neurogenesis by this gonadal steroid may also provide important clues about certain sexually dimorphic characteristics of mood disorders. These recent advances in our understanding of the factors that regulate cell survival and neurogenesis have led to considerable excitement about the prospect of pharmacologic intervention in the adult mammalian brain (Chen et al 2000; Kempermann and Gage 1999).

**The Discovery of bel-2 as a Therapeutically Relevant Target for the Actions of Lithium and Valproate: The Successful Application of a Concerted mRNA Differential Display Strategy to Identify Novel Target Genes**

It has become increasingly appreciated in recent years that the long-term treatment of mood disorders likely involves the strategic regulation of signaling pathways and gene expression in critical neuronal circuits (Hyman and Nestler 1996; Lenox et al 1998; Manji et al 1995; Wang and Young 1996). In this context, several independent laboratories have now demonstrated that lithium and valproate, at therapeutically relevant concentrations, produce com-
plex alterations in basal and stimulated DNA binding to activator protein 1 (AP-1) transcription factors (Asghari et al 1999; Chen et al 1999a; Jope 1999; Manji et al 2000a; Ozaki and Chuang 1997). Together, these data suggest that lithium and valproate, via their effects on the AP-1 family of transcription factors, may bring about strategic changes in gene expression in critical neuronal circuits, effects that may ultimately underlie its efficacy in the treatment of a very complex neuropsychiatric disorder. Although many genes that are the targets of long-term lithium and valproate have indeed been identified, it has been estimated that ∼10,000–15,000 genes may be expressed in a given cell at any time, and thus additional, novel methodologies are clearly required to study the complex pattern of gene expression changes induced by chronic drug treatment. One methodology that has been successfully utilized to identify the differential expression of multiple genes (e.g., in pathologic vs. normal tissue, or in control vs. treated tissue) is reverse transcription polymerase chain reaction messenger RNA (mRNA) differential display (Liang et al 1995). Using this method, Wang and Young (1996) were the first to make the novel observation that lithium increased 2′,3′-cyclic nucleotide 3′-phosphodiesterase mRNA levels in C6 glial cells. Subsequent studies have also utilized this methodology to identify novel genes that may be the targets for the actions of mood stabilizers. A major problem inherent in neuropharmacologic research, however, is the dearth of phenotypic changes clearly associated with treatment response, particularly for mood-stabilizing agents (Ikononov and Manji 1999). In the absence of clear-cut phenotypic changes, we have attempted to overcome this experimental hurdle by utilizing paradigms that involve the identification of common long-term molecular targets of structurally dissimilar mood-stabilizing agents (lithium and valproate) when administered chronically in vivo (Chen et al 1999c). These are two distinct agents that undoubtedly also exert a variety of dissimilar effects; however, the identification of genes affected by both agents, when administered in a variety of dissimilar effects; however, the identification of genes affected by both agents, when administered chronically in vivo (Chen et al 1999c). These are two distinct agents that undoubtedly also exert a variety of dissimilar effects; however, the identification of genes affected by both agents, when administered in a variety of dissimilar effects; however, the identification of genes affected by both agents, when administered chronically in vivo (Chen et al 1999c). It was further demonstrated that PEBP2β’s function (DNA binding of the PEBP2 αβ complex) was also clearly increased in the frontal cortex by both lithium and valproate, but not by chronic amphetamine or benzodiazepine. One critical gene whose expression is known to be regulated by PEBP2β is the major neuroprotective protein bcl-2; it was subsequently demonstrated that chronic treatment of rats with both agents resulted in a doubling of bcl-2 levels in the frontal cortex. Furthermore, immunohistochemical studies showed that chronic treatment of rats with lithium or valproate resulted in a marked increase in the number of bcl-2-immunoreactive cells in layers II and III of the frontal cortex. Interestingly, the importance of neurons in layers II–IV of the frontal cortex in mood disorders has recently been emphasized, since primate studies have indicated that these are important sites for connections with other cortical regions, and major targets for subcortical input (Rajkowska et al 1999). Chronic lithium also markedly increased the number of bcl-2–immunoreactive cells in the dentate gyrus and striatum (Manji et al 1999); detailed immunohistochemical studies following chronic valproate treatment are currently underway. It was subsequently demonstrated that lithium also increases bcl-2 levels in C57BL/6 mice (Chen et al 2000), and in human neuroblastoma SH-SY5Y cells in vitro (Manji et al 2000b); our demonstration of a lithium-induced increase in bcl-2 levels has also been convincingly replicated in rat cerebellar granule cells (Chen and Chuang 1999) in recent studies. This latter study was undertaken to investigate the molecular and cellular mechanisms underlying the neuroprotective actions of lithium against glutamate excitotoxicity (vide infra). These investigators found that lithium produced a remarkable increase in bcl-2 protein and mRNA levels. Moreover, lithium has very recently been demonstrated to reduce the levels of the pro-apoptotic protein p53 in both cerebellar granule cells (Chen and Chuang 1999) and SH-SY5Y cells (Lu et al 1999). Thus, overall the data clearly show that chronic lithium robustly increases the levels of the neuroprotective protein bcl-2 in areas of the rodent frontal cortex, hippocampus, and striatum in vivo, and in cultured cells of both rodent and human neuronal origin in vitro; furthermore, at least in cultured cell systems, lithium has also been demonstrated to reduce the levels of the proapoptotic protein p53.

Bcl-2 (an acronym for the B-cell lymphoma/leukemia-2 gene) was the first identified member of a large family of cellular and viral apoptosis-regulating proteins (Adams and Cory 1998; Bruckheimer et al 1998; Merry and Korsmeyer 1997). The bcl-2 family is the best characterized protein family involved in the regulation of apoptotic cell death, and consists of both antiapoptotic (e.g., bcl-2 and bcl-XL) and proapoptotic members (e.g., Bax and Bad), several of which are expressed in the rodent and mammalian CNS. bcl-2 attenuates apoptosis by sequestering proforms of death-driving cysteine proteases (called caspases), by preventing the release of mitochondrial apoptogenic factors such as calcium, cytochrome c, and apoptosis-inducing factor into the cytoplasm, and by enhancing mitochondrial calcium uptake (for reviews, see Adams and Cory 1998; Bruckheimer et al 1998). Increasing evidence suggests a critical role for the mitochondria
in the process of apoptosis, and studies have shown that mitochondria undergo major changes in membrane integrity before classic signs of apoptosis become manifest, leading to a disruption of the inner transmembrane potential ($\Delta \Psi_m$) and the release of intermembrane proteins through the outer membrane; bcl-2 acts on mitochondria to stabilize membrane integrity and to prevent opening of the permeability transition pore. Lithium, via its effects on bcl-2 and p53, may exert effects on the mitochondrial permeability transition pore, a key event in cell death. Lithium and VPA also inhibit glycogen synthase kinase 3B (GSK-3B), biochemical effects shown to have neuroprotective effects. Valproate also activates the ERK mitogen-activated protein (MAP) kinase pathway, an effect that may play a major role in neurotrophic effects and neurite outgrowth. BDNF, brain-derived neurotrophic receptor; trkB, tyrosine kinase receptor for BDNF; GRB2, scaffolding protein with src homology domains to coordinate MAP kinase signaling pathways; SOS, son of sevenless—facilitates guanine nucleotide exchange; GAPs, guanosine triphosphatase–activating proteins; Ras, Raf, MEK, ERK, and Elk1, components of the ERK MAP kinase pathway; RSK, ribosomal S-6 kinase; CREB, cyclic adenosine monophosphate response element–binding protein; PTP, mitochondrial permeability transition pore; p53 and BAX, proapoptotic proteins.

shown to protect neurons from a variety of insults both in vitro and in vivo (for reviews, see Adams and Cory 1998; Bruckheimer et al 1998; Table 2). Overexpression of bcl-2 in transgenic mice has been shown to prevent motor neuron and retinal ganglion death, to protect cells from the deleterious effects of N-methyl-D-aspartate (NMDA) or focal ischemia, to protect photoreceptor cells from inherited retinal degeneration, and to prolong survival and attenuate motor neuron degeneration in a transgenic animal model of amyotrophic lateral sclerosis (discussed in Adams and Cory 1998; Bruck-
Table 2. Neuroprotective Effects of bcl-2 and Lithium

<table>
<thead>
<tr>
<th>bcl-2</th>
<th>Lithium</th>
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<tbody>
<tr>
<td>Protects against the lethal</td>
<td>Protects cultured neurons against glutamate and NMDA-induced cell</td>
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<td>effects of a variety of</td>
<td>death</td>
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<tr>
<td>reactive oxygen species</td>
<td>Protects cerebellar granule cells from KCl deprivation and age- or</td>
</tr>
<tr>
<td>generating insults</td>
<td>drug-induced apoptosis</td>
</tr>
<tr>
<td>Protects against MPTP and</td>
<td>Protects SH-SYSY cells from ouabain toxicity</td>
</tr>
<tr>
<td>AMPA neurotoxicity</td>
<td>Protects SH-SYSY cells from Ca(^{2+}) and MPP(^{+}) toxicity</td>
</tr>
<tr>
<td>Protects against growth</td>
<td>Protects cells from deleterious effects of glycogen synthase kinase</td>
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<tr>
<td>factor deprivation</td>
<td>3β overexpression coupled to staurosporine addition</td>
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<tr>
<td>Protects against the</td>
<td>Protocols cultured rat cortical neurons from β-amyloid toxicity</td>
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<tr>
<td>effects of ionizing radiation</td>
<td>Reduces behavioral deficits and choline acetyltransferase activity</td>
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<td>induced by cholinergic lesions</td>
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<td></td>
<td>Reduces MCA occlusion-induced infarct size and neurologic deficits</td>
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<td></td>
<td>Protects neurons in the striatum from quinolinic acid toxicity</td>
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| ALS, amyotrophic lateral     | Does Inhibition of Glycogen Synthase Kinase 3β (GSK-3β) Play a Role in  |
| sclerosis (ALS), N-methyl-d- | the Therapeutic Effects of Mood Stabilizers?                           |
| aspartate (NMDA), MPP\(^{+}\), | Klein and Melton (1996) were the first to make the seminal observation |
| 1-methyl-4-phenylpyridinium   | that lithium, at therapeutically relevant concentrations, is an inhibitor |
| ion (MCA), middle cerebral    | of GSK-3β. Glycogen synthase kinase 3β not only controls developmental   |
| artery. Summarized from      | patterns in diverse organisms (including mammals), but also plays an    |
| Adams and Cory 1998;         | important role in the mature CNS, by regulating various cytoskeletal     |
| Alvarez et al 1999;          | processes as well as long-term nuclear events via phosphorylation of    |
| Arendt et al 1999; Bijur et  | ε-actinin, nuclear translocation of β-catenin, and nuclear export of    |
| al 2000; Bonfanti et al 1996;| nuclear factor of activated T cells (for reviews, see Dale 1998; Jope    |
| Bruckheimer et al 1998;      | 1999; Willert and Nusse 1998). Glycogen synthase kinase 3β has also     |
| Chen et al 1997; 2000;       | been implicated in regulating the phosphorylation of tau and β-catenin,  |
| Chuang et al 1999; D’Mello    | both of which have been implicated in certain types of disease-related   |
| et al 1994; Grignon et al    | neuronal death (discussed in Dale 1998; Manji et al 1999; Nishimura et  |
| al 1997; Li et al 1994;       | a catalytically active GSK-3 has been shown to induce apoptosis of PC12   |
| Lovestone et al 1999; Lu et al| cells, and dominant-negative GSK-3 prevents apoptosis following inhibition |
| et al 1999; Manji et al 1999;| of PI-3K (Pap and Cooper 1998). Furthermore, overexpression of GSK-3β   |
| Merry and Korsmeyer 1997;     | (to levels 3.5 times that in control cells) has been demonstrated to     |
| Munoz-Montano et al 1997;    | potentiate staurosporine-induced caspase activation as well as          |
| Nonaka and Chuang 1998;      | morphological changes indicative of apoptosis (Bijur et al 2000). Thus,   |
| Nonaka et al 1998a, 1998b;    | inhibition of GSK-3β by lithium may also afford protection against the   |
| Sparapani et al 1997;        | cell death induced by certain stimuli (Hetman et al 2000). In this       |
| Volonte and Rakenstein 1993. | context, several recent studies have found that inhibition of GSK-3β    |
| (Modified and reproduced with| by lithium reduces tau phosphorylation, effects that likely occur to    |
| permission from Manji et al  | some degree at therapeutically relevant lithium concentrations (see Jope |
| 1999.)                       | 1999 for an excellent discussion). Although many of the studies have     |
|                              | utilized lithium concentrations in excess of those utilized.            |

It is also noteworthy that recent studies have demonstrated that bcl-2 may mediate many of the downstream effects of several neurotrophic factors. Neurotrophic factors (e.g., nerve growth factor and brain-derived neurotrophic factor) are now known to promote cell survival by binding to membrane receptors (such as Trk A and Trk B) and regulating intracellular signal transduction pathways that can control apoptosis. The signal transduction cascades that have been identified include the mitogen-activated protein (MAP) kinase cascade and the phosphotyrosylinositol-3 kinase (PI-3K)/Akt pathway (Segal and Greenberg 1996; Tao et al 1998). Recent studies have demonstrated that the activation of the MAP kinase pathway can inhibit apoptosis by inducing the phosphorylation of Bad and increasing the expression of bcl-2, the latter effect likely involving the cAMP response element–binding protein (CREB; Bonni et al 1999; Riccio et al 1999). Phosphorylation of Bad occurs via activation of a downstream target of the MAP kinase cascade, ribosomal S-6 kinase (Rsk). Rsk phosphorylates Bad and thereby promotes its inactivation. Activation of Rsk also mediates the actions of the MAP kinase cascade and neurotrophic factors on the expression of bcl-2. Rsk can phosphorylate CREB, and this leads to induction of bcl-2 gene expression. Moreover, in addition to its neuroprotective effects, bcl-2 overexpression has also been shown to promote regeneration of axons in the mammalian CNS and regulate to neurite sprouting (Chen et al 1997). Thus, it has been convincingly argued that pharmacologic means of increasing CNS bcl-2 levels may represent a very effective therapeutic strategy for the treatment of many neurodegenerative diseases (Chen et al 1997).
therapeutically, the available data suggest that lithium, at concentrations of ~1 mmol/L does, indeed, reduce tau phosphorylation (Hong et al 1997; Jope 1999; Lovestone et al 1999; Munoz-Montano et al 1997). In view of the important role of GSK-3β in regulating cytoskeletal events and cell survival, a study was undertaken to determine if valproate also regulates GSK-3β; it was found that valproate also significantly inhibited GSK-3β at therapeutically relevant concentrations, and consistent with GSK-3β inhibition, incubation of SH-SY5Y cells with valproate resulted in a significant time-dependent increase in both cytosolic and nuclear β-catenin levels (Chen et al 1999b). Another independent laboratory has recently also demonstrated that valproate increases β-catenin levels and increases the expression of a reporter gene driven by β-catenin/lymphoid enhancer factor transcription factor (P.S. Klein, personal communication, March 2000). Most recently, it has been demonstrated that the chronic (3–4 week) administration of lithium and valproate also increase β-catenin levels in the rodent brain (G. Chen and H.K. Manji, unpublished observations), compatible with inhibition of GSK-3β during chronic in vivo administration of the agents under therapeutic paradigms.

Neuroprotective Effects of Lithium: Compelling Preclinical Evidence

Lithium’s robust effects on bcl-2 and GSK-3β in the mature CNS suggest that it may possess significant neuroprotective properties. Indeed, several studies conducted before the identification of bcl-2 or GSK-3β as targets for lithium’s actions had already demonstrated neuroprotective properties of lithium (Alvarez et al 1999; D’Mello et al 1994; Grignon et al 1996; Inouye et al 1995; Li et al 1994; Pascual and Gonzalez 1995; Volonte and Rukenstein 1993). The protective effects of lithium have been investigated in a number of in vitro studies, in particular using rat cerebellar granule cells, PC12 cells, and human neuroblastoma SH-SY5Y cells. In these studies, lithium has been shown to protect against the deleterious effects of glutamate, N-methyl-D-aspartate receptor activation, low potassium, and toxic concentrations of anticonvulsants (Table 2). Lithium also protects PC12 cells from serum/nerve growth factor deprivation (Volonte and Rukenstein 1993), protects both PC12 cells and human neuroblastoma SH-SY5Y cells from ouabain toxicity (Li et al 1994), and protects SH-SY5Y cells from both thapsigargin (which mobilizes intracellular Ca^{2+}) and 1-methyl-4-phenylpyridinium ion (MPP^+)–induced cell death (Figure 2). Most recently, lithium has been shown to protect cultured neurons from β amyloid–induced cell death (Alvarez et al 1999) and to protect against the deleterious effects of GSK-3β overexpression coupled to staurosporine addition (Bijur et al 2000; Table 2).

In addition to the demonstration of protective effects in vitro, a number of studies have also investigated lithium’s neuroprotective effects in vivo. In this context, studies have investigated the effects of lithium on the biochemical and behavioral manifestations of excitotoxic lesions of the cholinergic system (Arendt et al 1999; Pascual and Gonzalez 1995). These studies have demonstrated that lithium pretreatment attenuates both the behavioral deficits (passive avoidance and ambulatory behavior) and the reduction in choline acetyltransferase (ChAT) activity by forebrain cholinergic system lesions (Pascual and Gonzalez 1995). In a study that may have implications for the treatment of Alzheimer’s disease, rats received ibotenic acid lesions of cholinergic basal forebrain nuclei, resulting in a 30–40% depletion of both ChAT and acetylcholinesterase (AChE) activity (Arendt et al 1999). Lithium as well as tetrahydroaminoacridine, given separately either before or after the development of the lesion, had small but significant effects on the recovery of cortical ChAT and AChE activity. Intriguingly, when applied in combination the drugs clearly showed synergistic effects; however, considerable caution is required in the extrapolation of these results to the treatment of humans, since the coadministration of lithium and cholinesterase inhibitors has been shown to be capable of inducing seizures. In another study investigating lithium’s effects against excitotoxic insults, it was demonstrated that lithium attenuated the kainic acid–induced reduction in glutamate decarboxylase levels and ([3H]o)-aspartate uptake (Sparapani et al 1997). Chronic lithium has also been shown to exert dramatic protective effects against middle cerebral artery occlusion, reducing not only the infarct size (56%), but also the neurologic deficits (abnormal posture and hemiplegia; Nonaka and Chuang 1998). Most recently, the same research group has demonstrated that chronic in vivo lithium treatment robustly protects neurons in the striatum from quinolinic acid–induced toxicity, in a putative model of Huntington’s disease (Chuang et al 1999). Table 2 summarizes some of the most robust experimental evidence demonstrating neuroprotective effects of lithium in vitro and in vivo. In addition to its effects on bcl-2 and GSK-3β, lithium’s effects on other signaling pathways and transcription factors (Jope 1999; Manji et al 1995) may also contribute to its neuroprotective effects. In this context it is noteworthy that recent studies have shown that modulation of Akt-1 activity is involved in glutamate excitotoxicity, and may play a role in lithium’s neuroprotective effects in rat cerebellar granule cells (Chalecka-Franaszek and Chuang 1999).
Furthermore, Datta et al (1997) have demonstrated that Akt phosphorylation of BAD (a proapoptotic member of the bcl-2 family) blocks BAD-induced death of primary neurons. These results suggest that lithium’s effects on Akt-1 may also contribute to neuroprotective effects; however, such a contention awaits the demonstration of lithium-induced activation of Akt-1 in the CNS in vivo.

**Does Lithium Affect Neurogenesis?**

As discussed already, it has been shown, with a method for labeling cell division directly in the adult human brain, that the dentate gyrus (an area where robust lithium-induced increases in bcl-2 levels are observed) can produce new neurons during adulthood in humans. A large number of the newborn daughter cells are known to die rapidly, likely via apoptosis (Kempermann and Gage 1999). Thus, increasing bcl-2 levels could enhance the survival of the newborn cells,
allowing them to differentiate into neurons. Additionally, bcl-2 has been shown to have robust effects on the regeneration of CNS axons (Chen et al 1997). In view of bcl-2’s major neuroprotective and neurotrophic role, a study was undertaken to determine if lithium, administered at therapeutically relevant concentrations, affects neurogenesis in the adult rodent brain. Kempermann and Gage (1999) have suggested the use of neurogenesis to refer to a series of events (including proliferation of a neuronal precursor or stem cell, and survival of the daughter cells) that result in the appearance of a new neuron. To investigate the effects of chronic lithium on neurogenesis, mice were treated with “therapeutic” lithium (plasma levels 0.97 ± 0.20 mmol/L), for ~4 weeks. After treatment with lithium for 14 days, the mice were administered single doses of bromodeoxyuridine (BrdU), a thymidine analog that is incorporated into the DNA of dividing cells, for 12 consecutive days. Lithium treatment continued throughout the duration of the BrdU administration. Following BrdU immunohistochemistry (Chen et al 2000), three-dimensional cell counting was performed using a computer-assisted image analysis system (Rajkowska 2000). This system is based on the optical dissector method and estimates the number of cells independent of section thickness and cell shape. We found that chronic lithium administration does, indeed, result in an increase in the number of BrdU-positive cells in the dentate gyrus (Chen et al 2000). Moreover, approximately two thirds of the BrdU-positive cells also double stained with the neuronal marker NeuN, confirming their neuronal identity. Double staining of BrdU and bcl-2 was also observed, and studies using bcl-2 transgenic animals are currently underway to delineate the role of bcl-2 overexpression in the enhanced hippocampal neurogenesis observed.

Neurotrophic and Neuroprotective Effects of Valproate

Valproate’s effects on bcl-2 and GSK-3β suggest that this mood stabilizer may also possess neuroprotective/neurotrophic properties. Additionally, it has been recently demonstrated that valproate increases the expression of the molecular chaperone GRP78, a protein that binds Ca2+ in the endoplasmic reticulum, and protects cells from the deleterious effects of damaged proteins (Wang et al 1999). Although it is not as extensively studied as lithium, a growing body of data suggests that valproate does, indeed, exert neuroprotective effects (Bruno et al 1995; Mark et al 1995; Mora et al 1999). We have found that, similar to what has been observed in vivo, incubation of human neuroblastoma SH-SY5Y cells with either lithium (1.0 mmol/L) or valproate (0.6–1.0 mmol/L) significantly increases the levels of bcl-2. These results suggest that the in vivo increases in bcl-2 levels may be due, at least in part, to direct cellular effects of these agents (rather than secondarily due to alterations in synaptic input or long-loop feedback systems that are present in the in vivo condition). We have therefore utilized the SH-SY5Y model system to investigate valproate and lithium’s protective effects. SH-SY5Y cells were incubated with lithium (1.0 mmol/L) or valproate (0.6 mmol/L) for 3 days. Cells were then exposed to two different toxins—thapsigargin (which mobilizes intracellular calcium; 0.5 mmol/L for 16 hours) or MPP+ (25 μmol/L for 16 hours). The mitochondrial dehydrogenase activity that cleaves 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide was used to determine cell survival in a quantitative colorimetric assay. It was found that lithium and valproate treatment exerted significant protective effects against both toxins (Figure 2).

Robust Effects of Valproate on MAP Kinases

Although a growing body of data has shown that valproate increases AP-1 DNA binding activity and AP-1–mediated gene expression (vide supra), the mechanisms underlying these effects have not been fully elucidated. In this context, MAP kinases play a key role in the regulation of the AP-1 family of transcription factors (Gutkind 1998). Mitogen-activated protein kinases transmit extracellular signals to the nucleus, where the transcription of specific genes is induced by the synthesis, phosphorylation, and activation of transcription factors. Three distinct MAP kinase signal transduction pathways have been identified in mammalian cells, leading to activation of the MAP kinases—extracellular signal–regulated kinases (ERKs), c-Jun NH2-terminal kinases, and p38 (Gutkind 1998). Mitogen-activated protein kinases are abundantly present in the brain, and in recent years a broad role for the MAP kinase cascade in regulating gene expression in long-term forms of synaptic plasticity has been demonstrated (English and Sweatt 1997; Martin et al 1997; Roberson et al 1999). Thus, MAP kinases play important physiologic roles in the mature CNS and have been postulated to represent important targets for the actions of CNS-active agents (Nestler 1998; Yuan et al 1999; G. Chen et al, unpublished data).

In view of the important role of MAP kinases in mediating long-term neuroplastic events and in regulating AP-1 activity, we have undertaken a series of studies to determine if valproate regulates MAP kinases. We have found that valproate robustly activates the Ras/Raf/MEK/ERK MAP kinase pathway, as well as ERK/Elk-1–mediated gene expression (G. Chen et al, unpublished data). Since the ERK MAP kinases are known to mediate many of the effects of various neurotrophic factors (including...
Figure 3. Mechanism by which lithium may increase N-acetyl-aspartate (NAA) levels. Lithium, via its effects on bcl-2 and glycogen synthase kinase 3β (GSK-3β), may exert major neurotrophic effects, resulting in neuropil increases, accompanied by increases in NAA levels. (Modified and reproduced with permission from Manji et al 2000b.)

Figure 4. Brain gray matter volume is increased following 4 weeks of lithium administration at therapeutic levels in manic–depressive illness (MDI) patients. (a) A slice of the three-dimensional volumetric magnetic resonance imaging (MRI) data that was segmented by tissue type using quantitative methodology to determine tissue volumes at each scan time point. Brain tissue volumes were examined using high resolution three-dimensional MRI (124 1.5-mm–thick coronal T1-weighted spoiled gradient echo images) and validated quantitative brain tissue segmentation methodology to identify and quantify the various components by volume, including total brain white and gray matter content. Measurements were made at baseline (medication free, after a minimum 14-day washout) and then repeated after 4 weeks of lithium at therapeutic doses. Chronic lithium significantly increases total gray matter content in the human brain of patients with MDI. No significant changes were observed in brain white matter volume or in quantitative measures of regional cerebral water. (Modified and reproduced with permission from Moore et al, in press.)
nerve growth factor and brain-derived neurotrophic factor (BDNF) and to promote neurite outgrowth (Finkbeiner 2000; Segal and Greenberg 1996). We investigated valproate’s effects on the morphology of SH-SY5Y cells in detail. SH-SY5Y cells exposed to valproate’s (1.0 mmol/L) in serum-free media for 5 days exhibited prominent growth cones and long neurites. Growth cone–associated protein 43 (GAP-43) is a protein expressed at elevated levels during neurite growth during development or regeneration, and a greater than threefold increase in GAP-43 levels was observed after 5-day valproate exposure (G. Chen et al., unpublished data). bcl-2 is another protein known to play a key role in neuronal development and neurite growth, and 5-day valproate exposure resulted in greater than fivefold increase in bcl-2 levels. To further analyze the valproate-induced morphological changes, SH-SY5Y cells were seeded at a very low density and treated with valproate in serum-free media for 1 to 9 days. Valproate (0.5 mmol/L) dramatically increased neurite length approximately ninefold after 4-day incubation, and ~14-fold after 9-day incubation. In view of valproate’s apparent trophic effects, SH-SY5Y cells were grown in the presence of a therapeutic concentration of valproate without any additional neurotrophic factors. Remarkably, cells grown in the presence of valproate (but without other neurotrophic factors) continued to grow well for longer than 40 days.

As discussed, a variety of neurotrophins activate the ERK pathway via cell surface tyrosine kinase receptors (e.g., trkB), and ERK pathways are known to play a major role in neurotrophin-induced cell differentiation and neurite growth (Finkbeiner 2000; Segal and Greenberg 1996). Interestingly, recent studies have demonstrated that the chronic administration of a variety of antidepressants increases the expression of BDNF, neurotrophin-3, and trkB (Duman et al. 1997; Smith et al. 1995). It is thus noteworthy that valproate activates ERKs and promotes neurite growth in SH-SY5Y cells, effects that are characteristic of neurotrophic factors; these results, as well as the dramatic prolongation of the survival of SH-SY5Y cells grown in the absence of other trophic factors, suggests that valproate possesses neurotrophic properties and may thus have utility in the treatment of other neuropsychiatric disorders.

Can Lithium’s Neurotrophic/Neuroprotective Effects Be Demonstrated Longitudinally in Humans in the CNS In Vivo?

A clinical study was recently undertaken to determine if lithium also exerts neurotrophic/neuroprotective effects in the human brain in vivo. Proton magnetic resonance spectroscopy (MRS) is a tool that provides a noninvasive window to functional brain neurochemistry. N-Acetyl-aspartate (NAA) is one of the many neurochemical compounds that can be quantitatively assessed via MRS. N-Acetyl-aspartate is the predominant resonance in the proton MRS spectrum of the normal adult human brain, and although the functional role of this amino acid has not been definitively determined, NAA is a putative neuronal marker (Birken and Oldendorf 1989), localized to mature neurons and not found in mature glial cells, cerebrospinal fluid, or blood. A relative decrease in this compound may reflect decreased neuronal viability, neuronal function, or neuronal loss (for an excellent recent review of NAA, see Tsai and Coyle 1995). In a prospective longitudinal study, we have utilized quantitative in vivo proton MRS to test the hypotheses that 1) similar to the preclinical findings in the rodent brain and in human neuronal cells in culture, chronic lithium increases neuronal viability/function in the human brain in vivo, as evidenced by increased CNS levels of NAA, and 2) putative lithium-induced increases in NAA levels are positively correlated with regional brain gray matter content.

Proton MR spectra were acquired from 8-cm³ regions of interest (ROIs) in the frontal, temporal, parietal, and occipital lobes, with an acquisition time of 5 min per ROI (stimulated echo acquisition mode [STEAM] pulse sequence; echo time = 30 msec, modulation time = 13.7 msec, repetition time = 2000 msec; Moore et al. 1999). Two trained individuals analyzed the in vivo nuclear MR data with MRUI-VARPRO time domain spectral analysis software (deBeer et al. 1992; van den Boogaart et al. 1994); these individuals were blind to the study information and to each other’s results. After extensive validation of this method for in vivo measurement of regional NAA concentration, we have begun to apply this methodology in our studies of MDI patients and healthy volunteers undergoing lithium administration. It was found that chronic lithium administration at therapeutic doses increases NAA concentration in the human brain in vivo (Moore et al. 2000). These findings provide intriguing indirect support for the contention that, similar to the findings observed in the rodent brain and in human neuronal cells in culture, chronic lithium increases neuronal viability/function in the human brain. Furthermore, a striking ~.97 correlation between lithium-induced NAA increases and regional voxel gray matter content was observed (Moore et al. 2000), thereby providing evidence for colocalization with the regional specific bcl-2 increases observed (e.g., 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), but also exerts neurotrophic effects in humans (Figure 3).

In a follow-up study to the NAA findings, we hypothesized that, in addition to increasing functional neurochemical markers of neuronal viability, lithium-induced increases in bcl-2 would also lead to neuropil increases,
and thus to increased brain gray matter volume in MDI patients. In this clinical research investigation we examined brain tissue volumes using high resolution three-dimensional MRI (124 1.5-mm–thick coronal T1-weighted SPGR images) and validated quantitative brain tissue segmentation methodology to identify and quantify the various components by volume, including total brain white and gray matter content. Measurements were made at baseline (medication free, after a minimum 14-day washout) and then repeated after 4 weeks of lithium at therapeutic doses. This study revealed an extraordinary finding that chronic lithium significantly increases total gray matter content in the human brain of patients with MDI (Moore et al, in press; Figure 4). No significant changes were observed in brain white matter volume or in quantitative measures of regional cerebral water content, thereby providing strong evidence that the observed increases in gray matter content are likely due to neurotrophic effects as opposed to any possible cell swelling and/or osmotic effects associated with lithium treatment. A finer grained subregional analysis of this brain imaging data is ongoing. To our knowledge no other studies have examined the effects of chronic lithium treatment on brain tissue volume in MDI patients; however, the cross-sectional study by Drevets and associates demonstrating that lithium- or valproate-treated patients exhibited subgenual PFC gray matter volumes that were significantly higher than those of non–lithium- or non–VPA-treated patients, and the demonstration of gray matter–specific NAA increases with chronic lithium adds indirect support to these intriguing findings. Since it is believed that the majority of neuron-specific NAA is localized to the neurites rather than the cell body (Birken and Oldendorf 1989), the observed increase in NAA is likely due to expansion of neuropil content (Figure 3). Taken together, these exciting new results support the contention that lithium does indeed exert neurotrophic/neuroprotective events in the human brain in vivo.

Concluding Remarks

The evidence demonstrating the neurotrophic effects of lithium and valproate, the enhancement of hippocampal neurogenesis in the adult mammalian brain, and the growing appreciation that mood disorders are associated with cell loss and atrophy suggest that these effects may be very relevant for the long-term treatment of mood disorders. It should be emphasized that it is unclear if these neurotrophic effects are related to the acute treatment of mania or depression; it is quite likely that this enhancement of “cellular resiliency” is more related to long-term prophylactic effects and illness-related global deterioration. Does the long-term administration of these agents actually retard disease- or affective episode–induced cell loss or atrophy? As articulated, the distinction between disease progression and affective episodes per se is an important one, since it is quite possible that the neurotrophic effects of lithium or valproate may even be independent of their ability to treat or prevent affective episodes. There are currently no longitudinal studies that we are aware of that can fully address this question, but this is clearly a very important and fundamental issue worthy of investigation. The findings that lithium administration increases brain NAA levels and gray matter volumes, as well as the cross-sectional study demonstrating “normalized” subgenual PFC volumes in lithium- and valproate-treated patients, do provide indirect support for such a contention. Longitudinal studies investigating the potential long-term neurotrophic effects (using serial volumetric MRI scans or MRS quantitation of regional NAA levels, for example) of mood stabilizers are clearly warranted. The evidence also suggests that early and potentially sustained treatment may be necessary to adequately prevent many of the deleterious long-term sequellae associated with mood disorders. Although data suggest that hippocampal atrophy in depression is related to illness duration (Sheline 2000), it is currently not clear if the volumetric and cellular changes that have been observed in other brain areas (most notably the frontal cortex) are related to affective episodes per se. Indeed, some studies have observed reduced gray matter volumes and enlarged ventricles in mood disorder patients at first onset (Hirayasu et al 1998; Strakowski et al 1993). At this point, it is unclear if the cell death and atrophy in mood disorders occur because of the magnitude and duration of the biochemical perturbations, an enhanced vulnerability to the deleterious effects of these perturbations (due to genetic factors and/or early life events), or a combination thereof (Fig 1). In conclusion, emerging results from a variety of clinical and preclinical experimental and naturalistic paradigms suggest that a reconceptualization about the pathophysiology, course, and optimal long-term treatment of recurrent mood disorders is warranted. Optimal long-term treatment for these severe illnesses may only be achieved by the early use of agents with neurotrophic/neuroprotective effects, irrespective of the primary, symptomatic treatment. Such treatment modalities, via their effects on molecules involved in cell survival and cell death pathways, such as CREB, BDNF, bcl-2, and MAP kinases (Figure 1) would be envisioned as enhancing neuroplasticity and cellular resilience and modulating the long-term course of these devastating illnesses.

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References


Neurotrophic Effects of Mood Stabilizers


