Lithium Up-Regulates the Cytoprotective Protein Bcl-2 in the CNS In Vivo: A Role for Neurotrophic and Neuroprotective Effects in Manic Depressive Illness

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Although mood disorders have traditionally been conceptualized as "neurochemical disorders," considerable literature from a variety of sources demonstrates significant reductions in regional central nervous system (CNS) volume and cell numbers (both neurons and glia) in persons with mood disorders. It is noteworthy that recent advances in cellular and molecular biology have resulted in the identification of 2 novel, hitherto completely unexpected targets of lithium's actions, discoveries that may have a major impact on the future use of this unique cation in biology and medicine. Chronic lithium treatment has been demonstrated to markedly increase the levels of the major neuroprotective protein bcl-2 in rat frontal cortex, hippocampus, and striatum. Similar lithium-induced increases in bcl-2 are also observed in cells of human neuronal origin and are observed in rat frontal cortex at lithium levels as low as ~0.3 mM. Bcl-2 is widely regarded as a major neuroprotective protein, and genetic strategies that increase bcl-2 levels have demonstrated not only robust protection of neurons against diverse insults, but have also demonstrated an increase in the regeneration of mammalian CNS axons. Lithium has also been demonstrated to inhibit glycogen synthase kinase 3β (GSK-3β), an enzyme known to regulate the levels of phosphorylated *tau* and β-catenin (both of which may play a role in the neurodegeneration observed in certain forms of Alzheimer's disease). Consistent with the increases in bcl-2 levels and inhibition of GSK-3β, lithium has been demonstrated to exert robust protective effects against diverse insults both in vitro and in vivo. These findings suggest that lithium may exert some of its long-term beneficial effects in the treatment of mood disorders via underappreciated neurotrophic and neuroprotective effects. To date, lithium remains the only medication demonstrated to markedly increase bcl-2 levels in several brain areas; in the absence of other adequate treatments, an investigation of the potential efficacy of lithium in the long-term treatment of several neurodegenerative disorders is warranted. Additionally, we suggest that a reconceptualization of the use of lithium in mood disorders may be warranted—namely, that the use of lithium as a neurotrophic/neuroprotective agent should be considered in the long-term treatment of mood disorders, irrespective of the "primary" treatment modality being used for the condition.

(J Clin Psychiatry 2000;61[suppl 9]:82–96)

Manic depressive illness is a common, severe, chronic, and often life-threatening illness.[1] Suicide is the cause of death in 10% to 20% of individuals with manic depressive illness, and the risks of suicide in manic depressive illness may be higher than those in unipolar depression (reviewed in references 1 and 2). 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.[3,4] Despite well-established genetic diatheses and extensive research, the biochemical abnormalities underlying the predisposition to and the pathophysiology of manic depressive illness remain to be clearly established. Although mood disorders have traditionally been regarded as "good prognosis diseases," a growing body of data suggests that the long-term outcome is often much less favorable than previously thought. Indeed, according to the Global Burden of Disease Study, manic depressive illness is one of the leading causes of disability worldwide (discussed in reference 5). 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.[5]

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Supported by an unrestricted educational grant from Solvay Pharmaceuticals, Inc. The authors' research is supported by National Institute of Mental Health (RO1-MH57743, RO1-MH59107), the Theodore and Vada Stanley Foundation, NARSAD, and Joseph Young Sr. Research grants.

Outstanding editorial assistance was provided by Ms. Celia Knobelsdorf, B.S., B.A.

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ARE MOOD DISORDERS ASSOCIATED WITH REGIONAL VOLUMETRIC 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. In this context, it is noteworthy that although mood disorders have traditionally been conceptualized as "neurochemical disorders," considerable literature from a variety of sources demonstrates 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 neuroanatomical basis of mood disorders. Specifically, volumetric neuroimaging studies have demonstrated an enlargement of third and lateral ventricles in patients with manic depressive illness, although the lateral ventricle enlargement is not as consistently observed (see references 6–11 and references therein). Recent studies have also reported reduced basal ganglia volume and temporal lobe volume (including the hippocampus) in morphometric neuroimaging studies of mood disorders (references 6–11 and references therein). Within the frontal lobe, volumetric neuroimaging studies have also consistently shown reduced volumes in mood disorders. In particular, recent volumetric magnetic resonance imaging (MRI) studies in patients with familial bipolar and unipolar depression have demonstrated reductions in the mean gray matter volume of approximately 40% in the prefrontal cortex ventral to the genu of the corpus callosum.[12]

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 (reviewed in references 8 and 11). In addition to the accumulating neuroimaging evidence, several postmortem brain studies are now providing direct evidence for reductions in regional CNS volume and cell number in mood disorders. A recent study by Benes and coworkers[13] compared 4 brains of patients with manic depressive illness who were age- and postmortem interval–matched to 11 normal controls. They showed that nonpyramidal neurons were approximately 40% lower in CA2 of the hippocampal formation in the subjects with manic depressive illness compared with controls. Three recent postmortem studies of the prefrontal cortex have demonstrated reduced CNS volume and cell numbers in mood disorders. Rajkowska[14] has used 3-dimensional cell counting and morphological techniques to demonstrate decreased cortical and laminar thickness in subjects with manic depressive illness who completed suicide. Similar findings were also shown in a separate group of suicide victims with major depression. In an exciting recent study of several prefrontal cortical areas using similar methodologies, 12 subjects with major depressive disorder (without psychosis) demonstrated significantly reduced sizes and densities of both neurons and glia in several distinct areas compared with 9 matched control subjects.[15] Also in the prefrontal cortex, Ongur and colleagues[16] have recently reported a histologic study examining the cellular composition of area sg24 located in the subgenual prefrontal cortex. They found striking reductions in glial cell numbers in patients with familial major depression (24% reductions) and manic depressive illness (41% reductions) compared with controls. This is a particularly noteworthy finding because it is consistent with neuroimaging findings that show cortical volume loss in this same region on volumetric MRI in a similar diagnostic group.[12]

Together, the preponderance 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 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 (for example, 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 (discussed in references 10 and 17–19).

CAN DISEASE-RELATED CNS CELL DEATH OR ATROPHY BE ATTENUATED OR REVERSED?

It is now well established that in the developing nervous system, programmed cell death is responsible for the intricate matching of neurons to their targets and, as such, represents a tightly regulated set of cellular responses to both extrinsic and intrinsic signals. 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, however, has shown that many of the same pathways may also be involved in the cell death and atrophy that occurs *pathologically* in certain disorders. In recent years, considerable progress has been made in our understanding of the factors that regulate cell death and atrophy, as well as the mechanisms by which these changes occur. With the realization that these changes 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

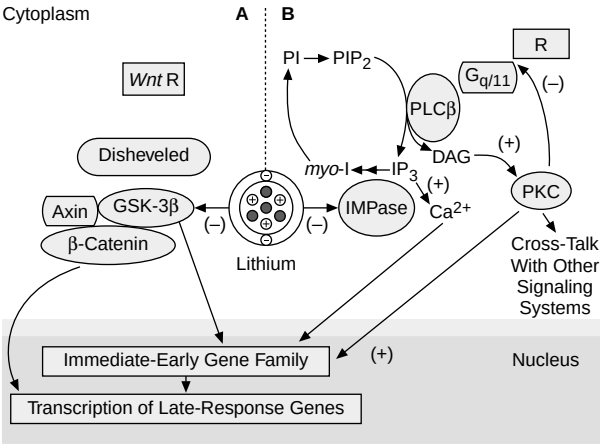
to be accepted as an unavoidable fate. This is particularly noteworthy since investigators at the Salk Institute have recently demonstrated that neurogenesis also occurs in the *adult* human brain,[20,21] and complementary studies have similarly demonstrated neurogenesis in the dentate gyrus of mature marmosets and macaques.[22] Most recently, Gould and colleagues[23] have demonstrated the presence of newborn neurons in the neocortex as well.

Besides overturning the earlier dogma that the adult human brain is incapable of cell division, this exciting work opens an avenue for therapeutic intervention that would take advantage of the replicative capacity of these neurons. Although further research is necessary to demonstrate that the new neurons are indeed capable of joining existing functional networks in the brain, the possibility of regulating neurogenesis in the human brain to correct disease-related pathophysiologic changes is a very exciting prospect indeed.[21,23] Elegant recent studies have greatly enhanced our understanding of the genetic and environmental factors regulating neurogenesis in the adult mammalian brain, leading to the exciting prospect that it may even be possible to pharmacologically regulate neurogenesis in the adult mammalian brain.[18,21,24,25]

LITHIUM: A UNIQUE CATION IN BIOLOGY AND MEDICINE

Lithium is an element discovered over 175 years ago (1817), but it was not until the seminal work of the Australian physician/scientist John Cade 50 years ago, and subsequent clinical studies by Mogens Schou, that lithium was seen by modern psychiatry as an effective treatment for manic depressive illness.[26,27] The discovery of lithium's efficacy as a mood-stabilizing agent revolutionized the treatment of patients with manic depressive illness—indeed, it is likely that the remarkable efficacy of lithium served to spark a revolution that has reshaped not only medical and scientific, but also popular concepts of severe mental illnesses.[28] After 3 decades of use in North America, lithium continues to be the mainstay of treatment for this illness, both for the acute manic phase and as prophylaxis for recurrent manic and depressive episodes.[1,27] Adequate lithium treatment, particularly in the context of a lithium clinic, is also reported to reduce the excessive mortality observed in the illness.[26,29–33] The effect on the broader community is highlighted by one estimation that the use of lithium saved the United States $4 billion in the period 1969 to 1979 by reducing associated medical costs and restoring productivity.[34] However, despite its role as one of psychiatry's most important treatments, the biochemical basis for the therapeutic effects of lithium remains to be fully elucidated.[35–38] Lithium has a variety of benefits in the treatment of mood disorders, including acute antimanic and antidepressant effects, antidepressant-potentiating effects, long-term prophylactic effects, and perhaps even independent antisuicidal effects (reviewed in references 1 and 33). It is unlikely that any single biochemical effect mediates all of lithium's clinical effects. In this context, considerable research has identified transmembrane cellular signaling pathways, in particular the protein kinase C signal transduction pathway, as therapeutically relevant targets for many of lithium's effects[37,39–48] (Figure 1). It is, however, intriguing that 50 years after John Cade's original report, advances in cellular and molecular biology have led to the identification of 2 novel, hitherto completely unexpected targets of lithium's actions, discoveries that may have a major impact on the future use of this unique cation in biology and medicine. Here we discuss the recent data demonstrating that lithium exerts major effects on the cytoprotective protein bcl-2 as well as on glycogen synthase kinase 3β (GSK-3β); these effects may be responsible, at least in part, for the growing body of data demonstrating that lithium exerts neuroprotective effects both in vitro and in vivo (Figure 2). It should be emphasized that it is not our con-

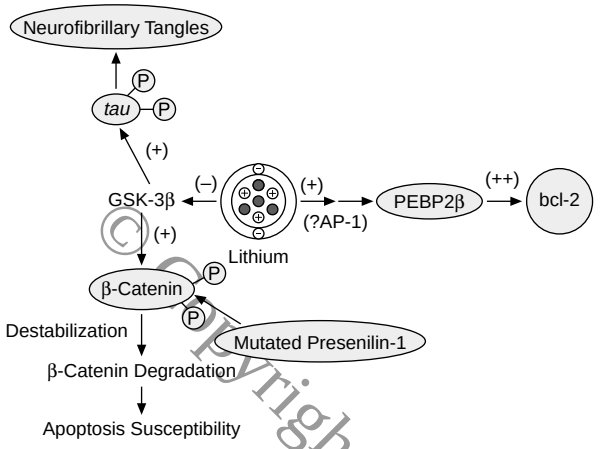
Figure 1. Molecular Mechanisms of Lithium's Action[a]



[a]Adapted with permission from Ikonomov and Manji.[49] Abbreviations: DAG = diacylglycerol, G = G protein, GSK = glycogen synthase kinase, IMPase = inositol monophosphatase, IP_3 = inositol 1,4,5-trisphosphate, myo-I = *myo*-inositol, P = phosphate, PI = phosphoinositide, PIP_2 = phosphatidylinositol 4,5-bisphosphate, PKC = protein kinase C, PLC = phospholipase C, R = receptor.

At therapeutic concentrations lithium directly inhibits 2 enzymes: GSK-3β and IMP. (A) In early *Xenopus* development, GSK-3β is in a cytosolic complex with other proteins: axin and β-catenin. β-Catenin is a transcription factor and the effector protein of the *Wnt* signaling pathway, which also involves extracellular ligands (encoded by *Wnt* gene family, not shown), *Wnt* membrane receptors (encoded by Frizzled gene family), and disheveled proteins. The *Wnt* signaling activation leads to a nuclear translocation of β-catenin, activation of c-*jun*, and dorsalization of the embryo. Short lithium exposure blocks the tonic inhibitory action of GSK-3β and activates the *Wnt* signaling pathway, an effect that could be imitated by a dominant negative GSK-3β mutant. (B) In the rodent brain, lithium inhibits inositol-1-phosphatase, thereby bringing about secondary changes in the PKC signaling cascade. Increased intracellular Ca^{2+} and PKC activate the transcription of immediate-early genes including the *fos* and *jun* family. Multiple late-response genes are known to be significantly affected by lithium treatment.

Figure 2. Molecular and Cellular Mechanisms Underlying the Neuroprotective Effects of Lithium[a]



[a]Adapted with permission from Manji et al.[50]
Abbreviations: AP = activator protein, bcl-2 = B-cell lymphoma/leukemia-2 gene, GSK = glycogen synthase kinase, P = phosphate, PEBP = polyomavirus enhancer binding protein.

Chronic lithium, at therapeutically relevant concentrations, increases the expression and function of the transcription factor PEBP2β, which results in a robust up-regulation of the major neuroprotective protein bcl-2 in the central nervous system. Lithium is also an inhibitor of GSK-3β. GSK-3β is known to phosphorylate *tau*, a major component of neurofibrillary tangles. It should be emphasized that GSK-3β is only one of the kinases involved in phosphorylating *tau*. Nevertheless, inhibition of GSK-3β by lithium may reduce levels of hyperphosphorylated *tau*. GSK-3β also regulates β-catenin levels, and inhibition of GSK-3β by lithium results in a stabilization of β-catenin. The lithium-induced stabilization of β-catenin may serve to offset the destabilizing effects of interactions of mutant presenilin-1 protein, thereby reducing neuronal vulnerability to apoptosis induced by amyloid-β protein.

tention that lithium's effects on bcl-2 and GSK-3β are responsible for all of lithium's therapeutic effects, but rather that these biochemical effects may play a major role in long-term neurotrophic/neuroprotective effects. Together with the exciting data demonstrating effects of antidepressants on neurotrophic factors,[51–53] these findings suggest that a reconceptualization of the cellular mechanisms underlying some of the long-term beneficial effects of lithium and antidepressants may be warranted.[37,50,53–57]

LITHIUM, SIGNAL TRANSDUCTION, AND GENE EXPRESSION

It has become increasingly appreciated in recent years that the long-term treatment of complex neuropsychiatric disorders such as manic depressive illness most likely involves the strategic regulation of signaling pathways and gene expression in critical neuronal circuits.[35,37,38,44,49,53,58] Effects of lithium on signal transduction pathways, in particular the protein kinase C signaling pathway, quite likely play a major role in the treatment of affective episodes.[35,37,39,42–48,59] In recent years, substantial progress has also been made in our understanding of the processes that convert short-term (sometimes very transient) second messenger–mediated events into long-term neuronal and physiologic phenotypic alterations. These advances have been particularly important for neurobiology, wherein we attempt to understand the mechanism(s) by which short-lived events (e.g., stressors) can have profound, long-term (perhaps lifelong) behavioral consequences and, importantly for the present discussion, help to unravel the processes by which a simple monovalent cation such as lithium may produce a long-term stabilization of mood in individuals vulnerable to an illness as complex as manic depressive illness.[8,60]

Effects of Lithium on Immediate-Early Genes

The transcriptional activation of immediate-early genes, including the *fos* and *jun* families, is a characteristic cellular response to extracellular stimuli such as hormones, growth factors, and neurotransmitters.[61,62] The transcriptional activation is followed by cytoplasmic translation of Fos, Jun, and other proteins, which translocate into the nucleus and form a variety of protein complexes. Activator protein 1 (AP-1) is a collection of homodimeric and heterodimeric complexes composed of products of *fos* and *jun* family members. These products bind to a common DNA site (12-O-tetradecanoylphorbol-13-acetate [TPA] response element [TRE]) in the regulatory domain of the gene and activate gene transcription. The genes regulated by AP-1 in CNS include genes for various neuropeptides, neurotrophins, receptors, transcription factors, enzymes involved in neurotransmitter synthesis, and proteins that bind to cytoskeletal elements.[62] The final result of this molecular cascade is alteration in the transcription of selected target genes bearing the specific DNA binding site on their regulatory regions.

Several independent laboratories have now demonstrated that lithium, at therapeutically relevant concentrations, produces complex alterations in basal and stimulated DNA binding of TRE to AP-1 transcription factors not only in human SH-SY5Y cells in vitro, but also in rodent brain after chronic, in vivo administration.[38,63–67] Paralleling an increase in basal AP-1 DNA binding activity, lithium has been shown to time- and concentration-dependently increase the expression of a luciferase reporter gene driven by an SV40 promoter that contains TREs; mutations in the TRE sites of the reporter gene promoter markedly attenuate the effects of lithium.[66,68] Importantly, lithium has also been demonstrated to increase the expression of endogenous proteins whose genes are known to be regulated by AP-1[69–71]; together, these results suggest that lithium may regulate gene expression (at least in part) through the AP-1 transcription factor pathway.[38,39,65–67] As discussed above, many of the genes known to be regulated by the AP-1 family of transcription factors in the brain include genes for various neuropep-

tides, neurotrophins, receptors, transcription factors, enzymes involved in neurotransmitter biosynthesis, and proteins that bind to cytoskeletal elements.[62] Together, these data suggest that lithium, via its 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.[35,38,49,58,65,67,72]

SUCCESSFUL APPLICATION OF A CONCERTED mRNA DIFFERENTIAL DISPLAY STRATEGY TO IDENTIFY NOVEL TARGET GENES

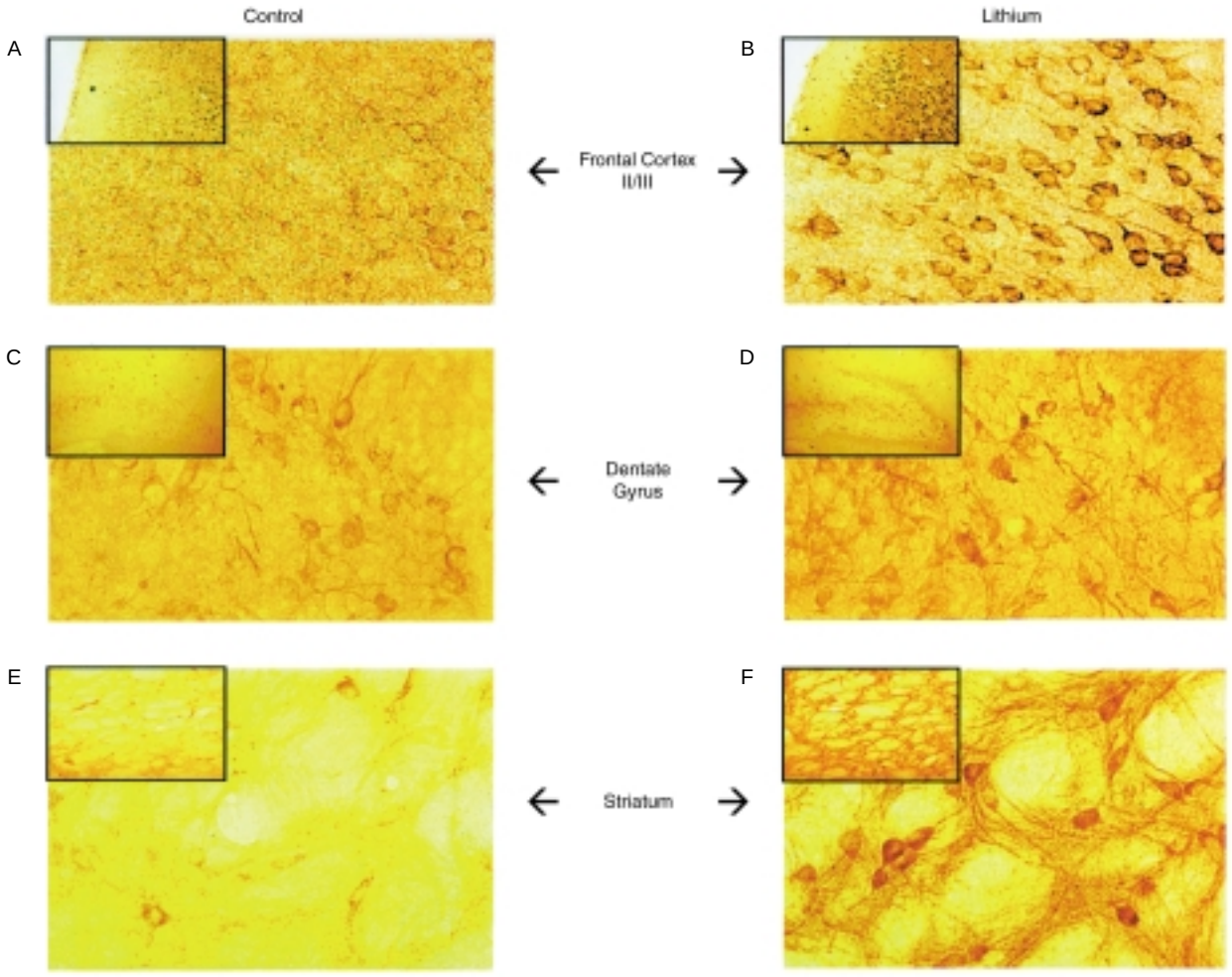
Bcl-2 as a Therapeutically Relevant Target for the Actions of Lithium

It is now clear that lithium, very likely via its effects on mitogen-activated protein (MAP) kinases[73] and GSK-3β (vide infra), exerts major effects on the AP-1 family of transcription factors (reviewed in references 37, 38, 54, 72, and 74), effects that have the potential to regulate the expression of a number of critical genes in the CNS. Although many genes that are the targets of long-term lithium treatment have indeed been identified, it has been estimated that approximately 10,000 to 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.[37,49,53,54,56]

In recent years, new methodologies have evolved to identify the differential expression of multiple genes (e.g., in pathologic vs. normal tissue, or in control vs. treated tissue); one such methodology that is being increasingly utilized is reverse transcription polymerase chain reaction mRNA differential display (RT-PCR DD).[75] Using this method, Wang and Young[76] were the first to make the novel observation that lithium increased 2',3'-cyclic nucleotide 3'-phosphodiesterase mRNA levels in C6 glioma cells. A major problem inherent in neuropharmacologic research, however, is the dearth of phenotypic changes clearly associated with treatment response, particularly for mood-stabilizing agents.[39,49] In the absence of suitable animal models, 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 when administered chronically in vivo. Thus, to identify changes in gene expression likely to be associated with components of the therapeutic efficacy of mood stabilizers, we have utilized RT-PCR DD to concurrently investigate the effects of lithium and valproate in the CNS, following chronic treatment of rodents in vivo.[77] These are 2 structurally highly dissimilar agents; although they most likely do not exert their therapeutic effects by precisely the same mechanisms, identifying the genes that are regulated in concert by these 2 agents, when administered in a therapeutically relevant paradigm, may provide important leads about the molecular mechanisms underlying mood stabilization. Inbred male Wistar Kyoto rats (selected to reduce potential false positives due to individual differences) were treated chronically with twice-daily intraperitoneal injections of lithium, valproate, or saline. Saline was provided ad libitum to the lithium-treated rats to reduce potential toxicity. The animals attained plasma drug concentrations similar to those attained clinically,[77] and no significant weight changes were observed with the chronic drug treatment. RNA was extracted from frontal cortices to study gene expression using RT-PCR DD.[75] One of the genes whose expression was markedly increased by the treatments is the transcription factor polyoma enhancer binding protein 2β (PEBP2β; GenBank Accession Number: AF087437, discussed in reference 77). After demonstrating that the function of PEBP2β (DNA binding of the PEBP2αβ complex) was also clearly increased by chronic lithium administration, we next investigated the effects of lithium on the levels of a critical protein known to be regulated by PEBP2β—the major neuroprotective protein bcl-2[78]—and found that chronic treatment of rats with lithium resulted in a doubling of bcl-2 levels in frontal cortices.

Additional studies were subsequently undertaken to further localize lithium's effects on CNS bcl-2 levels. In these studies, inbred male Wistar rats were once again used. The mean ± SD plasma lithium levels were 0.7 ± 0.3 mM, and no significant weight changes were observed with 4 weeks of treatment. Immunohistochemical studies showed that chronic treatment of rats with lithium resulted in a marked increase in the number of bcl-2 immunoreactive cells in layers II and III of frontal cortices. Interestingly, the importance of neurons in layers II through IV of the frontal cortices 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 (discussed in reference 15). Chronic administration of lithium at therapeutically relevant concentrations also resulted in a marked increase in the number of bcl-2 immunoreactive cells in the dentate gyrus and striatum (Figure 3).[50] To determine if lithium also increases bcl-2 levels in human cells of neuronal origin, human neuroblastoma SH-SY5Y cells were treated with 1.0 mM of lithium for 6 days. Similar to the situation observed in rat brain in vivo, chronic lithium administration produced a marked increase in bcl-2 levels in SH-SY5Y cells (Figure 4). We have subsequently demonstrated that lithium also increases bcl-2 levels in C57BL/6 mice (G. Chen, M.D., Ph.D.; F. Du, Ph.D.; H.K.M., manuscript submitted), and our demonstration of a lithium-induced increase in bcl-2 levels has also been convincingly replicated in rat cerebellar granule cells in a recent study.[79] This latter study was undertaken to investigate the molecular and cellular mechanisms underlying the neuroprotective actions of lithium against glutamate excitotoxicity

Figure 3. Effects of Chronic Lithium on the Immunolabeling of Bcl-2 in Rat Brain[a]



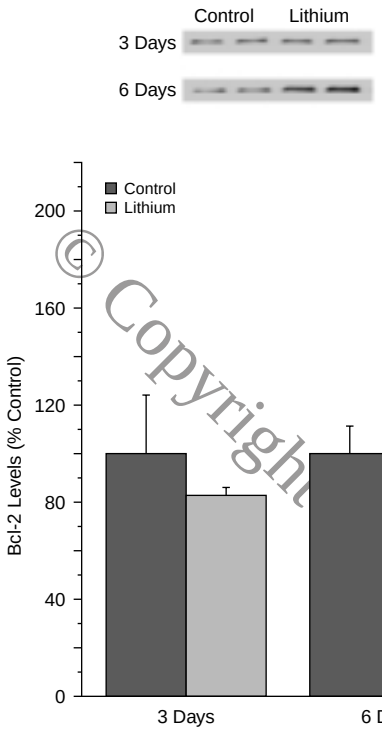
[a]Adapted with permission from Manji et al.[50] Inbred male Wistar Kyoto rats were treated with either Li_2CO_3 or saline by twice-daily i.p. injections for 4 weeks. There was no significant weight loss observed with chronic lithium administration. Trunk blood was collected for determination of lithium levels (mean ± SD = 0.7 ± 0.3 mM). The rats' brains were cut at 30 µm; serial sections were cut coronally through the anterior portion of the brain, mounted on gelatin-coated glass slides, and stained with thionin. The sections of the second and third sets were incubated free-floating for 3 days at 4°C in 0.01 M phosphate buffered saline containing a polyclonal antibody against bcl-2 (N-19, 1:3000, Santa Cruz Biotechnology, Santa Cruz, Calif.), 1% normal goat serum, and 0.3% Triton X-100 (Sigma, St. Louis, Mo.). Subsequently, the immunoreaction product was visualized according to the avidin-biotin complex method. The figure shows immunohistochemical labeling of bcl-2 in saline- (A, C, and E) and lithium- (B, D, and F) treated rats, in layers 2 and 3 of frontal cortex (A and B), hippocampus (C and D), and striatum (E and F). Main photographs were obtained with 40× magnification; insets with 10× magnification.

(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 both in cerebellar granule cells[79] and SH-SY5Y cells.[80] Thus, overall, the data clearly show that chronic lithium administration robustly increases the levels of the neuroprotective protein bcl-2 in areas of rodent frontal cortices, 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 pro-apoptotic protein p53.

Bcl-2, Mediator of Cellular Life and Death: Clinical Implications of the Lithium-Induced Increases

Extensive research efforts aimed at elucidating the signaling pathways and proteins involved in regulating physiologic and pathophysiologic cell death have revealed critical roles for mammalian proteins that show considerable homology to the "*C. elegans* death proteins"—the bcl-2 family of proteins. Bcl-2 is the acronym for B-cell lymphoma/leukemia-2; the gene for this protein was first discovered because of its involvement in B-cell malignancies, where chromosomal translocations activate the gene in the majority of follicular non-Hodgkin B-cell lymphomas (references 81–85 and references therein). Bcl-2 was

Figure 4. Effects of Lithium on Bcl-2 Levels in Human Neuroblastoma SH-SY5Y Cells[a]



[a]Human SH-SY5Y neuroblastoma cells were incubated with lithium (1 mM) for 3 days or 6 days in inositol-free minimal essential medium (MEM). Immunoblotting was conducted using established methods with monoclonal antibodies against bcl-2 (1 in 100 dilution; Santa Cruz Biotechnology, N-19, Santa Cruz, Calif.) Incubation of SH-SY5Y cells with lithium for 6 days (but not 3 days) resulted in significant increases in the levels of bcl-2.
*$p < .05$ compared with control.

the first identified member of a large family of cellular and viral apoptosis-regulating proteins (reviewed in references 82–85). These proteins appear to regulate common pathways for apoptosis and programmed cell death, with several functioning as "protectors" (e.g., bcl-2, bcl-XL) and others as "executioners" (e.g., Bax, Bak, Bad). In a number of cases, these proteins interact with each other in a complex network of homodimers and heterodimers (references 81–85 and references therein).

Bcl-2 is expressed in the rodent and mammalian nervous system and is localized to the outer mitochondrial membrane, endoplasmic reticulum, and nuclear membrane. Although the precise mechanisms of action of bcl-2 are unknown, it is now clear that bcl-2 is a protein that inhibits both apoptotic and necrotic cell death induced by diverse stimuli (references 81–83 and references therein). It is likely that several cellular mechanisms are involved in mediating the protective effects of bcl-2, including sequestering the proforms of caspases, inhibiting the effects of caspase activation, providing antioxidant effects, enhancing mitochondrial calcium uptake, and attenuating

Table 1. Mechanisms Underlying the Neuroprotective Effects of Bcl-2[a]

Blocks cytochrome c release from mitochondria
Acts as an antioxidant or reduces free-radical production
Regulates calcium homeostasis
Regulates other gene products that promote apoptosis, eg, forms heterodimers with the pro-apoptotic protein Bax
Inhibits effects of cysteine protease activation
Maintains the mitochondrial membrane potential and prevents activation of the mitochondrial permeability transition
Detoxifies or decreases the production of reactive oxygen species
Causes a redistribution of glutathione to the nucleus
Enhances the mitochondrial membrane potential and improves ATP/ADP ratios
Enhances the mitochondrial calcium uptake potential of neural cells
Inhibits mitochondrial release of calcium

[a]Data summarized from Merry and Korsmeyer,[81] Adams and Cory,[82] Bruckheimer et al.,[83] Sadoul,[84] and Li and Yuan.[85]

the release of calcium and cytochrome c from mitochondria (reviewed in references 82–85; Table 1).

A role for bcl-2 in protecting neurons from cell death is now supported by abundant evidence; thus, bcl-2 has been shown to protect neurons from a variety of insults in vitro including growth-factor deprivation, glucocorticoids, ionizing radiation, and oxidant stressors such as hydrogen peroxide, *tert*-butylhydroperoxide, reactive oxygen species, and buthionine sulfoxamine.[82,83] In addition to these potent in vitro effects, bcl-2 has also been shown to prevent cell death in numerous studies in vivo. In the absence of pharmacologic means of increasing CNS bcl-2 expression (until now), all the studies have hitherto utilized transgenic mouse models or viral vector-mediated delivery of the bcl-2 gene into the CNS. In these models, bcl-2 overexpression has been shown to prevent motor neuron death induced by facial nerve axotomy and sciatic nerve axotomy, to save retinal ganglion cells from axotomy-induced death, to protect cells from the deleterious effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or focal ischemia, and to protect photoreceptor cells from 2 forms of inherited retinal degeneration. Interestingly, neurons that survive ischemic lesions or traumatic brain injury in vivo show up-regulation of bcl-2 (references 81, 84, 86–90 and references therein). Overexpression of bcl-2 has also recently been shown to prolong survival and attenuate motor neuron degeneration in a transgenic animal model of amyotrophic lateral sclerosis[91] (Table 2).

Most recently, it has been clearly demonstrated that not only does bcl-2 overexpression protect against apoptotic and necrotic cell death, it can also promote regeneration of axons in the mammalian CNS, leading to the intriguing postulate that bcl-2 acts as a major regulatory switch for a genetic program that controls the growth of CNS axons.[88] Since bcl-2 has also recently been shown to promote neurite sprouting, it has been convincingly argued that increasing CNS bcl-2 levels may represent a very effective therapeutic strategy for the treatment of many neurodegenerative diseases.[88] As articulated already, the only

Table 2. Neuroprotection by Bcl-2[a]

Protects neuronal cells from the lethal effects of a variety of stimuli that generate reactive oxygen species
Protects neuronal cells in culture from glutamatergic toxicity and growth factor deprivation–induced cell death
Gene transfer of bcl-2 enhances survival of cultured neurons exposed to glutamate and hypoglycemia and protects against focal ischemia in the striatum
Transgenic mice overexpressing bcl-2 demonstrate protection against axotomy-induced neonatal motor neuron death
Transgenic mice overexpressing bcl-2 demonstrate protection against apoptotic cell death after traumatic brain injury
Transgenic mice overexpressing bcl-2 demonstrate reduced infarct sizes after focal cerebral ischemia
Transgenic mice overexpressing bcl-2 crossed into a transgenic mouse model of amyotrophic lateral sclerosis show enhanced survival
Transgenic mice overexpressing bcl-2 show marked resistance to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity and are protected against acute MPTP-induced dopamine depletion
Transgenic mice overexpressing bcl-2 show enhanced regeneration of severed central nervous system axons, independent of the antiapoptotic effects of bcl-2

[a]Adapted with permission from Manji et al.[50] Summarized from Merry and Korsmeyer,[81] Adams and Cory,[82] Bruckheimer et al.,[83] Sadoul,[84] Li and Yuan,[85] Bonfanti et al.,[86] Lawrence et al.,[87] Chen et al.,[88] Raghupathi et al.,[89] Yang et al.,[90] and Kostic et al.[91]

means of therapeutically increasing CNS bcl-2 levels in the adult brain has heretofore been by the use of complex gene transfer methodologies; thus, pharmacologic means of robustly increasing CNS bcl-2 levels represent a major advance for the long-term treatment of certain neurodegenerative disorders.

GSK-3β: Another Novel Cellular Target for the Actions of Lithium

In recent years, another hitherto unexpected target for the effects of lithium has been identified. Klein and Melton[92] were the first to make the seminal observation that lithium's effects on the dorsalization of the *Xenopus* embryo had attributes that suggested the activation of another evolutionarily conserved signal-transduction pathway—the *Wnt* (secreted extracellular ligands) signaling pathway (see Figure 1). They further showed that the selective inhibition of inositol monophosphatase (IMPase) with other agents did not mimic lithium's effects in *Xenopus*, leading to their studies demonstrating that lithium, at therapeutically relevant concentrations, is an inhibitor of GSK-3β.[92] Glycogen synthase kinase (GSK-3) is an evolutionarily highly conserved kinase, known to play a key role in regulating developmental patterns in diverse organisms (including mammals), as well as regulating important neuronal functions.[93–96] During normal development, GSK-3β (zeste-white3/shaggy) inhibits the transduction of the *Wnt* signal from the *Wnt* membrane receptors (encoded by Frizzled protein family) to the nucleus. Other important intracellular molecules in *Wnt* signaling are disheveled, axin, β-catenin, and possibly other yet unidentified interacting proteins.[93–97] Exposure to lithium leads to a nuclear translocation of β-catenin (a final result of *Wnt* signaling activation) and duplication of the dorsal embryonic axis.[92,98] Most pertinent for the present discussion, it is now known that GSK-3β plays a critical role in the CNS, by regulating various cytoskeletal processes via its effects on *tau* and synapsin I, as well as long-term nuclear events via phosphorylation of c-*jun*, nuclear translocation of β-catenin, and nuclear export of NF-ATc (reviewed in references 93–96). Thus, lithium's inhibition of GSK-3β may underlie some of its transcriptional and posttranscriptional actions in the brain, and thereby potentially some of its long-term therapeutic effects.[54,96,99,100]

As discussed above, lithium is known to bring about a variety of biochemical effects, and in the absence of clear-cut phenotypic changes associated with therapeutic response,[49] it is unclear if inhibition of GSK-3β represents a therapeutically relevant effect. On the basis of similar clinical efficacy of lithium and sodium valproate as antimanic agents (and potentially as mood stabilizers) in manic depressive illness, we investigated if valproate also regulates GSK-3β, and found significant valproate-induced inhibition of GSK-3β at therapeutically relevant concentrations.[101] Consistent with GSK-3β inhibition, incubation of SH-SY5Y cells with valproate results in a significant time-dependent increase in both cytosolic and nuclear β-catenin levels.[101] These data indicate that GSK-3β is a common target for both mood stabilizers and support its potential role in the beneficial long-term action of lithium. Importantly for the present discussion, GSK-3β is known to phosphorylate the immediate-early gene c-*jun* at 3 sites adjacent to its DNA binding domain, thereby reducing AP-1 binding.[74,102] Thus, the acute inhibition of GSK-3β by lithium and valproate has the potential to bring about long-term changes in the CNS via the transcriptional activity of both β-catenin and AP-1[49] (see Figure 1). These original seminal studies by Klein and Melton[92] have resulted in a number of follow-up studies, and have generated considerable excitement about the possibility of developing novel GSK-3β modulators as potential new therapeutics for bipolar disorder.[74,99]

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 *tau* phosphorylation, an effect that very likely occurs to some degree at therapeutically relevant lithium concentrations (see reference 37 for an excellent discussion). Although many of the studies have utilized lithium concentrations in excess of those utilized therapeutically, the available data suggest that lithium, at concentrations of ~1 mM does, indeed, reduce *tau* phosphorylation.[37,103–105] For the purposes of the present discussion, it is noteworthy that 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 microtubule-associated protein *tau*. Hyperphosphorylation of *tau* is an

early event in the course of Alzheimer's disease and may precede the disruption of the microtubule cytoskeleton. Studies with transgenic mice have suggested that GSK-3β may play an important role in *tau* phosphorylation; thus, although GSK-3β is undoubtedly only one of several kinases involved in the aberrant hyperphosphorylation, inhibition of GSK-3β represents a potential mechanism to reduce the accumulation of hyperphosphorylated *tau,* which is found in neurofibrillary tangles (see Figure 2).

As discussed, GSK-3β also plays a major role in regulating β-catenin levels; inhibition of GSK-3β results in β-catenin accumulation, quite likely due to a decrease in the rate of β-catenin protein degradation.[93,94] In this context, it is noteworthy that recent studies have shown that presenilin-1 forms a complex with β-catenin in vivo, leading to an increase in β-catenin stability.[106] Furthermore, mutations in the presenilin-1 gene (which have been found in many patients with a rare form of familial Alzheimer's disease) have been shown to reduce the ability of presenilin-1 to stabilize β-catenin, thereby leading to increased degradation of β-catenin in the brains of transgenic mice. Presenilin mutations associated with Alzheimer's disease have also recently been demonstrated to cause defective intracellular trafficking of β-catenin, a component of the presenilin protein complex.[107] Moreover, β-catenin levels are reduced in the brains of patients who have Alzheimer's disease with presenilin-1 mutations, and loss of β-catenin signaling appears to increase neuronal vulnerability to apoptosis induced by amyloid-β protein.[106] Thus, inhibition of GSK-3β (for example by lithium) may serve to offset the β-catenin destabilizing effects of mutated forms of presenilin-1, and thereby reduce the vulnerability of affected neurons to apoptosis induced by β-amyloid protein (see Figure 2).

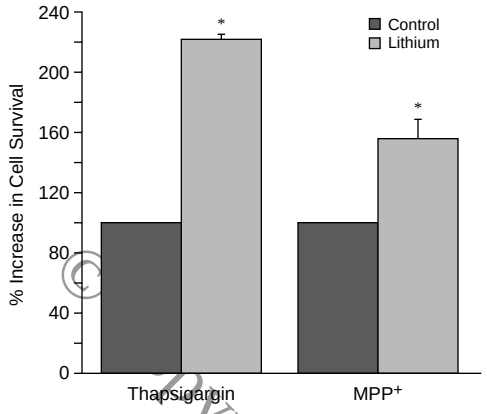
NEUROPROTECTIVE EFFECTS OF LITHIUM

Compelling Preclinical Evidence

Robust effects of lithium on bcl-2 and GSK-3β (see Figure 2) in the mature CNS suggest that this cation, at therapeutically relevant concentrations, may also possess significant neuroprotective properties. Indeed, although the effects of lithium on GSK-3β and, in particular, on bcl-2 are very recent observations, several earlier studies had already demonstrated neuroprotective properties of lithium.[108–114] More recently, a growing body of evidence is convincingly demonstrating that lithium does truly exert neuroprotective effects both in vitro and in vivo. The protective effects of lithium have been investigated in a number of in vitro studies using rat cerebellar granule cells. When switched to nondepolarizing medium after maturation in vitro, cerebellar granule cells have been shown to undergo massive apoptotic cell death. Lithium has been shown to robustly protect the cells in this paradigm, and interestingly, lithium's effects require new protein synthesis.[114] Other independent laboratories have also utilized the cerebellar granule cell model and have demonstrated that lithium robustly protects against the toxic effects of a variety of insults, including glutamate, *N*-methyl-D-aspartate (NMDA) receptor activation, low potassium, and toxic concentrations of anticonvulsants.[109,115,116] The protective effects of lithium against the deleterious effects of glutamate and NMDA receptor activation have also been demonstrated to occur in hippocampal and cortical neurons in culture. In addition to these "harsh insults," lithium has also been shown to exert protective effects in a more "naturalistic" paradigm: age-induced cerebellar granule cell death.[116] Other in vitro studies have utilized cultured cells with properties of catecholaminergic neurons, and it has been demonstrated that lithium induces the survival of PC12 cells after serum/nerve growth factor deprivation,[108] protects both PC12 cells and human neuroblastoma SH-SY5Y cells from ouabain toxicity,[111] and protects SH-SY5Y cells from both thapsigargin- (which mobilizes intracellular Ca^{++}) and 1-methyl-4-phenylpyridinium ion (MPP^{+})-induced cell death (Figure 5). Most recently, lithium has been shown to prevent the enhanced phosphorylation of *tau* protein at critical sites when cultured rat cortical neurons are incubated with β-amyloid.[111] Moreover, in this study lithium also significantly protected the cultured neurons from β-amyloid–induced cell death.[111]

In addition to the demonstration of protective effects in vitro, a number of studies have also investigated the neuroprotective effects of lithium in vivo. Thus, Inouye and associates[112] exposed newborn mice to gamma irradiation, focusing on proliferating cells of the external granular layer, which are known to be highly sensitive to ionizing radiation. They found that lithium pretreatment delayed radiation-induced apoptosis in these cells. Studies have also investigated the effects of lithium on the biochemical and behavioral manifestations of excitotoxic lesions of the cholinergic system.[113] These studies have demonstrated that lithium pretreatment attenuated both the behavioral deficits (passive avoidance and ambulatory behavior) and the reduction in choline acetyl transferase activity by forebrain cholinergic system lesions.[113] In another study investigating the effects of lithium against excitotoxic insults, it was demonstrated that lithium attenuated the kainic acid–induced reduction in glutamate decarboxylase levels and [^{3}H]D-aspartate uptake.[117] Chronic lithium has recently been shown to exert dramatic protective effects against middle cerebral artery occlusion, reducing not only the infarct size (by 56%), but also the neurologic deficits (abnormal posture and hemiplegia).[118] Most recently, the same research group has demonstrated in a putative model of Huntington's disease that chronic in vivo lithium treatment robustly protects neurons in the striatum from quinolinic acid–induced toxicity.[119] Table 3 summarizes some of the most robust experimental evidence demonstrating neuro-

Figure 5. Neuroprotective Effects of Lithium In Vitro[a]



[a]Human SH-SY5Y neuroblastoma cells were incubated with lithium (1 mM) for 6 days in inositol-free MEM, and were then exposed to 2 different toxins: thapsigargin (which mobilizes intracellular calcium) or 1-methyl-4-phenylpyridinium ion (MPP^+). The mitochondrial dehydrogenase activity that cleaves 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was used to determine cell survival in a quantitative colorimetric assay. The tetrazolium ring of MTT is cleaved by various dehydrogenase enzymes in active mitochondria, forming a blue-colored insoluble product, formazan. Cells were incubated with MTT (125 µg/mL) added to the growth medium for 1 h at 37°C. The medium was then aspirated, and the formazan product was dissolved in dimethyl sulfoxide and quantified spectrophotometrically at 540 nm. The results are expressed as a percentage of control culture viability. Six days of lithium treatment exerted significant protective effects against both toxins.
*$p < .05$.

Table 3. Experimental Paradigms in Which Lithium Has Been Demonstrated to Exert Neuroprotective Effects[a]

Protects cultured neurons against glutamate and *N*-methyl-D-aspartate–induced cell death
Protects cerebellar granule cells from KCl deprivation and anticonvulsant- or age-induced apoptosis
Induces survival of PC12 cells after serum/neurotrophic growth factor deprivation
Protects PC12 and SH-SY5Y cells from ouabain toxicity
Protects cultured neurons from β-amyloid toxicity
Delays radiation-induced apoptosis in external granule cells of mouse cerebellum
Protects SH-SY5Y cells from Ca^{++} and 1-methyl-4-phenylpyridinium ion (MPP^+) toxicity
Attenuates behavioral deficits and choline acetyltransferase activity reduction by forebrain cholinergic system lesions
Reduces middle cerebral artery occlusion–induced infarct size and neurologic deficits
Protects striatal neurons against quinolinic acid–induced toxicity

[a]Adapted with permission from Manji et al.[50] Summarized from Jope,[37,38] Manji et al.,[39] Lu et al.,[80] Munoz-Montano et al.,[103] Lovestone et al.,[105] Volonte et al.[108] D'Mello et al.,[109] Li et al.,[110] Alvarez et al.,[111] Grignon et al.,[114] Nonaka et al.,[115,116] Sparapani et al.,[117] Nonaka and Chuang,[118] and Chuang et al.[119]

protective effects of lithium in vitro and in vivo. It must be acknowledged at this point that the regulation of cell survival and cell death is a complex process involving multiple interacting signaling pathways, transcription factors, and gene expression. Thus, the effects of lithium on other signaling pathways and transcription factors[35,37] may also contribute to its neuroprotective effects. However, in view of the major neuroprotective role of bcl-2 in a variety of in vitro and in vivo experimental paradigms (references 81–83 and references therein), lithium's robust up-regulation of bcl-2 levels at therapeutically relevant concentrations very likely plays a major role in its neuroprotective effects.

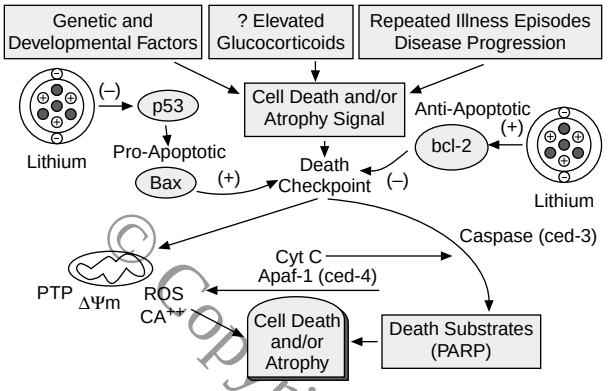
Does Lithium Affect Neurogenesis?

As discussed already, utilizing a method for labeling cell division directly in the adult human brain, Eriksson and colleagues[20] have shown 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. In view of the robust effects of bcl-2 on the regeneration of CNS axons[88] in addition to its potent neuroprotective effects, we have recently undertaken a study to determine if lithium administration results in an increased number of BrdU- (bromodeoxyuridine, a thymidine analog that is incorporated into the DNA of dividing cells) positive neurons in the hippocampus of adult rodents. We have found that 2 to 3 weeks of lithium administration does, indeed, result in an increase in the number of BrdU-positive neurons in the dentate gyrus (G. Chen, M.D.; F. Du, Ph.D.; H.K.M., manuscript submitted). Overall, the data clearly suggest that the effects of lithium on CNS bcl-2 levels (and accompanying neurotrophic/neuroprotective effects, vide infra) may be of considerable importance in the long-term treatment of mood disorders (Figure 6). To more definitively make such an assertion, it is clearly necessary to demonstrate in a longitudinal study that lithium treatment does indeed reduce or delay CNS cell death and/or atrophy in patients with mood disorder. Unfortunately, such data are presently not available; however, subsequent to the demonstration of lithium's robust effects on CNS bcl-2 levels, Drevets and associates have reanalyzed the volumetric data from their cohort of patients with familial bipolar and unipolar depression. Intriguingly, they have found that the lithium-treated subjects exhibit smaller reductions in frontal cortex volumes than the non–lithium-treated patients (W. Drevets, M.D., written communication, March 1999), findings that are compatible with a neurotrophic/neuroprotective effect of chronic lithium treatment.

Can the Neurotrophic/Neuroprotective Effects of Lithium Be Demonstrated Longitudinally in Humans in the CNS in Vivo?

We have recently undertaken a clinical study to determine if lithium may also exert 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

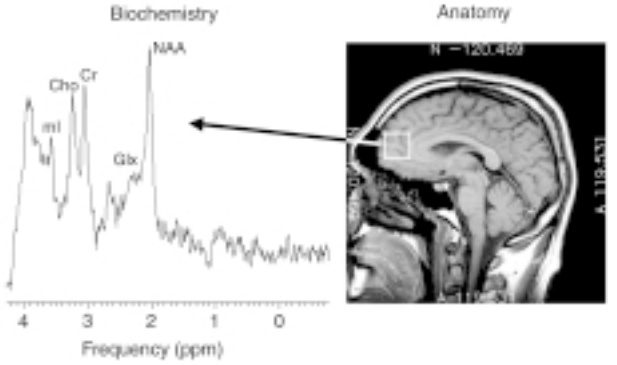
Figure 6. Neuroprotective Effects of Lithium: The Role of Bcl-2[a]



[a]Derived from Merry and Korsmeyer,[81] Adams and Cory,[82] Bruckheimer et al.,[83] Sadoul,[84] and Li and Yuan.[85] Abbreviations: Apaf-1 = apoptotic protease-activating factor-1, Bax = a pro-apoptotic protein from the bcl-2 family, bcl-2 = an anti-apoptotic protein from the bcl-2 (B-cell lymphoma/leukemia-2 gene) family, ced = antiapoptotic gene that encodes caspase proteins, Cyt C = cytochrome c, ΔΨm = mitochondrial transmembrane potential, PARP = poly(ADP-ribose) polymerase, p53 = pro-apoptotic protein, PTP = permeability transition pore, ROS = reactive oxygen species.

This figure depicts the manner by which lithium may exert neurotrophic/neuroprotective effects in the long-term treatment of mood disorders. Genetic/neurodevelopmental factors, repeated affective episodes (and likely elevations of glucocorticoids), and illness progression may all contribute to the volumetric reductions and cell death/atrophy observed in mood disorders. Lithium, via its effects on bcl-2 and p53, may exert effects on the mitochondrial permeability transition pore, a key event in cell death.

Figure 7. Quantitation of Lithium-Induced Changes in *N*-Acetyl-Aspartate (NAA) Levels Using Proton Magnetic Resonance Spectroscopy (MRS)[a]



[a]Adapted with permission from Manji et al.[74] Abbreviations: Cho = choline compounds, Cr = creatine/phosphocreatine, Glx = glutamate/glutamine, mI = *myo*-inositol, ppm = parts per million.

The Biochemistry portion of this figure is the typical proton MRS spectrum from the frontal lobe of a patient with bipolar disorder. Proton MRS is a tool that provides a noninvasive window to functional brain neurochemistry. NAA is one of the many neurochemical compounds that can be quantitatively assessed via MRS. NAA is the predominant resonance in the proton MRS spectrum of the normal adult human brain, and while the functional role of this amino acid has not been definitively determined, NAA is a putative neuronal marker.

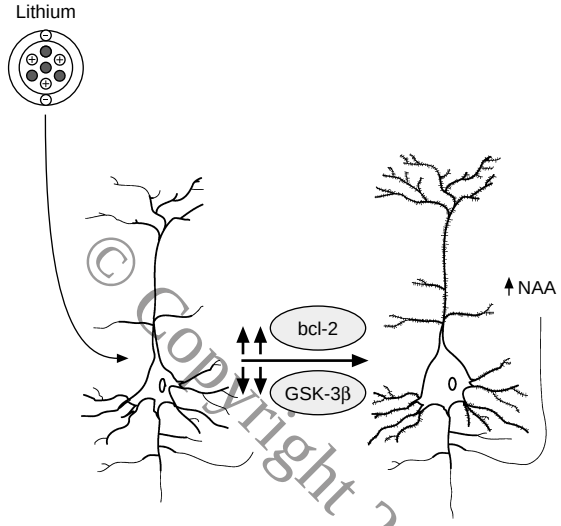
The Anatomy portion of this figure has the voxel placement in regions of interest in the frontal, temporal, occipital, and parietal cortex in a longitudinal study investigating the effects of lithium on regional NAA levels.

via MRS (Figure 7). NAA is the predominant resonance in the proton MRS spectrum of the normal adult human brain, and while the functional role of this amino acid has not been definitively determined, NAA is a putative neuronal marker,[120] 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 or function or neuronal loss. (For an excellent recent review of NAA, see reference 121.) In a prospective longitudinal study, we have utilized quantitative in vivo proton MRS to test the hypothesis 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 in both medication-free patients who have manic depressive illness and healthy subjects, and
2. putative lithium-induced changes in NAA levels are related to the gray matter content of different brain regions.

Proton MRS spectra are acquired from 8-cc regions of interest (ROIs) in the frontal, temporal, parietal, and occipital lobes, with an acquisition time of 5 min/ROI (stimulated echo acquisition mode pulse sequence echo time = 30 msec, modulation time = 13.7 msec, repetition time = 2000 msec).[122] Two trained individuals analyzed the in vivo nuclear magnetic resonance data with MRUI-VARPRO time domain spectral analysis software[123,124]; 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 patients who have manic depressive illness and healthy volunteers undergoing lithium administration. We have demonstrated for the first time that chronic lithium administration at therapeutic doses increases NAA concentration in the human brain in vivo.[125,126] 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, we observed a striking ~0.97 correlation between lithium-induced NAA increases and voxel gray matter content.[126] Together, these exciting new results support the contention that some of the long-term beneficial effects of lithium may be mediated by neurotrophic/neuroprotective events (Figure 8).

Figure 8. Mechanism by Which Lithium May Increase *N*-Acetyl-Aspartate (NAA) Levels[a]



[a]Lithium, via its effects on bcl-2, may exert major neurotrophic effects, resulting in neuropil increases, accompanied by increases in NAA levels.

CONCLUDING REMARKS

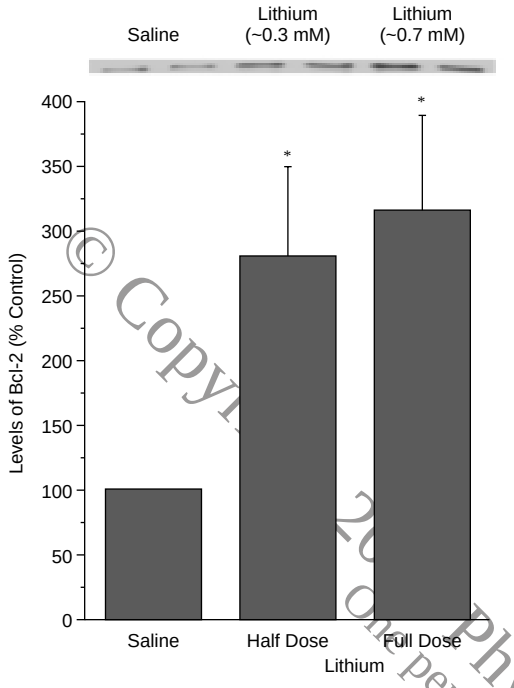
To date, lithium remains the only medication that has been demonstrated to produce such robust increases in the levels of bcl-2 in areas of frontal cortex, hippocampus, and striatum in vivo. The clear evidence for lithium's neuroprotective effects, as well as the growing appreciation that mood disorders are associated with cell loss and/or atrophy, suggests that these effects may be very relevant for the long-term treatment of mood disorders. Does long-term lithium treatment actually retard disease- or affective episode–induced cell loss or atrophy? The distinction between disease progression and affective episodes per se is an important one, since it is quite possible that the cytoprotective effects of lithium may be independent of its ability to treat or prevent affective episodes. There are presently no longitudinal studies that we are aware of which can adequately address this question, but this is clearly a very important and fundamental issue worthy of investigation. Thus, longitudinal studies comparing the long-term beneficial effects (using serial volumetric MRI scans or MRS quantitation of NAA levels for example) of lithium and anticonvulsants that do not share lithium's effects on bcl-2 or GSK-3β are clearly warranted. Similarly, the data suggest that the potential protective effects of lithium in conditions associated with high glucocorticoid levels such as Cushing disease may also be worthy of investigation.

The robust increases in bcl-2 levels and the inhibition of GSK-3β (and accompanying effects on *tau* and β-catenin) as well as the clear evidence for neuroprotective effects all suggest that the potential efficacy of lithium in the long-term treatment of various neurodegenerative disorders should be investigated. Although there have been some major breakthroughs in the identification of the genetic and pathogenic causes of many neurodegenerative diseases, the currently available therapies for nearly all of these disorders are clearly quite inadequate. Increasing knowledge of etiology and pathogenesis will provide future opportunities to develop specific therapies aimed at protecting neurons from underlying degenerative processes. The data reviewed in this article suggest that we may have overlooked the potential of a simple monovalent cation that has been used therapeutically for other CNS disorders for decades. An extensive literature search revealed no data to support or refute the contention that chronic lithium administration to patients with manic depressive illness results in a reduction in the incidence and/or severity of neurodegenerative disorders in this population. The only indirect human data that we are aware of are the data from Drevets and associates demonstrating smaller subgenual prefrontal cortex volume decrements in lithium-treated subjects (W. Drevets, M.D., written communication, March 1999) and the longitudinal study[126] demonstrating lithium-induced increases in NAA levels (vide supra). Many questions still to be answered include the identification of the biochemical and morphological identity of the cells in which lithium brings about the most robust increases in bcl-2 levels.

We fully agree with the absolute need for carefully controlled studies and the need to refrain from exaggerated, unsubstantiated claims[127]; however, while we search for the improved therapeutics of the future, we suggest that the efficacy of lithium in retarding disease progression clearly needs to be investigated. It is clear that lithium will very likely have no benefit in the acute treatment of various neurodegenerative disorders, and the increased sensitivity (with respect to side effects) of individuals with these illnesses suggests that lithium may even cause an acute worsening in some cases. However, studies in our laboratory have shown that chronic treatment of rodents with low doses of lithium (resulting in plasma concentrations ~ 0.3 mM) also robustly increases bcl-2 levels in the frontal cortex (Figure 9). Thus, although more detailed neuroanatomical studies are required, the requisite "bcl-2 up-regulating dose" of lithium may, in fact, be quite tolerable for most patients. In sum, recent advances in cellular and molecular biology have facilitated the identification of 2 novel, unexpected targets for the actions of lithium; these targets appear to play a major role in lithium's neuroprotective effects. We suggest that a reconceptualization of the long-term use of lithium may be warranted—namely, that the use of lithium should be considered as a long-term neurotrophic/neuroprotective agent in the treatment of mood disorders, irrespective of the "primary" treatment modality being used for the condition.

Disclosure of off-label usage: The authors have determined that, to the best of their knowledge, no investigational information about pharmaceutical agents has been presented in this article that is outside U.S. Food and Drug Administration–approved labeling.

Figure 9. Effects of Low-Dose Lithium Treatment on Bcl-2 Levels in Rat Frontal Cortex[a]



[a]Inbred male Wistar Kyoto rats were treated with Li_2CO_3 at "full dose" (resulting in plasma levels of ~0.7 mM) or "half dose" (resulting in plasma levels of ~0.3 mM) for 3 to 4 weeks. Immunoblotting of bcl-2 in frontal cortex was conducted using established methods with monoclonal antibodies against bcl-2 (1 in 100 dilution; Santa Cruz, Biotechnology, N-19, Santa Cruz, Calif.). Treatment of rats with either full-dose or half-dose lithium for 3 to 4 weeks resulted in significant increases in the levels of bcl-2.
*p < .05 compared with control.

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