

Overview of the Mechanism of Action of Lithium in the Brain: Fifty-Year Update

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© Since its discovery, lithium has been shown to act upon various neurotransmitter systems at multiple levels of signaling in the brain. Lithium, affecting each neurotransmitter system within complex interactive neuronal networks, is suggested to restore the balance among aberrant signaling pathways in critical regions of the brain. Recent molecular studies have revealed the action of lithium on signal transduction mechanisms, such as phosphoinositide hydrolysis, adenylyl cyclase, G protein, glycogen synthase kinase-3 β , protein kinase C, and its substrate myristoylated alanine-rich C kinase substrate. Such effects are thought to trigger long-term changes in neuronal signaling patterns that account for the prophylactic properties of lithium in the treatment of bipolar disorder. Through its effects on glycogen synthase kinase-3 β and protein kinase C, lithium may alter the level of phosphorylation of cytoskeletal proteins, which leads to neuroplastic changes associated with mood stabilization. Chronic lithium regulates transcriptional factors, which in turn may modulate the expression of a variety of genes that compensate for aberrant signaling associated with the pathophysiology of bipolar disorder. Future studies on long-term neuroplastic changes caused by lithium in the brain will set the stage for new drug-discovery opportunities. *(J Clin Psychiatry 2000;61[suppl 9]:5-15)*

As we reflect upon the first 50 years since the discovery of the efficacy of lithium for the treatment of manic-depressive illness, we have made significant strides in laying the foundation for our understanding of the mechanism of action of lithium in the brain. In many ways, the knowledge that we have accumulated has directly paralleled the advancement of the field of neuroscience and the experimental strategies developed over this past half century. While the generation of experimental evidence for the action of lithium has shifted from ion transport and presynaptic neurotransmitter-regulated release to postsynaptic receptor regulation, to signal transduction cascades, to gene expression and neuroplastic changes in the neuropil, the focus of research has remained on the ability of the monovalent cation to uniquely

alter signaling in critical regions of the brain. As we have noted previously, proper interpretation of these data has at times been limited by experimental designs, which have often ignored not only the clinically relevant therapeutic range of concentration and onset of action of lithium but also the critical control studies defining the specificity of action as compared with other monovalent cations and classes of psychopharmacologic agents. As we review the progress made over the past 50 years, we will highlight some of the most robust data that not only define the multiple sites wherein lithium can exert its action but also will ultimately lead to new insights over the next 50 years.

LITHIUM AND ION TRANSPORT

Ion-gated channels, which are driven by either adenosine triphosphate (ATP) or net free energy of transmembrane concentration gradients, regulate the distribution of lithium across the cell membrane. These transport systems are crucial for the regulation of resting lithium in the bulk cytoplasm, as they essentially regulate all steady-state intracellular ion concentrations. Lithium, in particular, has the highest energy of hydration of all the alkali metal series, which accounts for its similarity in size to divalent cations such as calcium (Ca⁺⁺) and magnesium (Mg⁺⁺). In red blood cells (RBCs), which have been used as a cell model for the study of lithium transport properties, the influx of lithium into the cell occurs predominantly through passive diffusion, but also appears to in-

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volve a Na (Li) K cotransport pathway. Extrusion of lithium from the cell occurs primarily through the sodium-lithium countertransport pathway.^{1,2} On the other hand, the gating of lithium through the ion channels that accept the lithium ion as a substitute for their normal ionic substrates is vital to the regulation of intracellular lithium.

Na,K-ATPase pump, being involved in altering the neuronal excitability, has been extensively studied in relation to the membrane transport of lithium as well as the therapeutic effect of lithium (see reviews in references 3 and 4). When measured in peripheral neurons as well as synaptosomal membrane fractions from brain, chronic lithium treatment was found to decrease Na,K-ATPase activity, particularly in hippocampus.⁵ Various groups have studied Na,K-ATPase activity in patients with mood disorders and have reported alterations of the RBC to plasma lithium concentration ratio in bipolar patients as a function of clinical state and genetic aspects of the illness. Although a large interindividual variation and confounding clinical parameters precluded consistent replication of these findings, the investigations led to the hypothesis that the pathogenesis of affective disorder may be related to membrane dysfunction.^{6,7} A number of groups have reported that Na,K-ATPase activity in RBCs from patients is reduced in the depressed phase of both unipolar and bipolar patients, which may account for the increased retention of sodium that had been reported by many groups (see review in reference 3). In addition, in light of the fact that free Ca⁺⁺ concentration parallels free sodium, the reduced Na,K-ATPase activity in patients may, in part, account for more recent findings that the intracellular Ca⁺⁺ is increased in peripheral blood cells of bipolar patients.⁸ Interestingly, when bipolar patients were treated with lithium, Na,K-ATPase activity was found to be increased, which is consistent with observations of reduced Ca⁺⁺ following treatment.

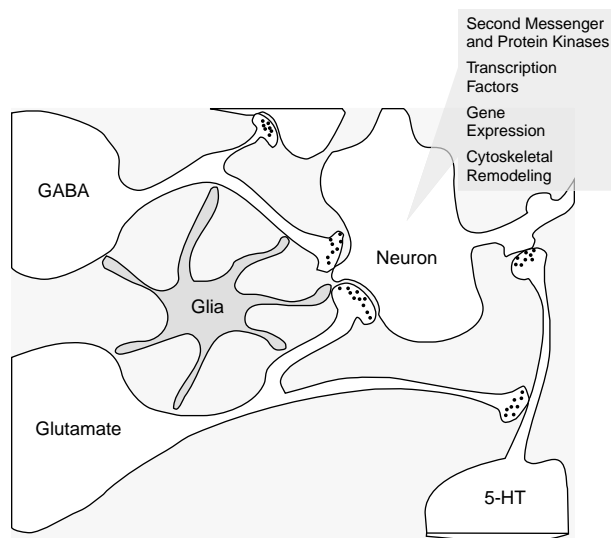
While a balance of resting lithium conductance and net transport-efflux mechanisms regulates the steady-state lithium homeostasis, the ligand-gating of ion channels on the time scale of the channel activity may play a more significant role in the regulation of intracellular lithium concentration within regulatory sites of an excitable cell such as the neuron. Recently, it was shown that lithium concentration in the local environment of dendritic spine can rise by as much as 5-fold to 10-fold from resting intracellular lithium following a train of synaptic stimuli.⁹ In *Xenopus* oocyte, by expressing glutamate receptors, one synaptic current can increase the local lithium concentration in dendritic spine up to several-fold higher than the extracellular concentration of lithium. It is via such a mechanism that lithium can accumulate more selectively in neurons of higher synaptic activity, and such an activity-dependent accumulation of lithium may be crucial for lithium's therapeutic specificity and its ability to regulate synaptic function in the brain.¹⁰

BALANCE OF NEUROTRANSMITTER SIGNALING

Neurotransmitter and Neuropeptide Systems

In search of a link between the mechanism of action of lithium and neurotransmission, the effect of lithium has been extensively studied on virtually every neurotransmitter system. Historically, earlier studies were focused on the modulation of presynaptic components, including the synthesis, release, turnover, and reuptake of the neurotransmitters. In recent years, the focus has shifted to postsynaptic events, such as the regulation of the signal transduction mechanisms (see reviews in references 10 and 11). Despite the fact that some of the results of the presynaptic and postsynaptic investigations are not in full agreement at present, the evidence supports the action of lithium at multiple sites that modulate neurotransmission. Lithium appears to reduce presynaptic dopaminergic activity and postsynaptically to prevent the development of receptor up-regulation and supersensitivity. In the cholinergic system, lithium enhances receptor-mediated responses at neurochemical, electrophysiologic, and behavioral levels. Lithium increases the activity of γ -aminobutyric acid (GABA)ergic-mediated inhibition and has been shown to reduce excitatory glutamatergic neurotransmission. While lithium modulates each neurotransmitter system through complex mechanisms, the net effect of chronic lithium appears to be its ability to alter the balance among neurotransmitter/neuropeptide signaling pathways as noted in Figure 1.

Dopamine. Presynaptically, lithium has been shown to increase the dopamine turnover in a region-specific manner (see review in reference 12) and decrease the formation of the neurotransmitter.^{13,14} On the basis of the hypothesis that mania might be associated with supersensitive dopamine receptors, the effect of lithium on dopamine receptor supersensitivity has been studied. Chronic lithium treatment prevents behavioral and biochemical manifestations of haloperidol-induced dopamine receptor supersensitivity. It was shown that lithium treatment decreases haloperidol-induced supersensitivity to iontophoretically applied dopamine¹⁵ and partially prevents the development of electrical intracranial self-stimulation following haloperidol treatment in rats (see review in reference 16). While the effects of lithium on supersensitive dopamine receptors were well demonstrated by functional measures, lithium treatment has not been shown to result in any consistent regulation of D₁ or D₂ receptors, suggesting a possible involvement of a postreceptor site of lithium action. Interestingly, lithium also appears to block amphetamine-induced behavioral changes such as increased locomotor activity in animals (see reviews in references 12 and 16) as well as euphoriant effects in depressed patients.^{17,18} Many groups have studied dopamine and its metabolites in the cerebrospinal fluid (CSF) of patients, but the results have been confounded by mood and activity state (see review in reference 10).

Figure 1. Lithium Regulation of Signaling in Brain^a

^aAbbreviations: 5-HT = serotonin, GABA = γ -aminobutyric acid. Since its discovery as a treatment for manic-depressive illness over 50 years ago, lithium has been proposed to act upon different neurotransmitter systems at multiple levels of signal transduction. By virtue of its ability to modulate signaling within complex interactive neuronal networks, it is suggested that lithium can restore the balance among aberrant signaling pathways in critical limbic and limbic-related regions of the brain. Accumulated data over the past 2 decades support an action of lithium within signal transduction cascades that are shared among neuronal populations. Recent data also point to a role for glia in the regulation of the levels of neurotransmitters, e.g., glutamate, functionally available for interneuronal signaling. Such effects of lithium are thought to trigger long-term changes in neuronal signaling patterns that account for the prophylactic properties of lithium that underlie the clinical efficacy of lithium in preventing the recurrent affective episodes of manic-depressive illness. Chronic lithium has been shown to alter the activity of transcription factors and subsequent regulation of gene expression, which appear to set in motion its potential role in neuroprotection and cytoskeletal remodeling. These neuroplastic changes may lay the foundation leading to the mood-stabilizing properties of lithium and represent a target for new drug-discovery opportunities.

Norepinephrine. The presynaptic effect of lithium on norepinephrine appears to be biphasic; early increases in norepinephrine uptake and synthesis are followed by a return to baseline level after chronic treatment. The effect of lithium on norepinephrine receptor binding has been generally inconclusive (see review in reference 3). However, it has been shown that lithium treatment enhances receptor subsensitivity following antidepressant treatment.¹⁹ In addition, lithium treatment prevents β -adrenergic supersensitivity induced by neurotransmitter depletion.^{20,21} A consistent observation in vivo as well as in vitro, however, is that β -adrenergic receptor-mediated accumulation of cyclic adenosine monophosphate (cAMP) is decreased by acute lithium treatment. Following chronic treatment, the release of norepinephrine is facilitated, possibly via the effects on the presynaptic α_2 autoreceptors.²² In clinical investigations, variable results have been reported in plasma

and urinary norepinephrine metabolite levels after lithium treatment.

Serotonin. Preclinical studies have shown that lithium affects the serotonergic neurotransmission at various levels. Presynaptically, lithium appears to enhance the serotonergic neurotransmission. In rat brain slices, it has been shown that chronic treatment of lithium increases basal and stimulation-induced serotonin release. There is accumulating evidence that lithium treatment produces a subsensitivity of presynaptic inhibitory 5-HT_{1A} receptors, which can result in a net increase of 5-HT released per impulse.^{23,24} Furthermore, by using electrophysiologic recordings to measure the effects of lithium on the serotonin system, it was demonstrated that short-term lithium treatment enhances the effect of activation of the ascending (presynaptic) 5-HT pathway, while the responsiveness of the postsynaptic neuron to serotonin was not affected.^{25,26} These findings are consistent with the observations that short-term lithium enhances the efficacy of the presynaptic 5-HT system and have formed the basis for a series of clinical investigations demonstrating the efficacy of lithium as an adjunct to antidepressants in the treatment of refractory depression.

Receptor-binding studies have shown regionally specific effects with significant variability, except that many groups reported a consistent decrease in 5-HT₁ or 5-HT₂ receptors in rat hippocampus (see review in reference 12). Multiple groups have investigated the levels of 5-HT and metabolites in CSF from human subjects following lithium treatment, but the results have been confounded by changes in affective state and remain inconclusive.

Acetylcholine. Neurochemical, behavioral, and electrophysiologic studies have all suggested that the cholinergic system is involved in affective illness²⁷ and that lithium enhances the synaptic processing of acetylcholine in rat brain. Chronic lithium treatment enhances various behavioral responses that are cholinergically mediated. In pilocarpine-induced catalepsy, hypothermia, and seizure, lithium enhances the behavioral effects of treatment with this cholinergic agonist.²⁷ Interestingly, the effect of lithium in potentiating pilocarpine-induced seizure is attenuated by central *myo*-inositol administration, suggesting an involvement of receptor-mediated phosphoinositide (PI) signaling.^{28,29} In addition, studies have shown that protein kinase C (PKC) may mediate presynaptic facilitation of excitatory neurotransmission that may contribute to the reduction of seizure threshold. The regulation of muscarinic receptors by lithium has been reported to increase, decrease, or have no effect on the density of receptors in various regions of the brain. However, similar to the effect in dopaminergic and β -adrenergic receptors, lithium can block the development of receptor supersensitivity in the cholinergic system. Chronic treatment with atropine results in an up-regulation of muscarinic receptors and supersensitivity of PI response in rat hippocampus. When

lithium was coadministered with atropine, however, the treatment prevented the development of supersensitivity of muscarinic receptor PI response without significantly affecting the receptor up-regulation,^{30,31} suggesting a post-receptor site of action.

γ -Aminobutyric acid. GABA is the major inhibitory neurotransmitter in brain and has been implicated in the etiology of affective disorders (see review in reference 32). In plasma and CSF of human subjects as well as various regions of animal brain, lithium treatment has been shown to increase the level of GABA. Following lithium treatment, GABA level, which was observed to be reduced in bipolar patients compared with healthy control subjects, appears to normalize³³ in CSF. Chronic lithium administration was shown to decrease low-affinity GABA binding in the corpus striatum and the hypothalamus.³⁴ However, when the regulation of GABA receptors by lithium was studied by using specific agonists for GABA_A and GABA_B receptors, GABA_B receptors were found to be increased in hippocampus³⁵ following chronic treatment with lithium as well as with anticonvulsant mood stabilizers.

Glutamate. More recently, studies have implicated lithium in the modulation of glutamatergic neurotransmission. In monkey and mouse cerebrocortical slices, acute lithium resulted in enhanced glutamate release, which accompanied *N*-methyl-D-aspartate (NMDA) receptor-mediated IP₃ (inositol 1,4,5-triphosphate) accumulation.³⁶⁻³⁸ The increase in glutamate release by lithium appeared to result from inhibition of presynaptic uptake of glutamate.³⁹ Following chronic lithium treatment however, glutamate uptake into synaptosomes was significantly increased,³⁹ suggesting that increased glutamate uptake by chronic lithium may be related to its antimanic effect. Furthermore, as described below in more detail, the proposed neuroprotective effect of lithium has implicated NMDA receptors.⁴⁰ Thus, up-regulation of glutamate uptake might be involved in the neuroprotective effect of lithium treatment.

Neuropeptides. Various neuropeptide systems have been investigated for diverse effects of lithium, including the opioid peptides, substance P, tachykinin, neuropeptide Y, neurokinin A, and calcitonin gene-related peptide. The levels of dynorphin, substance P, tachykinin, neuropeptide Y, and neurokinin A were found to be increased following lithium treatment in certain regions of the brain. In the case of dynorphin and tachykinin, the increase in the immunoreactivity for the neuropeptide was accompanied by a parallel increase in mRNA, suggesting that lithium, at least in part, regulates the gene expression pretranslationally.^{41,42} In hypothalamic slices, acute administration of lithium enhances the release of several opioid peptides, suggesting an action of lithium at the level of presynaptic autoreceptor.⁴³ Interestingly, however, when CSF levels of various pro-opiomelanocortin peptides were examined in bipolar patients before and after lithium treatment, lithium

had no significant effect on the CSF levels of any of the peptides.^{44,45} Enkephalin binding sites in neuronal membranes were found to be decreased after 3 weeks of treatment with lithium,⁴⁶ but overall, the regulation of receptors for neuropeptides by lithium has yet to be fully investigated.

Summary. While our understanding of the molecular substrates for mood syndromes, such as mania or depression, is far from complete, it is highly likely that they involve multiple neurotransmitter systems. Lithium affects various neurotransmitter systems at multiple levels. By increasing or decreasing the regulation of presynaptic release or postsynaptic receptor sensitivity, the balance of neurotransmitter signaling can be modulated. Furthermore, as noted below, lithium may achieve this modulation on a more chronic basis by altering signal transduction cascades and gene expression, resulting in long-term changes in neural plasticity and cytoskeletal remodeling. Thus, analogous to a conductor, lithium orchestrates an altered pattern of signaling throughout critical regions of the brain, and this may underlie its efficacy as a mood stabilizer in preventing the recurrent episodes of mania and depression in bipolar disorder.

Lithium and Signal Transduction

Phosphoinositide cycle. Since it was discovered that lithium is a potent inhibitor of intracellular enzyme, inositol monophosphatase ($K_i = 0.8$ mM), which converts inositol monophosphates to inositol,^{47,48} receptor G protein-coupled PI hydrolysis has been extensively investigated as a site for the action of lithium as a mood stabilizer. Since brain has limited access to inositol other than that derived from recycling of inositol phosphates, blockade of inositol monophosphatase by lithium treatment can deplete cells of inositol, which is necessary for the resynthesis of the PI substrate PIP₂ (phosphatidylinositol-4,5-bisphosphate).⁴⁹ Furthermore, since the mode of enzyme inhibition is uncompetitive, likely through interaction with Mg⁺⁺ binding sites,⁵⁰ the effects of lithium have been postulated to be most pronounced in systems undergoing the highest rate of PIP₂ hydrolysis.^{50,51} Thus, Berridge and associates⁵² first proposed that the physiologic consequence of the action of lithium is derived through a depletion of free inositol and that the selectivity of lithium could be attributed to its preferential action on the most overactive receptor-mediated neuronal pathways.⁵² However, it has been difficult to demonstrate that lithium treatment reduces resynthesis of PIP₂ in all cell systems, leading to a series of studies providing evidence that selective cell populations may be predisposed to inositol reductions in the presence of lithium. Such studies might provide support for the specificity of lithium action in brain signaling pathways regulating mood (see reviews in references 3 and 11). In such cell systems, including cerebral cortical slices of chronically treated rats, effects of lithium on receptor-

coupled PI signaling can be prevented or reversed by high concentration of *myo*-inositol.⁵³⁻⁵⁵ Moreover, the effect of chronic lithium on embryonic development was also rescued by the presence of *myo*-inositol.^{56,57}

Interestingly, however, in studies examining the in vivo physiologic effects of lithium, such as polyuria or enhancement of cholinergically induced seizures, addition of *myo*-inositol reduced but did not fully reverse the lithium-induced effects.^{28,29} When regional brain *myo*-inositol levels were measured by quantitative proton magnetic resonance spectroscopy in bipolar patients, *myo*-inositol levels were observed to be significantly decreased in the right frontal lobe.⁵⁷ However, the reduction in *myo*-inositol preceded the improvement in mood symptoms, showing a temporal dissociation between the changes in *myo*-inositol and the clinical improvement.⁵⁸ As described below, *myo*-inositol depletion by lithium appears to trigger long-term events through modulation of downstream components of intracellular signaling pathways, such as PKC. In fact, the regulation of expression of a major substrate of PKC, myristoylated alanine-rich C kinase substrate (MARCKS), in the presence of chronic lithium in hippocampal cells is dependent on the concentration of *myo*-inositol and is reversed in the presence of elevated *myo*-inositol concentration.^{57,59} These data suggest that lithium does cause a relative depletion of *myo*-inositol in selective regions of the brain, dependent on cell type and level of activation of signaling pathways, but its physiologic effects that may be therapeutically relevant cannot be accounted for solely by a reduction in *myo*-inositol.

Adenylyl cyclase. The other major receptor-coupled second-messenger system in which lithium has been shown to have significant effects is the adenylyl cyclase (AC) system. Studies in a variety of cell systems including the human brain have demonstrated that lithium attenuates receptor-coupled activation of the cAMP pathway at IC₅₀ concentrations that range from 1 to 5 mM (see review in reference 10). Lithium, in vitro, inhibits AC activity stimulated by GTP (guanosine triphosphate) analogue or calcium-calmodulin, which interacts directly with AC.⁶⁰⁻⁶² Interestingly, these inhibitory effects of lithium are antagonized by Mg⁺⁺, which suggests that the action of lithium on the AC system is mediated by direct competition with Mg⁺⁺.⁶¹ However, attenuation of AC activity following chronic lithium treatment in rat cortical membranes was not antagonized by Mg⁺⁺ alone but was reversed by increased concentrations of GTP, implying that the effect of chronic lithium treatment may be mediated at the level of G proteins.^{60,62}

Lithium appears to have dual effects on the accumulation of cAMP. While lithium decreases receptor-coupled stimulation of AC, lithium increases basal levels of cAMP formation.⁶³ In vivo microdialysis of cAMP from prefrontal cortex of rats that were treated with lithium for 4 weeks showed that lithium significantly increased basal

cAMP.⁶⁴ The increased basal level of cAMP in lithium-treated rats was accompanied by enhanced in vitro pertussis toxin-catalyzed [³²P]adenosine 5'-diphosphate (ADP) ribosylation of G α_i (the inhibitory G protein), suggesting that lithium interferes with the dissociation of G α_i into its active components, thereby increasing basal cAMP by removing a tonic inhibition on AC. Thus, it has been suggested that the action of lithium on the AC system is dependent on state of activation, i.e., under basal conditions, where tonic inhibition of cAMP formation through G α_i is predominant, levels of cAMP are increased, whereas during receptor activation of AC mediated by G α_s , cAMP formation is attenuated. This has been referred to recently as the *bimodal model* for the mechanism of action of lithium, which might in part account for its therapeutic efficacy in depression and mania.¹¹ While this would appear to be overly simplistic, the clinical relevance to side effects of lithium, such as nephrogenic diabetes insipidus and subclinical hypothyroidism, have generally been attributed to inhibition of vasopressin- or thyrotropin-sensitive AC. The therapeutic relevance of AC for lithium in the treatment of bipolar disorder at this time, however, remains unclear.

G Proteins. G protein is a family of GTP binding proteins that transduce the intracellular signaling between the receptor stimulation and the activation of downstream effectors, and might provide a reasonable target for the action of psychotropic drugs (see review in reference 65). Since lithium has been shown to interact with the 2 major receptor G protein-coupled signaling pathways, i.e., PI and AC, studies have examined the direct effects of lithium on G protein activity. While there have been reports of direct interaction with the GTP binding site in tissue from rat brain as well as platelets from patients, such studies have been difficult to replicate and appear to be physiologically inconsistent.⁶⁶ There is evidence that lithium can reduce PI signaling via alteration in G protein function in cell preparations,⁶⁷⁻⁶⁹ but these data are not replicated in rat or monkey brain.^{37,70} Currently, it is thought that the effects of chronic lithium may in part be mediated through posttranslational modifications of G protein that affect its coupling to receptor and/or second-messenger systems as noted below.¹⁰

A number of investigators have examined the effects of chronic lithium on the expression of G proteins at both protein and mRNA levels in brain. While the studies of protein have remained conflicting,^{64,71,72} at the mRNA level, there is evidence that G α_s , G α_{11} , and G α_{12} may be down-regulated in rat cerebral cortex following chronic lithium.⁷¹⁻⁷³ These effects are, however, modest in nature, and it is still unclear as to their physiologic significance. As noted above, posttranslational modification of G protein alters the equilibrium of the active/inactive states of G protein conformation, which might be an important mechanism by which chronic lithium can affect receptor-mediated signaling in multiple pathways. One such post-

translational modification is ADP ribosylation of the G_i protein, which shifts equilibrium of the G_i protein complex to its undissociated inactive form, $\alpha\beta\gamma$ heterotrimer. In rat brain, chronic lithium treatment was found to increase endogenous ADP-ribosylation activity.⁷⁴ In addition, in C6 glioma cells, ADP-ribosylation of $G\alpha_s$ was markedly increased after lithium treatment, while anticonvulsant treatment either decreased ADP-ribosylation or had no effect.⁷⁵ As noted previously, it has been suggested that this mechanism may account for the effect of chronic lithium in enhancing basal levels of cAMP by tonic inhibition of G_i and reducing receptor-mediated activation of AC through attenuation of G_s coupling.⁷⁶ Another lithium-mediated posttranslational modification of G protein is also possible via phosphorylation mechanisms as discussed below. Given the relative abundance of G protein, the impact of the level of posttranslational changes induced by therapeutic levels of lithium on the balance of receptor-mediated signaling in brain is yet to be determined.

Protein kinases and protein kinase C substrates. A crucial component of cAMP signaling is protein kinase A (PKA), which is a principal mediator of cAMP action in the CNS. Chronic lithium treatment has been shown to increase the regulatory and catalytic subunits of PKA in rat brains, which appears to result in increased cAMP binding.⁷⁷ Postmortem studies of subjects with bipolar illness have shown changes in cAMP binding, as well as in PKA activity in temporal cortex.^{78,79} These findings suggest that alterations in PKA activity are associated with the action of lithium.

On the basis of the action of lithium in the PI signaling pathway as discussed earlier, it became apparent that the long-term prophylactic effects of lithium in stabilizing dysregulated signaling in regions of the brain associated with the limbic system might be mediated via the diacylglycerol (DAG) arm of the PIP_2 hydrolytic pathway through an action on the regulation of PKC and specific phosphoprotein substrates.^{80,81} Accumulating studies have provided the evidence that PKC plays a crucial role in mediating the action of chronic lithium in a variety of cell systems including primary and immortalized neurons in culture as well as rat brain (see reviews in references 81 and 82). PKC represents a large family of at least 12 isozymes that are closely related in structure but differ in several ways—intracellular and regional distribution in the brain, second-messenger activators, and substrate affinities—all suggesting distinct cellular functions for these isozymes. PKC isozymes are highly expressed in the brain, with the γ isoform expressed exclusively, and are localized both presynaptically and postsynaptically. PKC is located in the cytoplasmic and membrane compartments of cells, and its activation requires its translocation from the cytosol to the membrane. Translocation from the cytosol to the membrane is most often associated with acti-

vation of the enzyme, which is followed by autocatalysis and down-regulation of the enzyme.

Studies of chronic lithium administration in the rat have demonstrated a 30% reduction in membrane-associated PKC α and ϵ in the subiculum and in CA1 regions of the hippocampus.⁸³ In brain slices from lithium-treated rats exposed to phorbol ester, a known activator of PKC, there was a marked reduction in the translocation of PKC activity from the cytoplasm to the membrane, and this was accompanied by a reduction in phorbol ester-induced serotonin release.⁸⁴ Studies of chronic lithium in both C6 glioma cells and immortalized hippocampal cells in culture also demonstrate a reduction in the expression of these same PKC isozymes (see review in reference 85). This is interesting in light of data demonstrating an enhancement of PKC activity in platelets of patients during a manic episode.⁸⁵ Moreover, in rat *in vivo* studies, administration of *myo*-inositol was able to reverse the down-regulation of PKC ϵ in brain by chronic lithium, consistent with the role of inositol in the downstream action of lithium on regulation of PKC by DAG. These studies have led to a pilot clinical study of the use of tamoxifen, a drug known to inhibit PKC *in vitro*, in the treatment of acute mania.⁸⁶ While the results of this preliminary study appear interesting and consistent with the hypothesis, the sample size was small, and it is unknown whether this drug *in vivo* inhibited PKC isozymes and/or whether its other properties, *i.e.*, anti-estrogenic, played a role.

The activation of PKC results in the phosphorylation of a number of membrane-associated phosphoprotein substrates, the most prominent of which in brain is the MARCKS. Direct activation of PKC by phorbol esters in immortalized hippocampal cells effectively down-regulates the MARCKS protein.⁸⁷ Chronic lithium administered to rats over a period of 4 weeks in clinically relevant concentrations dramatically reduces the expression of MARCKS in the hippocampus, and these findings have been replicated and extended in immortalized hippocampal cells in culture.^{59,88,89} Studies in the hippocampal cells have demonstrated that the extent of down-regulation of MARCKS expression following chronic lithium exposure is dependent on both the inositol concentration and activation of receptor-coupled PI signaling, consistent with the hypothesis as stated above. Moreover, this action of lithium in the brain and hippocampal cells is apparent only after chronic administration, not acute administration, and persists beyond abrupt discontinuation of the drug for an extended period of time, paralleling the clinical time course for the therapeutic effects of lithium during initial treatment as well as its discontinuation. Subsequent studies have discovered that this property of reducing the expression of MARCKS in hippocampal cells is shared by the anticonvulsant valproic acid and not by other classes of psychotropic agents.⁹⁰ The altered expression of MARCKS further supports the role of PI signaling and

PKC in the action of chronic lithium in the brain and may serve to provide insight regarding a role for neuroplasticity in the long-term treatment of bipolar disorder as discussed below.

GENE EXPRESSION AND NEUROPLASTICITY

Gene Expression

The clinical data indicating that the therapeutic effect of lithium requires days to weeks of lag time and that its reversal on discontinuation of lithium occurs over a period of weeks to months suggests that the therapeutically relevant action of lithium in the brain involves long-term neuroplastic changes mediated by gene regulation. Evidence has accumulated that lithium can regulate gene expression via nuclear transcriptional factors. *C-fos*, one of the immediate-early genes, works as a master switch of gene regulation, through interacting with *cis*-acting elements and other transcriptional factors. Lithium has been shown to alter the expression of *c-fos* in various cell systems⁹¹ as well as in the brain⁹²⁻⁹⁴; however, its effects have been variable depending on brain region, cell type, and time course examined.⁹⁵⁻⁹⁸

c-Fos is known to interact with jun family members to form activator protein 1 (AP-1), which binds to a common DNA site. An alternative experimental strategy has utilized studies examining the effect of lithium on AP-1 DNA binding activity. In rat cerebellar granule cells that were treated with 0.5 to 1 mM of lithium, AP-1 binding activity was increased consistently during the treatment (up to 7 days), which was accompanied by increases in the protein levels of *c-Fos*, *c-Jun*, and phosphorylated cAMP-responsive element binding protein (CREB).⁹⁹ In vivo, similar increases in AP-1 binding activity along with phosphorylated CREB, Jun D and Fos family proteins were observed following chronic dietary treatment with lithium (for 4 weeks) in rat frontal cortex, hippocampus, amygdala, and cerebellum.⁹⁹ To examine if the increased AP-1 activity by lithium actually mediates transcriptional regulation of the genes, tissue culture cells were transfected with a reporter gene vector driven by SV40 promoter that contains AP-1 site, and it was shown that the activity of the reporter gene increased in a time and concentration-dependent manner.¹⁰⁰⁻¹⁰² Interestingly, however, when AP-1 binding activity was measured following receptor stimulation, lithium treatment attenuated the induced AP-1 DNA binding activity.^{103,104} These seemingly contradictory findings may suggest that the effect of lithium on gene transcription is dependent on the activity level of the neurons. By increasing AP-1 binding activity at the basal level, but decreasing it when stimulated, lithium can constrain the overall magnitude of fluctuations of gene expression as a function of neuronal activity. Another mood stabilizer, valproic acid, has been shown to have similar effects on the activity of AP-1,^{100,102,105} which lends support

to the possibility that gene regulation through AP-1 may represent a target for mood stabilizers. It must be kept in mind, however, that AP-1 binding activity is responsive to a multitude of signals and is unlikely to define specificity of action for the therapeutic effect of lithium in the treatment of bipolar disorder.

Lithium-induced alteration in gene expression may also account for recent findings that have demonstrated a neuroprotective effect in some cell systems. A number of groups have shown the neuroprotective effect of lithium in both in vivo and in vitro systems^{40,106,107} against a variety of insults including glutamate-induced excitatory apoptosis. It is well established that neuronal survival during apoptosis, or programmed cell death, is dependent on the relative expression of "executioner" proteins and "protector" proteins and presence of neurotrophic factors. Bcl-2 (B-cell lymphoma/leukemia-2 gene), abundantly present in mammalian neurons, is one of the protector proteins that inhibits the apoptosis and cell death under a variety of circumstances. Recent studies have demonstrated that polyomavirus enhancer-binding protein 2 β gene expression, which regulates bcl-2 expression, was markedly increased in rat brain following chronic lithium (as well as valproic acid) treatment.¹⁰⁸ Subsequently, it was found that chronic treatment of rats with lithium increases bcl-2 immunoreactive cells in layers II and III of frontal cortex, dentate gyrus, and striatum.¹⁰⁹ In cultured cerebellar granule cells, long-term treatment with lithium induces a concentration-dependent decrease in mRNA and protein levels of p53 and Bax (apoptotic genes), but a remarkable increase in bcl-2 at both mRNA and protein levels.¹¹⁰ To what extent this neuroprotective effect may be related to lithium's long-term prophylactic effect in stabilizing the course of bipolar disorder and reported morphological changes in brain observed in neuroimaging studies remains to be demonstrated.

Neuroplasticity and Cytoskeletal Remodeling

Recent studies in a number of laboratories have provided evidence that long-term lithium treatment may alter molecular substrates underlying neuroplastic changes in brain that mediate alterations in interneuronal connectivity. Developmental studies in the *Xenopus* embryo have recently provided evidence that lithium can act as an inhibitor of glycogen synthase kinase (GSK)-3 β , a component of the *Wnt* signaling pathway, at concentrations that may be relevant to clinical treatment.¹¹¹ GSK-3 β , originally known as an enzyme to phosphorylate glycogen synthase, also phosphorylates various proteins, including microtubule-associated proteins (MAPs) such as *tau* and MAP-1B, known to regulate neuronal cytoskeletal network. Several groups have reported that inhibition of GSK-3 β by lithium reduces phosphorylation of *tau* protein in different cell systems, which acts to enhance the binding of *tau* to microtubules and promote microtubule as-

sembly.^{112,113} Lithium treatment also decreases phosphorylation of MAP-1B, a microtubule-associated protein involved in axonal outgrowth. Lithium-induced dephosphorylation of MAP-1B reduces its ability to bind to microtubules and in cerebellar granule neurons; this reduction was accompanied by axonal spreading and increases in growth cone area and perimeter.^{114,115} Thus, it is possible under the appropriate conditions that inhibition of GSK-3 β by lithium can induce significant changes in microtubule assembly resulting in changes in the association dynamics among cytoskeletal proteins mediating neuroplastic changes in regions of the brain.

The significance of neuronal cytoskeletal restructuring in the effect of lithium is also supported by a series of studies demonstrating that chronic lithium down-regulates the expression of the PKC substrate MARCKS in brain as discussed previously. MARCKS is a complex protein that will bind calmodulin in a calcium-dependent manner as well as bind and cross-link filamentous actin in a mutually exclusive fashion. Following phosphorylation of its phosphorylation site domain in the presence of activated PKC, MARCKS translocates from the plasma membrane and will neither bind calmodulin nor cross-link actin. This protein is in a key position to transduce extracellular signals to alterations in the conformation of the actin cytoskeleton, which is critical to cellular processes including morphogenesis and secretion. MARCKS is enriched in neuronal growth cones, developmentally regulated, and necessary for normal brain development.^{116–118} MARCKS expression remains elevated in specific regions of the hippocampus and limbic-related structures, which retain the potential for plasticity in the adult rat^{119,120} and human brain,¹²¹ and its expression is induced in the mature CNS during axonal regeneration.¹²² Accumulating data has implicated MARCKS in cellular processes associated with cytoskeletal restructuring and signaling that include receptor-mediated signal transduction and neurotransmitter release. Adult mutant mice expressing MARCKS at 50%, but without apparent morphological abnormalities in brain, exhibit significant spatial learning deficits that are transgenically “rescued.”¹¹⁶ Finally, the induction of long-term potentiation, thought to be a physiologic component of learning and memory, elevates MARCKS phosphorylation.¹²³ These data reveal that MARCKS plays an important role in the mediation of neuroplastic processes in the developing and mature CNS. This down-regulation of its expression following long-term lithium administration may therefore play a role in altering presynaptic/postsynaptic membrane structure in order to stabilize aberrant neuronal activity in key brain regions.⁸⁷

CONCLUSION

A full understanding of the therapeutically relevant mechanism of action of lithium in the brain for the treat-

ment of bipolar disorder will ultimately result from integrating the multiple molecular targets that underlie the pathophysiology of the clinical manifestation of the disease. At such a time, we must account for the neurovegetative symptomatology at the system response level, e.g., sleep/wake cycle dysregulation, psychomotor activity, cognitive disturbance. This integration will be most meaningful once we have identified the susceptibility genes responsible for the full expression of the pathogenesis predisposing to the recurrent affective episodes. For example, a more complete understanding of the well-established effects of lithium on circadian rhythm in plants and animals in the coming years may offer us better insight into the role of biological rhythms in the physiologic expression of bipolar disorder. We are currently still at the stage of identifying the pieces of the lithium puzzle; within the next 50 years, we will be putting the puzzle together.

Drug names: tamoxifen (Nolvadex), valproic acid (Depakene).

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.

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