

# Lithium at 50: Have the Neuroprotective Effects of This Unique Cation Been Overlooked?

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

*Recent advances in cellular and molecular biology have resulted in the identification of two 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 mmol/L. 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 the regeneration of mammalian CNS axons. Lithium has also been demonstrated to inhibit glycogen synthase kinase 3 $\beta$  (GSK-3 $\beta$ ), an enzyme known to regulate the levels of phosphorylated tau and  $\beta$ -catenin (both of which may play a role in the neurodegeneration observed in Alzheimer's disease). Consistent with the increases in bcl-2 levels and inhibition of GSK-3 $\beta$ , 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 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, the potential efficacy of lithium in the long term treatment of certain neurodegenerative disorders may be warranted. Biol Psychiatry 1999;46:929–940 © 1999 Society of Biological Psychiatry*

**Key Words:** Lithium, manic-depressive illness, bcl-2, neuroprotection, GSK-3 $\beta$ , neurogeneration

## Introduction

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 (MDI, Bipolar Affective Disorder). The discovery of lithium's efficacy as a mood-stabilizing agent revolutionized the treatment of patients with MDI—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 (Goodwin and Ghaemi 1999). After three 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 (Goodwin and Jamison 1990). Adequate lithium treatment, particularly in the context of a lithium clinic, is also reported to reduce the excessive mortality observed in the illness (Baldessarini et al 1999). 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 1969–1979, by reducing associated medical costs and restoring productivity (Reifman and Wyatt 1980). Despite its role as one of psychiatry's most important treatments, however, the biochemical basis for lithium's therapeutic effects remains to be fully elucidated (Jope 1999; Manji et al 1995). 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 Baldessarini et al 1999; Goodwin and Jamison 1990). 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 (Jope 1999; Manji et al 1995). 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 two novel, hitherto completely unexpected targets of lithium's actions, discoveries that may have a major impact on the future use of

From the Laboratory of Molecular Pathophysiology, Department of Psychiatry and Behavioral Neurosciences, and Cellular and Clinical Neurobiology Program, Wayne State University School of Medicine, Detroit, Michigan  
Address reprint requests to Husseini K. Manji, MD, FRCPC, Director, Laboratory of Molecular Pathophysiology, Department of Psychiatry and Behavioral Neurosciences, WSU School of Medicine, UHC 9B, 4201 St. Antoine Blvd., Detroit, MI 48201

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this unique cation in biology and medicine. In this article, we discuss the recent data demonstrating that lithium exerts major effects on the cytoprotective protein, bcl-2, as well as on GSK-3 $\beta$  (glycogen synthase kinase 3 $\beta$ ); 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. It should be emphasized that it is not our contention that lithium's effects on bcl-2 and GSK-3 $\beta$  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 (Duman et al 1997; Nibuya et al 1996; Smith et al 1995), 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 (Duman et al 1997; Jope 1999; Manji et al 1999).

### Lithium, Signal Transduction and Gene Expression: the Identification of Novel Targets

It has become increasingly appreciated in recent years that the long term treatment of complex neuropsychiatric disorders like MDI likely involves the strategic regulation of signaling pathways and gene expression in critical neuronal circuits (Duman et al 1997; Jope 1999; Hyman and Nestler 1996; Manji et al 1995). Lithium's effects on signal transduction pathways, in particular the protein kinase C signaling pathway, likely plays a major role in the treatment of affective episodes (Bebchuk 1999; Jope 1999; Manji and Lenox 1999). Substantial progress has been made both in identifying the genes responsive to transsynaptic stimulation, as well as in elucidating the processes that convert ephemeral second messenger-mediated events into long-term cellular phenotypic alterations. This has 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 life-long) behavioral consequences, and more 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 MDI. In this context, lithium has been shown to exert major effects on the AP-1 family of transcription factors (reviewed in Jope 1999; Manji et al 1999; Wang et al 1997), 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 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 (Jope 1999; Manji et al 1999; Nestler 1998). In recent years, new methodologies have evolved to identify the differential expression of multiple genes (e.g., in pathological 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, Liang et al 1995). Using this method, Wang and Young (1996) were the first to demonstrate that lithium increased 2',3'-cyclic nucleotide 3'-phosphodiesterase mRNA levels in C6 glioma cells. A major problem inherent in neuropharmacologic research is that the dearth of phenotypic changes clearly associated with treatment response, particularly for mood-stabilizing agents (Ikonov and Manji 1999). 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 (VPA) in the CNS, after chronic treatment of rodents in vivo (Chen et al 1999). These are two structurally highly dissimilar agents; although they likely do not exert their therapeutic effects by precisely the same mechanisms, identifying the genes that are regulated in concert by these two 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, VPA or saline. Saline was provided ad lib. to the lithium treated rats to reduce potential toxicity. The animals attained plasma drug concentrations similar to those attained clinically (Chen et al 1999), and no significant weight changes were observed with the chronic drug treatment. RNA was extracted from frontal cortices (FCx) to study gene expression using RT-PCR DD (Liang et al 1995). One of the genes whose expression was markedly increased by the treatments is the transcription factor, PEBP2 $\beta$  (GenBank Accession Number: AF087437, discussed in Chen et al 1999). After demonstrating that PEBP2 $\beta$ 's *function* (DNA binding of the PEBP2  $\alpha\beta$  complex) was also clearly increased by chronic lithium, we next investigated lithium's effects on the levels of a critical protein known to be regulated by PEBP2 $\beta$ —the major neuroprotective protein

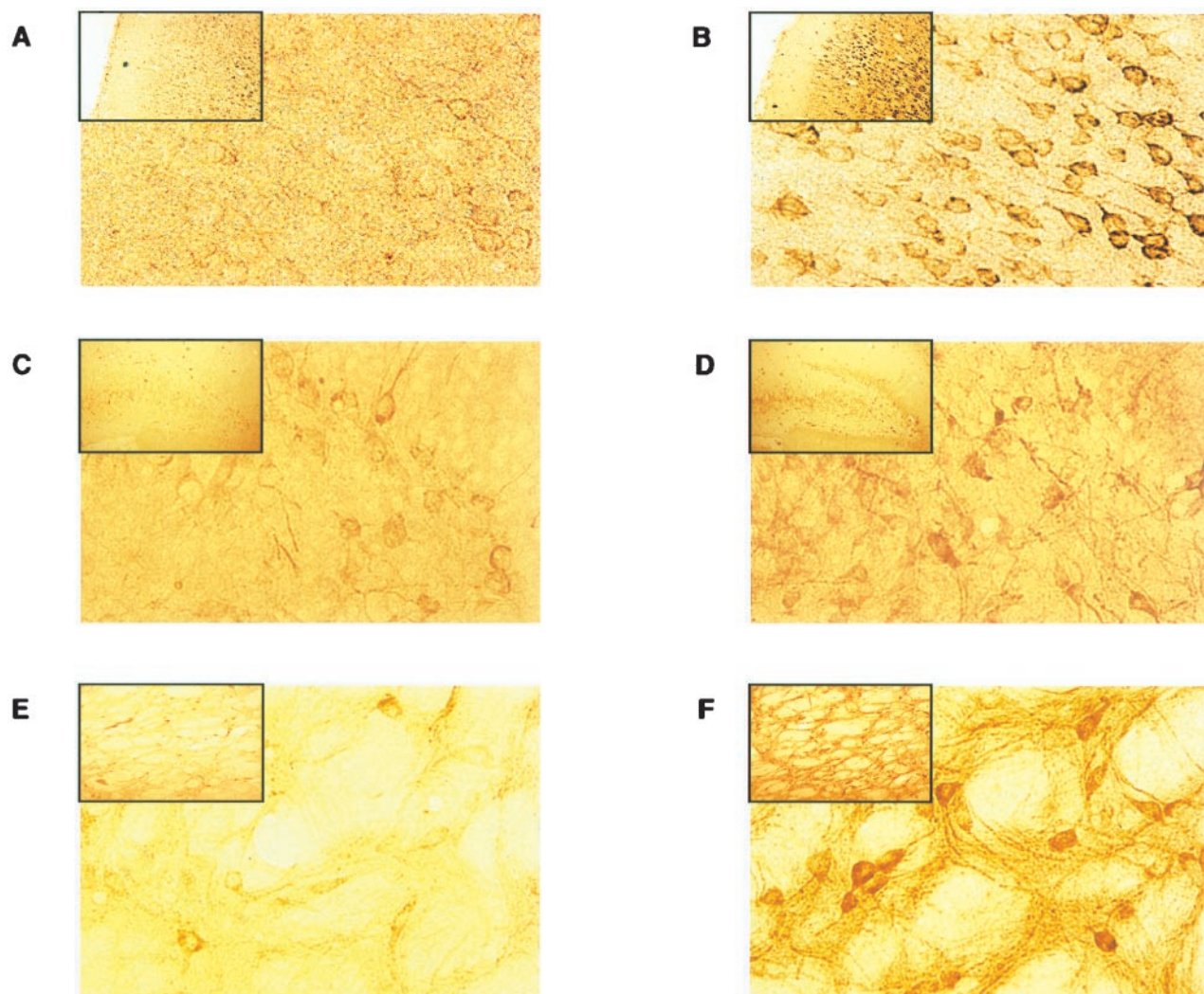


Figure 1. Effects of chronic lithium on the immunolabeling of bcl-2 in rat brain. Inbred male Wistar Kyoto rats (starting weight 150–200 g) were treated with either  $\text{Li}_2\text{CO}_3$  (4 mEq/kg/day) or saline by twice daily i.p. injections for 4 weeks. There was no significant weight loss observed with chronic lithium administration. Trunk blood was collected for determination of lithium levels ( $0.7 \pm 0.3$  mmol/L). Rats brains were cut at 30  $\mu\text{m}$ ; serial sections were cut coronally through the anterior portion of the brain, mounted on gelatin-coated glass slides and were 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 PBS containing a polyclonal antibody against bcl-2 (N-19, Santa Cruz Biotechnology, Santa Cruz, CA; 1:3000), 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, E) and lithium (B, D, F) treated rats, in layers 2 and 3 of frontal cortex (A, B), hippocampus (C, D) and striatum (E, F). Main photographs were obtained with 40 $\times$  magnification; insets with 10 $\times$  magnification.

**bcl-2** (Klampfer et al 1996), and found that chronic treatment of rats with lithium resulted in a doubling of bcl-2 levels in FCx. 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 plasma lithium levels were  $0.7 \pm 0.3$  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 immunoreac-

tive cells in layers II and III of FCx. Interestingly, the importance of neurons in layers II–IV of the FCx in mood disorders has recently been emphasized, because primate studies have indicated that these are important sites for connections with other cortical regions, and major targets for subcortical input (discussed in Rajkowska et al 1999). 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 (see Figure 1). 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 lithium for 6 days. Similar to the situation observed in rat brain in vivo, lithium produced a marked increase in bcl-2 levels in SH-SY5Y cells (Chen and Manji unpublished observations). Interestingly, lithium has very recently also been demonstrated to increase bcl-2 levels in rat cerebellar granule cells in a recent study (Chen and Chuang 1999). 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 (Chen and Chuang 1999). Moreover, lithium has very recently been demonstrated to reduce the levels of the pro-apoptotic protein p53 both in cerebellar granule cells (Chen and Chuang 1999) and SH-SY5Y cells (Lu et al 1999). Thus, overall the data clearly shows that chronic lithium robustly increases the levels of the neuroprotective protein bcl-2 in areas of rodent FCx, 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.

### The Bcl-2 Family of Proteins: Mediators of Cellular Life and Death

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 neurodegenerative 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, 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. Extensive research efforts aimed at elucidating the signaling pathways and proteins involved in regulating physiological and pathophysiological cell death has revealed critical roles for mammalian proteins that show consider-

able homology to the "*C. Elegans* death proteins"—the bcl-2 family of proteins.

Bcl-2 is the acronym for the B-cell lymphoma/leukemia-2 gene; this gene was first discovered because of its involvement in B-cell malignancies, where chromosomal translocations activate the gene in the majority of follicular non-Hodgkin's B-cell lymphomas (Merry and Korsmeyer 1997; Adams and Cory 1998; Bruckheimer et al 1998; Sadoul 1998; Li and Yuan 1999 and references therein). Bcl-2 was the first identified member of a large family of cellular and viral apoptosis-regulating proteins (reviewed in Adams and Cory 1998; Bruckheimer et al 1998; Li and Yuan 1999; Sadoul 1998). These proteins seem to regulate common pathways for apoptosis and programmed cell death, with several functioning as "protectors" (Bcl-2, Bcl-XL, Mcl-1, Ced-9, BHRF-1, E1b-19 kDa, A1, ASFV-5HL, Bcl-W, and NR13) and others as "executioners" (Bax, Bcl-XS, Bak, Bad, Bik, and Bid). In a number of cases, these proteins interact with each other in a complex network of homodimers and heterodimers (Adams and Cory 1998; Bruckheimer et al 1998; Li and Yuan 1999; Merry and Korsmeyer 1997; Sadoul 1998 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 (Adams and Cory 1998; Bruckheimer et al 1998; Merry and Korsmeyer 1997; Wilson 1998 and references therein). It is likely that several cellular mechanisms are involved in mediating bcl-2's protective effects, including sequestering the proforms of caspases, inhibiting the effects of caspase activation, antioxidant effects, enhancing mitochondrial calcium uptake, and attenuating the release of calcium and cytochrome c from mitochondria (reviewed in Adams and Cory 1998; Bruckheimer et al 1998; Li and Yuan 1999; Sadoul 1998).

A role for bcl-2 in protecting neurons from cell death is now supported by abundant evidence (Table 1); 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 sulfoximine (Adams and Cory 1998; Bruckheimer et al 1998). 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 pharmacological 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,

Table 1. Neuroprotection by Bcl-2

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- \*Protects neuronal cells from the lethal effects of a variety of stimuli generating 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 MPTP neurotoxicity, and are protected against acute MPTP-induced dopamine depletion
- \*Transgenic mice overexpressing bcl-2 show enhanced *regeneration* of severed CNS axons, independent of bcl-2's anti-apoptotic effects

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Summarized from Adams and Cory 1998; Bonfanti et al 1996; Bruckheimer et al 1998; Chen et al 1997; Kostic et al 1997; Lawrence et al 1996; Li and Yuan 1999; Merry and Korsmeyer 1997; Raghupathi et al 1998; Sadoul 1998; Yang et al 1998.

bcl-2 over-expression 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 MPTP or focal ischemia, and to protect photoreceptor cells from two forms of inherited retinal degeneration; interestingly, neurons that survive ischemic lesions or traumatic brain injury in vivo show upregulation of bcl-2 (Bonfanti et al 1996; Lawrence et al 1996; Chen et al 1997; Merry and Korsmeyer 1997; Raghupathi et al 1998; Sadoul 1998; Yang et al 1998, and references therein). Over expression 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 (Kostic et al 1997).

Most recently, it has been clearly demonstrated that not only does bcl-2 over expression 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 (Chen et al 1997). Because 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 (Chen et al 1997). As articulated already, the only means of therapeutically increasing CNS bcl-2 levels in the adult brain has heretofore been by the use of complex gene transfer methodologies; thus, pharmacological means of robustly increasing CNS bcl-2 levels represents a major advance for the long term treatment of certain neurodegenerative disorders.

### **Are Lithium-Induced Increases in CNS Bcl-2 Levels Relevant in the Long-Term Treatment of Mood Disorders?**

The evidence reviewed above strongly suggests that the robust lithium-induced increases in CNS bcl-2 levels

should result in neuroprotective effects. Are such putative neuroprotective effects relevant for mood disorders? There is now considerable literature support from a variety of sources demonstrating significant reductions in regional CNS volume and cell numbers (both neurons and glia) associated with mood disorders. One line of evidence comes from structural imaging studies that 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 MDI patients, although the lateral ventricle enlargement is not as consistently observed (reviewed by Ketter et al 1997; Soares and Mann 1997). Recent studies have also reported reduced basal ganglia volume in mood disorders, and reduced temporal lobe volume (including the hippocampus) is one of the more consistent structural neuroimaging findings in mood disorders (reviewed in Ketter et al 1997; Soares and Mann 1997). Within the frontal lobe, volumetric neuroimaging studies have also consistently shown reduced volumes in mood disorders. In particular, recent volumetric 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 ventral to the genu of the corpus callosum (Drevets et al 1997). 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 Ketter et al 1997). 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 (1998) compared 4 brains of MDI patients 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 MDI subjects compared to controls. Three recent

postmortem studies of the prefrontal cortex have also demonstrated reduced CNS volume and cell numbers in mood disorders. Rajkowska (1997) has used 3 dimensional cell counting and morphological techniques to demonstrate decreased cortical and laminar thickness in MDI subjects completing 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 to 9 matched control subjects (Rajkowska et al 1999). Intriguingly, neuronal diminution was especially pronounced in layer II of the rostral orbitofrontal region (Rajkowska et al 1999), an area where we have observed amongst the largest lithium-induced increases in bcl-2 immunoreactivity (Chen et al 1999). Also in the prefrontal cortex, Ongur and colleagues (1998) have recently reported a histological 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 MDI (41% reductions) compared to controls. This is a particularly striking finding as it is consistent with neuroimaging findings showing cortical volume loss in this same region on volumetric MRI in a similar diagnostic group (Drevets et al 1997).

Together, the preponderance of the data from the neuroimaging studies and the growing body of postmortem evidence presents 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 of glia and neurons, or the sequelae of the biochemical changes (for example, in glucocorticoid and catecholamine 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 Gould et al 1998; McEwen and Magarinos 1997). Most recently, it has been demonstrated that stress inhibits the proliferation of granule cell precursors in three different mammalian species—rat, tree shrew, and marmoset monkey—suggesting that this phenomenon is a common characteristic of all animals that produce granule neurons in adulthood (Gould et al 1998). These findings are particularly noteworthy because, utilizing a method for labeling cell division directly in the

adult human brain, Ericksson and colleagues 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 (Chen et al 1997), 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 2–3 weeks of lithium administration does, indeed, result in an increase in the number of BrdU positive neurons in the dentate gyrus (Chen and Manji 1999). Overall, the data clearly suggests that lithium's effects 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. 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 or atrophy in mood disorder patients. Unfortunately, such data is presently not available; however, subsequent to the demonstration of lithium's robust effects on CNS bcl-2 levels, Drevets and associates have re-analyzed the volumetric data from their cohort of familial bipolar depressives and familial unipolar depressives. Intriguingly, they have found that the lithium-treated subjects exhibit smaller reductions in FCx volumes than the non-lithium treated patients (personal communication from W. Drevets to H.K. Manji, March 1999), findings that are compatible with a neurotrophic/neuroprotective effect of chronic lithium. Additional indirect support comes from our recent study in which we have investigated the effects of chronic lithium administration on CNS *N*-acetylaspartate (NAA, a putative marker of neuronal viability; see Tsai and Coyle 1995 for an excellent review) levels. Using magnetic resonance spectroscopy, we have found that chronic lithium significantly increases brain NAA levels, and furthermore observe a striking ( $r \sim .97$ ) correlation between voxel grey matter content and the lithium-induced NAA increases (Moore et al, unpublished data 1999).

### **GSK-3 $\beta$ : A Cellular Target for the Actions of Lithium**

Remarkably, in addition to the robust effects on bcl-2, another completely unexpected target for the action of lithium has recently been identified, one that may also afford protection against certain types of cell death. Klein and Melton (1996) were the first to demonstrate that lithium, at therapeutically relevant concentrations, is an inhibitor of GSK-3 $\beta$ . Glycogen synthase kinase 3 $\beta$  (GSK-

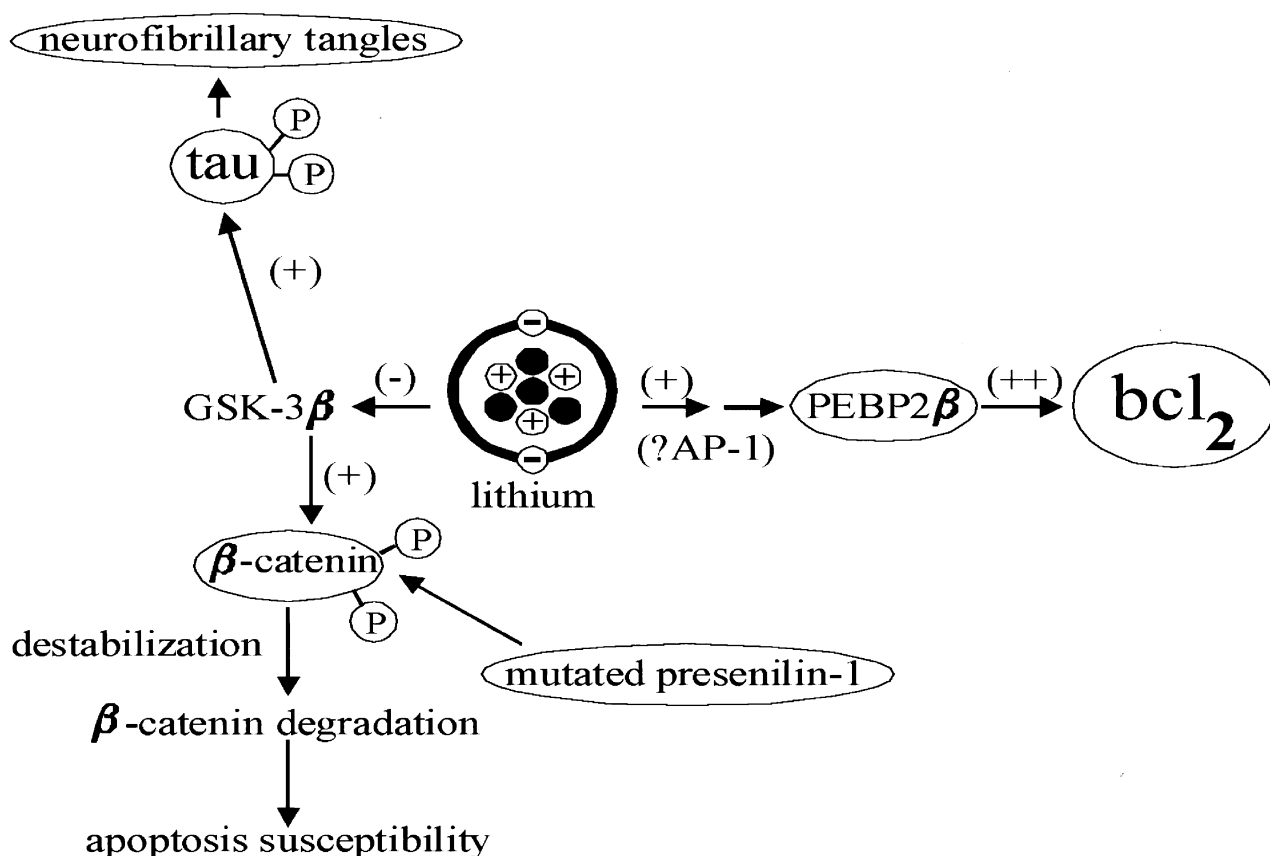


Figure 2. Molecular and cellular mechanisms underlying lithium's neuroprotective effects. Chronic lithium, at therapeutically relevant concentrations, increases the expression and function of the transcription factor PEBP2 $\beta$ , that results in a robust upregulation of the major neuroprotective protein, bcl-2 in the CNS. Lithium is also an inhibitor of glycogen synthase kinase GSK-3 $\beta$  (GSK-3 $\beta$ ). GSK-3 $\beta$  is known to phosphorylate  $\tau$ , a major component of neurofibrillary tangles. It should be emphasized that GSK-3 $\beta$  is only one of the kinases involved in phosphorylating  $\tau$ . Nevertheless, inhibition of GSK-3 $\beta$  by lithium may reduce levels of hyperphosphorylated  $\tau$ . GSK-3 $\beta$  also regulates  $\beta$ -catenin levels, and inhibition of GSK-3 $\beta$  by lithium results in a stabilization of  $\beta$ -catenin. The lithium-induced stabilization of  $\beta$ -catenin may serve to offset the destabilizing effects of interactions of mutant presenilin-1 protein, thereby reducing neuronal vulnerability to apoptosis induced by amyloid- $\beta$ -protein.

3 $\beta$ ) is an evolutionarily highly conserved enzyme, originally identified as a regulator of glycogen synthesis. It is now known to play 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, and nuclear translocation of  $\beta$ -catenin (reviewed in Dale 1998; Willert and Nusse 1998). These original seminal studies by Klein and Melton have resulted in a number of follow-up studies, and have generated considerable excitement about the possibility of developing novel GSK-3 $\beta$  modulators as potential new therapeutics for bipolar disorder (Hedgpeth et al 1997; Manji et al 1999).

Unlike many protein kinases, GSK-3 $\beta$  is highly active in resting cells and is primarily regulated by inactivation. Several recent studies have found that inhibition of GSK-3 $\beta$  by lithium reduces  $\tau$  phosphorylation, effects that

likely occur at therapeutically relevant CNS concentrations (see Jope 1999 for an excellent discussion). Although many of the studies have utilized lithium concentrations in excess of those utilized therapeutically, the available data suggests that lithium, at concentrations of  $\sim 1$  mmol/L does, indeed, reduce  $\tau$  phosphorylation (Hong et al 1997; Lovestone et al 1999; Munoz-Montano et al 1997; reviewed in Jope 1999). For the purposes of the present discussion, it is noteworthy that the intracellular neurofibrillary tangles (NFTs, that have been shown to directly correlate with the severity of dementia) 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 that may precede the disruption of the microtubule cytoskeleton, and studies with transgenic

mice have shown that GSK-3 $\beta$  may play a major role in  $\tau$  phosphorylation. Thus, although GSK-3 $\beta$  is undoubtedly not the only kinase involved in the aberrant hyperphosphorylation, inhibition of GSK-3 $\beta$  (for example by lithium) represents an attractive potential mechanism to reduce the accumulation of hyperphosphorylated  $\tau$  that is found in NFTs (Figure 2).

GSK-3 $\beta$  also plays a major role in regulating  $\beta$ -catenin levels; inhibition of GSK-3 $\beta$  results in  $\beta$ -catenin accumulation, likely due to a decrease in the rate of  $\beta$ -catenin protein degradation (Dale 1998; Willert and Nusse 1998). In this context, it is noteworthy that recent studies have shown that presenilin-1 forms a complex with  $\beta$ -catenin *in vivo* leading to an increase in  $\beta$ -catenin stability (Zhang et al 1998). Furthermore, mutations in the presenilin-1 gene (that have been found in many patients with familial Alzheimer's disease) have been shown to reduce the ability of presenilin-1 to stabilize  $\beta$ -catenin, thereby leading to increased degradation of  $\beta$ -catenin in the brains of transgenic mice. Presenilin mutations associated with Alzheimer disease have also recently been demonstrated to cause defective intracellular trafficking of  $\beta$ -catenin, a component of the presenilin protein complex (Nishimura et al 1999). Moreover,  $\beta$ -catenin levels are markedly reduced in the brains of Alzheimer's disease patients with presenilin-1 mutations, and loss of  $\beta$ -catenin signaling seems to increase neuronal vulnerability to apoptosis induced by amyloid- $\beta$  protein (Zhang et al 1998). Thus, inhibition of GSK-3 $\beta$  (for example by lithium) may serve to offset the  $\beta$ -catenin destabilizing effects of mutated forms of presenilin-1, and thereby reduce the vulnerability of affected neurons to apoptosis induced by amyloid- $\beta$  protein (Figure 2).

### Does Lithium Actually Have Neuroprotective Effects?

Perhaps the most intuitively obvious interpretation of the lithium-induced increases in bcl-2 levels is that they may underlie, at least in part, the well known phenomenon of lithium-induced leukocytosis; indeed this is a very plausible and heuristic hypothesis currently under investigation. Lithium's robust effects on bcl-2 and GSK-3 $\beta$  (see Figure 2) in the mature CNS suggests that this cation, at therapeutically relevant concentrations, may also possess significant neuroprotective properties. Indeed, although the effects of lithium on GSK-3 $\beta$  and, in particular, on bcl-2 are very recent observations, several earlier studies had already demonstrated neuroprotective properties of lithium (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). 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 non-depolarizing 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 (Grignon et al 1996). 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, NMDA receptor activation, low potassium, and toxic concentrations of anticonvulsants (D'Mello et al 1994; Nonaka et al 1998a, 1998b). Lithium's protective effects against the deleterious effects of glutamate and NMDA receptor activation have also been demonstrated to occur in hippocampal and cortical neurons in culture, and 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 (Nonaka et al 1998b). 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 (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 (that mobilizes intracellular Ca<sup>++</sup>) and MPP<sup>+</sup> induced cell death (Chen and Manji, unpublished observations).

In addition to the demonstration of protective effects *in vitro*, a number of studies have also investigated lithium's neuroprotective effects *in vivo*. Thus, Inouye and associates (1995) exposed newborn mice to gamma irradiation, focusing on cells of the external granular layer that 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 (Pascual and Gonzalez 1995). 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 (Pascual and Gonzalez 1995). 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 [<sup>3</sup>H]D-aspartate uptake (Sparapani et al 1997). Chronic lithium has recently been shown to exert dramatic protective effects against

Table 2. Experimental Paradigms in which Lithium Has Been Demonstrated to Exert Neuroprotective Effects

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- \*Protects cultured neurons against glutamate and NMDA-induced cell death<sup>a</sup>
  - \*Protects cerebellar granule cells from KCl deprivation and anticonvulsant- or age-induced apoptosis<sup>ab</sup>
  - \*Induces survival of PC12 cells after serum/NGF deprivation<sup>c</sup>
  - \*Protects PC12 & SY5Y cells from ouabain toxicity<sup>d</sup>
  - \*Delays radiation-induced apoptosis in external granule cells of mouse cerebellum<sup>e</sup>
  - \*Protects SY5Y cells from Ca<sup>++</sup> and MPP<sup>+</sup> toxicity<sup>f</sup>
  - \*Attenuates behavioral deficits and ChAT activity reduction by forebrain cholinergic system lesions<sup>g</sup>
  - \*Reduces middle cerebral artery occlusion induced infarct size and neurological deficits<sup>h</sup>
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<sup>a</sup>Nonaka et al 1998a.<sup>b</sup>Nonaka et al 1998b.<sup>c</sup>Volonte and Rukenstein 1993.<sup>d</sup>Li et al 1994.<sup>e</sup>Inouye et al 1995.<sup>f</sup>Chen and Manji unpublished observations 1999.<sup>g</sup>Pascual and Gonzales 1995.<sup>h</sup>Nonaka and Chuang 1998.

middle cerebral artery occlusion, reducing not only the infarct size (56%), but also the neurological deficits (abnormal posture and hemiplegia) (Nonaka and Chuang 1998). Most recently, in an elegant series of studies, 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 (personal communication from D.M. Chuang to H.K. Manji, March 1999). Table 2 summarizes some of the most robust experimental evidence demonstrating neuroprotective effects of lithium in vitro and in vivo. It must be acknowledged at this point that the regulation of cell survival and cell death are complex processes involving multiple interacting signaling pathways, transcription factors and gene expression. Thus, lithium's effects on signaling pathways and transcription factors (Jope 1999; Manji et al 1995) 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 (Adams and Cory 1998; Bruckheimer et al 1998; Merry and Korsmeyer 1997 and references therein), lithium's robust upregulation of bcl-2 levels at therapeutically relevant concentrations likely plays a major role in its neuroprotective effects. It is also important to emphasize that in addition to neuroprotective effects, lithium is well known to exert toxic effects at higher concentrations in a variety of experimental conditions (Hasegkar et al 1996; Madiehe et al 1995) as well as in humans (Goodwin and Jamison 1990; Lenox and Manji 1998). In general, lithium's toxicity seems to be related to dose, maturity of the cells, and interactions with other pharmacologic agents such as neuroleptics and cholinomimetics (D'Mello et al 1994; Jope et al 1986; Lenox and Manji 1998).

## Conclusions

Nineteen ninety-nine marks the 50th anniversary of John Cade's seminal report of lithium's efficacy, but we con-

tinue to discover exciting new biochemical properties that this unique monovalent cation possesses. 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 or atrophy, suggests that these effects may be very relevant for the long term treatment of mood disorders. These results suggesting potential neuroprotective effects of lithium follow the exciting findings from Duman and associates demonstrating that chronic administration of a variety of antidepressants increases the expression of BDNF (brain derived neurotrophic factor), and its receptor trkB in certain populations of neurons in the hippocampus and cerebral cortex (Duman et al 1997; Nibuya et al 1996). Because stress has been shown to decrease the expression of BDNF, it has been postulated that the antidepressant-induced increases in BDNF and trkB expression may increase the survival of hippocampal neurons, and perhaps even promote the sprouting of critical neurons that innervate the hippocampus (Duman et al 1997). These exciting findings have thus led to a heuristic molecular and cellular hypothesis of depression, that posits that stress-induced vulnerability and the therapeutic action of antidepressant treatments occur via intracellular mechanisms regulating neurotrophic factors necessary for the survival and functioning of critical neurons (Duman et al 1997). Intriguingly, Smith and associates (1995) have also recently demonstrated opposite effects of stress and antidepressants on the expression of another neurotrophic factor, neurotrophin 3 in the locus coeruleus. Additionally, Duman and associates have recently demonstrated that chronic administration of a variety of antidepressants enhances neurogenesis in regions of the hippocampus in the mature rodent (Duman 1998). Together, the results suggest that antidepressants and lithium may exert some of their long term beneficial effects via

underappreciated neurotrophic/neuroprotective effects, and represents an exciting area for future medication development (Nestler 1998). 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, because 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 that 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 for example) of lithium and anticonvulsants that do not share lithium's effects on bcl-2 or GSK-3 $\beta$  are clearly warranted. Similarly, the data suggests that the potential protective effects of lithium in conditions associated with high glucocorticoid levels such as Cushing's disease may also be worthy of investigation.

The robust increases in bcl-2 levels, the inhibition of GSK-3 $\beta$  (and accompanying effects on *tau* and  $\beta$ -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. *Science's* recent issue focusing on neurodegeneration (Nov. 6, 1998) highlights the devastating impact that neurodegenerative disorders have on the lives of millions. 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 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; however, as articulated by Shoulson (1998), there is a mounting sense of urgency and desperation among patients and families to develop new "wonder drugs" for some of society's most devastating illnesses. Ironically, the data reviewed in this article suggests that, in our efforts to develop novel "magic bullets," 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 did not reveal any data to support or refute the contention that chronic lithium administration to bipolar patients results in a reduction in the incidence or severity of neurodegenerative disorders in this population. The only indirect human data that we are aware of is the data from Drevets and associates demonstrating smaller subgenual prefrontal cortex volume decrements in lithium-treated subjects, and the longitudinal study demonstrating lithium-induced increases in NAA

levels (vide supra). Clearly many questions still to be answered, including the identification of the biochemical and morphological identity of the cells in which lithium brings about the most robust increases in bcl-2 levels; additionally, although cholinomimetics and lithium can be judiciously safely co-administered, their cavalier concomitant administration should clearly be avoided because of the possibility of inducing seizures.

We fully agree with the absolute need for carefully controlled studies, and the need to refrain from exaggerated, unsubstantiated claims (Shoulson 1998); 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 investigating. It is clear that lithium will 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 acutely even cause a worsening in some cases. Studies in our laboratory have shown, however, that chronic treatment of rodents with low doses of lithium (resulting in plasma concentrations  $\sim$ 0.3 mmol/L) also robustly increases bcl-2 levels in the frontal cortex; thus, although more detailed neuroanatomical studies are required, the requisite "bcl-2 upregulating dose" of lithium may, in fact, be quite tolerable for most patients. In sum, recent advances in cellular and molecular biology has facilitated the identification of two novel, unexpected targets for lithium's actions; these discoveries should serve to spark further research into the mechanisms, neuroanatomic specificity, and potential "insult or illness specificity" of lithium's neuroprotective effects, and may have a major impact on the future use of this unique cation in medicine.

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