Neurobiology of Lithium: An Update

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Lithium remains a first-line approach for the treatment of acute mania and the prophylactic management of manic-depressive illness, yet the underlying neurobiological mechanisms remain as yet undefined. In this paper we critically examine the accumulated preclinical and clinical evidence for the action of lithium in the brain and suggest areas that may be most productive for future investigation, i.e., membrane transport systems, neurotransmitter receptor regulation, second messenger generating systems, protein kinase C (PKC) regulation, and gene expression. In their experimental design, preclinical investigations have often jeopardized the physiologic relevance of their studies by a relative lack of attention to issues such as therapeutic concentrations, acute versus chronic exposure, and a lack of adequate cation and/or psychotropic controls. Future studies should account for the established prophylactic efficacy of lithium, the higher risk for relapse into mania after abrupt discontinuation, the ability of lithium to stabilize recurrent depression associated with unipolar disorder, and the efficacy of lithium in the treatment of refractory major depressive disorder in the presence of an antidepressant. Studies of the action of lithium in receptor mediated phosphoinositide signaling in the brain over the past several years have opened up heuristic lines of investigation that stem from lithium’s uncompetitive inhibition of the enzyme inositol monophosphatase. Subsequent studies involving regulation of inositol transport, PKC isozymes and activity, and the expression of the major PKC substrate MARCKS (myristoylated alanine-rich C-kinase substrate) have offered potential avenues for understanding the complexity of the action of long-term lithium in the brain. These studies will offer us a better understanding of the neuroanatomical sites of action of lithium and together with ongoing clinical investigations using brain imaging in patients with manic-depressive illness a more complete understanding of the pathophysiology of this disease.

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Within decades of its discovery, lithium was found to be the most soluble salt of uric acid and early observations of Alexander Ure in the 1840s addressed the potential medicinal properties of lithium in gout. Sir Alexander Garrod was the first to introduce the oral use of lithia salts as a treatment for gout or “uric acid diathesis,” which was believed to encompass affective symptoms of both mania and depression.1–3 However, it wasn’t until the observations of the American physician John Aulde and the Danish internist Carl Lange in the 1880s that lithium was considered to be a treatment of recurrent symptoms associated with depression independent of gout.4–5 After falling into disrepute as a medication because of serious toxicity associated with its widespread use in elixirs and tonics as well as a salt substitute, it was the rediscovery by Cade 48 years ago and seminal clinical studies by Schou in the 1950s that positioned lithium as an effective antimanic treatment and prophylactic therapy for manic-depressive illness.6–7 Since Cade’s observations in 1949, numerous studies have explored potential mechanisms for the therapeutic action of lithium in the treatment of manic-depressive illness. Early studies focused on lithium’s property of being a monovalent cation, its role in sodium transport, and its effect on electrophysiologic properties of cells. As neurotransmitter systems in brain were discovered over the past 45 years, the effects of lithium on these systems and their receptors have been examined in both animal and clinical investigations. Moreover, it has become increasingly apparent that the underlying pathophysiology of manic-depressive illness derives from a dysregulation of multiple signaling pathways in limbic and limbic-related regions of the brain, which results in recurrent clinical affective symptomatology.8–11 Unfortunately, the majority of these studies have suffered from a number of limitations, and interpretation has been confounded by several issues including a lack of attention in

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Presented at the symposium “Lithium in the Treatment of Manic-Depressive Illness: An Update” held May 30–31, 1997, Sea Island, Ga., and supported by an educational grant from Solvay Pharmaceuticals, Inc. Studies supported in part by grant MH-50105 from the National Institute of Mental Health, Bethesda, Md., and The Stanley Foundation grants (Drs. Lenox and Manji) and an MRC/CIBA-GEIGY Training Fellowship (Dr. McNamara).

The authors thank Ms. S. Heini for help in the preparation of this manuscript.

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experimental design to acute versus chronic effects as well as to clinically relevant lithium concentrations; a lack of specificity of pharmacologic agonists and antagonists; examination of one neurotransmitter system to the exclusion of others; overinterpretation in clinical studies of the relevance of peripheral findings to the CNS; and often small effects that have been difficult to replicate. In this review, we will focus upon several areas of research that have provided a strategically important direction or hold the promise of a new perspective for understanding the therapeutic action of lithium in the brain.

Several points regarding the clinical action of lithium in the treatment of manic-depressive illness are worthy of note as related to its mechanism of action in the brain. It is generally agreed that the action of lithium that is most efficacious in the treatment of manic-depressive illness is its prophylactic efficacy in preventing the recurrence of affective episodes and stabilizing the course of the illness during long-term treatment. Upon even rapid discontinuation of lithium in patients who have undergone chronic treatment for many years, symptomatology does not immediately return, but there appears to be a significantly increased risk for relapse over the ensuing months, highly suggestive of a withdrawal-like physiologic response. The observation that there is a predisposition during the withdrawal period to relapse into a manic episode should be of considerable interest to investigators. Lithium is also effective in the treatment of acute mania, but its action occurs over the course of 1 week to 10 days. In the treatment of unipolar illness, lithium has been shown to possess therapeutic benefit in the prevention of recurrent episodes of major depressive disorder and in the treatment of refractory episodes of major depressive disorder. In the latter case, the effect of lithium appears to occur in the presence of an already existing antidepressant and is apparent over a period of days to weeks, often at plasma concentrations that are significantly less than those used in the treatment of manic-depressive illness. As we examine the mechanism of action of lithium in the brain, it will be important to note that while it is highly likely that more than one molecular target in the brain accounts for these clinical properties, common sites of action may trigger events that result in both enhancement of antidepressant activity as well as stabilization of dysregulation in multiple signaling pathways in the brain. Moreover, such sites of action for lithium should account in some way for its specificity of action at therapeutic concentrations in critical regions of the brain.

**LITHIUM AND MEMBRANE TRANSPORT**

Both membrane transport systems and ion channels play roles in the regulation of intracellular lithium. Transport systems may be driven by either ATP (adenosine triphosphate), e.g., the Na,K-ATPase, or by the net free energy of transmembrane concentration gradients, e.g., the sodium-calcium exchanger. These transport systems are likely to be crucial for the regulation of resting lithium in the bulk cytoplasm, as they essentially regulate all steady-state intracellular ion concentration. While membrane transport systems may exist that specifically recognize lithium and regulate its transmembrane concentration, e.g., a gradient-dependent sodium-lithium exchange process, it is arguably more likely that the primary regulation of lithium is affected by transport systems that accept the lithium ion as a substitute for their normal ionic substrates. While clinical studies over the years have been constrained by relatively small and often variable findings, there is evidence that Na,K-ATPase activity may be reduced especially in the depressed phase of both unipolar depression and manic-depressive illness, which is associated with an increase in sodium retention (see reviews in references 9 and 18). Furthermore, chronic lithium treatment has been observed to result in an increased accumulation of lithium and activity of Na,K-ATPase in erythrocyte membranes and concomitant reduction of intraerythrocyte sodium and calcium in patients with manic-depressive illness. Since free calcium ion concentration tends to parallel free sodium concentration, this may account for observations that intracellular calcium is increased in patients with manic-depressive illness. These data related to Na,K-ATPase should be viewed with caution, however, since recent data also support the evolution of specific gene products expressed and posttranslationally regulated and unique not only to neurons but among brain regions. Thus, while the erythrocyte may be used as a peripheral model for lithium transport, extrapolations to lithium homeostasis in the brain or as a potential genetic model for variations in ion homeostatic processes in the brain underlying the pathophysiology of a disease such as manic-depressive illness remain highly speculative.

Since most data seem to indicate that lithium achieves an approximately equal distribution across the plasma membrane barrier while leading to only small depolarizations, it should be appreciated that the predominant form of lithium transport must be efflux to oppose any resting conductances that would tend to concentrate positively charged ions within the negatively charged intracellular milieu, which is reflected in reported brain:serum ratios in the range of 0.76-0.8. While it is the balance of resting lithium conductance and net transport-efflux mechanisms that regulate steady-state lithium homeostasis, the gating of ion channels on the time scale of the channel activity will alter this homeostasis to varying degrees. Thus, activation of voltage-dependent sodium channels may play a significant role in the regulation of intracellular lithium concentration in neurons, such that lithium concentration might be elevated in active neurons, on the basis of the influx of lithium through sodium channels. However, simple calculation reveals that the flux of lithium through voltage-dependent sodium channels is unlikely to be im-
portant in the regulation of lithium homeostasis, even on a short-term basis or as a consequence of high levels of neuronal activity. Moreover, in the local environment of a dendritic spine, the surface area to volume ratio becomes relatively large, such that the lithium component of a synaptic current can cause very significant increases in the local lithium concentration—as much as a fivefold to tenfold increase in intracellular lithium following a train of synaptic stimuli. Such an activity-dependent mechanism for creating focal increases of intracellular lithium at sites of high synaptic activity may be crucial for lithium’s therapeutic specificity and ability to regulate synaptic function in the brain.

**LITHIUM AND NEUROTRANSMITTER AND NEUROPEPTIDE SYSTEMS**

Over the past 30 years, clinical and preclinical studies have focused upon the effects of both acute and chronic lithium on the regulation of monoamine neurotransmitter systems in brain; modest attention has been given to amino acid and neuropeptide neurotransmitters (reviewed in references 9, 12, 28, and 29). More recently attention has turned to lithium-induced changes in second messenger systems and gene expression, which have served to form heuristic lines of investigation actively being pursued.

**Norepinephrine**

Findings in clinical studies of lithium treatment have generally been confounded by changes in affective state and associated changes in activity level, arousal, and sympathetic outflow and have provided conflicting evidence for an effect of lithium on norepinephrine levels and turnover in brain. On the other hand, preclinical studies appear to support an action of acute lithium in reducing the β-adrenergic stimulated AC (adenylyl cyclase) response, and chronic lithium in facilitating the release of norepinephrine, possibly via effects on the presynaptic α2 autoreceptor, as well as blockade of the β-adrenergic receptor supersensitivity after presynaptic depletion of norepinephrine (see review in reference 9).

**Dopamine**

Clinical studies of dopamine metabolites in the periphery of bipolar patients have similarly been confounded by mood and activity state. Chronic lithium has been reported to prevent haloperidol-induced dopamine receptor up-regulation and induce supersensitivity to iontophoretically applied dopamine or intravenous apomorphine. Interestingly, a number of studies have reported a lack of effect if lithium is administered after the induction of dopamine supersensitivity suggesting that in this model, lithium exerts its greatest effects prophylactically. Lithium also appears to block amphetamine-induced behavioral changes in both animals and humans.

**Serotonin**

Preclinical studies indicate that the effects of lithium on serotonin (5-HT) function may occur at multiple levels and result in an enhancement of serotonergic neurotransmission, although its effects on 5-HT appear to vary depending on brain region, length of treatment, and 5-HT receptor subtype (see review in reference 9). Interpretation of studies attempting to clarify the roles of presynaptic versus postsynaptic receptors in mediating the effects of lithium on 5-HT function have been confounded by the relative lack of understanding of the numerous receptor subtypes and their distribution, as well as the existence of subtype-specific agonists and antagonists. However, there is accumulating evidence that lithium produces a subsensitivity of presynaptic inhibitory 5-HT1A receptors, which can result in a net increase of the amount of 5-HT released per impulse. These findings are consistent with observations that short-term lithium enhances the efficacy of the ascending (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.

**Acetylcholine**

Neurochemical, behavioral, and physiologic studies have all suggested that the cholinergic system is involved in affective illness and that lithium enhances the synaptic processing of acetylcholine (ACh) in rat brain. Notably, lithium effectively potentiates seizures induced by a muscarinic agonist which are markedly attenuated by central muscarinic agonist administration, consistent with effects of lithium on receptor-mediated PI (phosphoinositide) signaling discussed below. In addition, studies by Evans and colleagues have suggested that the role of lithium in the pilocarpine seizure model appears to occur through a presynaptic facilitation of excitatory neurotransmission mediated by protein kinase C (PKC). Thus, lithium may target cholinergic neurotransmission and chronically may play a role in preventing muscarinic receptor supersensitivity through interaction within PI signaling systems. These findings are consistent with an effect of chronic lithium in preventing receptor-mediated supersensitivity as observed in both the dopaminergic and noradrenergic systems and suggest a putative site of action for lithium beyond the receptor at receptor-effector coupling and/or intracellular second messenger systems.

**Amino Acids**

While relatively less attention has been paid to effects of lithium on amino acid and neuropeptide regulation in brain, dysregulation of GABAergic neurotransmission (gamma-aminobutyric acid [GABA] is the major inhibitory neurotransmitter in brain) has been postulated to play a role in the etiology of affective disorders. In an albeit limited series of patients, GABA has been observed to be re-
duced in CSF of depressed patients\textsuperscript{57–59} and in the plasma of bipolar patients.\textsuperscript{60} Previously low levels of plasma and CSF GABA appear to normalize in bipolar patients being treated with lithium,\textsuperscript{61,62} though baseline GABA levels appear unrelated to clinical responsiveness to lithium.\textsuperscript{63} While preclinical studies have been constrained by the limitations in design and methodology noted earlier, investigators have reported that lithium produced elevations in GABA in the striatum and midbrain,\textsuperscript{64–66} GABA turnover in the hippocampus and striatum,\textsuperscript{67} and a potentiation of kainic acid-evoked [$^3$H]GABA release in striatal neurons.\textsuperscript{68} The effect of lithium on glutamate, a major excitatory neurotransmitter in brain, has been studied more recently by using monkey cerebral cortical slices—lithium was found to stimulate glutamate release at doses ranging from 1.5 to 25 mM.\textsuperscript{69} It is of interest that the proconvulsant action of lithium in pilocarpine-treated rats described earlier is blocked by the noncompetitive NMDA (N-methyl D-aspartate) receptor antagonist, MK-801.\textsuperscript{70} However, chronic in vivo lithium treatment results in a transient impairment of ACh-induced potentiation of current responses elicited by NMDA in hippocampal CA1 neurons.\textsuperscript{71}

**Neuropeptides**

Preclinical studies of neuropeptides have revealed diverse effects of lithium on multiple systems including the opioid peptides, substance P, tachykinin, neuropeptide Y, neuropeptide A, and calcitonin gene-related peptide. Comparisons between studies are difficult due to differences in time and dose of lithium administration; however, for the most part, an increase in neuropeptide levels in brain has been observed and in the case of both dynorphin and tachykinin, the increase is associated with an increase in mRNA.\textsuperscript{72–75} It has also been reported that chronic lithium abolishes both the secondary reinforcing effects of morphine and the aversive effects of the opioid antagonist, naloxone.\textsuperscript{74–78} However, in one of the few applicable clinical studies, CSF levels of various pro-opiomelanocortin peptides were examined in euthymic bipolar patients before and during lithium treatment; no significant effects of lithium on the CSF levels of any of the peptides were observed.\textsuperscript{79,80} Alterations in the regulation of these neuropeptides, particularly in basal ganglia, may be of interest in regard to the commonly observed lithium-induced side-effect of tremor.

**LITHIUM AND SIGNAL TRANSDUCTION**

**Phosphoinositide Cycle**

Lithium, at therapeutically relevant concentrations in brain, is a potent inhibitor of the intracellular enzyme, inositol monophosphatase ($K_i = 0.8$ mM), which plays a major role in the recycling of inositol phosphates.\textsuperscript{81,82} Since the brain has limited access to inositol other than that derived from recycling of inositol phosphates, the ability of a cell to maintain sufficient supplies of myo-inositol can be crucial to the resynthesis of the PIs and the maintenance and efficiency of signaling.\textsuperscript{83–85} Furthermore, since the mode of enzyme inhibition is uncompetitive,\textsuperscript{86,87} the effects of lithium have been postulated to be most pronounced in systems undergoing the highest rate of PIP$_2$ (phosphatidylinositol 4,5-bisphosphate) hydrolysis. Thus, Berridge and associates\textsuperscript{88,89} first proposed that the physiologic consequence of lithium’s action is derived through a depletion of free inositol, and that the selectivity of lithium could be attributed its preferential action on the most overactive receptor-mediated neuronal pathways. Since several subtypes of adrenergic, cholinergic, and serotonergic receptors are coupled to PIP$_2$ turnover in the CNS, such an hypothesis offers a plausible explanation for the therapeutic efficacy of lithium in treating the dysregulation of neurotransmitter signaling as underlying the pathophysiology of bipolar illness.\textsuperscript{90–92} The preponderance of data indicates that the effects of lithium on PI signaling can be prevented and reversed in the presence of high concentrations of exogenous myo-inositol.\textsuperscript{52,84,90–92} In a recent preliminary clinical investigation that used proton magnetic resonance spectroscopy (1H-MRS), lithium administration to bipolar depressed and manic patients produced a significant reduction in the levels of myo-inositol in frontal (but not occipital) cortex; these changes are observed after 5 days of treatment and persist for at least 3 to 4 weeks.\textsuperscript{93} The action of lithium in inhibiting the recycling of inositol through the receptor-mediated hydrolysis of PIP$_2$ is thought to cause an accumulation of critical pools of diacylglycerol resulting in activation of PKC isozymes responsible for mediating long-term alterations in cell function.\textsuperscript{8,10} In fact, downstream effects of chronic lithium on embryonic development and regulation of PKC and MARCKS protein triggered by changes in PI signaling have also been shown to be dependent upon myo-inositol availability.\textsuperscript{95–97} Studies wherein the effects of myo-inositol have been difficult to demonstrate may be attributable, at least in part, to significant differences in inositol transport and accumulation among cell types.\textsuperscript{85} Thus, overall, both the preclinical and clinical data suggest that although lithium does bring about a relative depletion of myo-inositol in the brain, its therapeutic effects are likely mediated by a secondary cascade of signaling changes, rather than reductions in myo-inositol per se.\textsuperscript{9,10,88}

**Adenyl Cyclase**

The other major receptor-coupled second messenger system in which lithium has been shown to have significant effects is adenyl cyclase (AC), which generates cyclic adenosine monophosphate (cAMP). cAMP accumulation by various neurotransmitters and hormones is reported to be inhibited by lithium at high therapeutic concentrations and above both in vivo and in vitro, but the sensitivity, especially in brain, appears to be less than that...
observed in the PI system. In fact, lithium inhibition of vasopressin-sensitive or thyroid-stimulating-hormone-sensitive AC is generally believed to underline two of lithium’s more common side effects, namely nephrogenic diabetes insipidus and hypothyroidism. 

Norepinephrine- and adenosine-stimulated cAMP accumulation in rat cortical slices is inhibited significantly by 1 to 2 mM of lithium; in human brain tissue, the IC50 for lithium inhibition of norepinephrine-stimulated cAMP accumulation is approximately 5 mM. Prolonged lithium exposure produces little effect on β-adrenergic-stimulated AC but an increase in basal cAMP has been observed. Data suggest that lithium’s inhibition of AC in vitro may be due to competition with Mg++ for a binding site on the catalytic unit of AC. However, the inhibitory effects of chronic lithium treatment on rat brain AC are not reversed by Mg++, and these effects still persist after washing of the membranes but are reversed by increasing concentrations of GTP (guanosine triphosphate). These results suggest that the physiologically relevant effects of chronic lithium may be exerted at the level of signal-transducing G proteins at a GTP-responsive step.

G Proteins

Since lithium has been shown to affect both PI turnover and AC activity, a series of studies have focused on mechanisms shared by these two major second messenger generating systems, namely the signal-transducing G proteins. As noted above, experimental evidence has shown that lithium may alter receptor coupling to PI turnover in the absence of consistent changes in the density of the receptor sites themselves. When fluoride ion or GTP analogs that directly activate G protein-coupled second messenger responses were used, studies in rat brain after chronic lithium exposure have revealed either no change or an attenuation of PI turnover. While there have been reports of a direct effect of lithium on receptor-mediated G protein binding of guanine nucleotides in brain membranes, such data have been difficult to replicate and are physiologically inconsistent.

Investigations have also addressed the role of G proteins in the action of lithium-induced attenuation of receptor-mediated AC activity in both rodents and humans. These studies have revealed evidence for an increase by chronic lithium of pertussis toxin-catalyzed [32P]ADP-ribosylation of inhibitory G proteins in both rat brain as well as platelets from volunteer subjects treated for 2 weeks. These data provide no evidence for a change in the amount of the G proteins and suggest a stabilization of the inactive undissociated αβγ heterotrimeric form of the inhibitory G protein. This was accompanied by an enhancement of both basal and postreceptor-stimulated AC in both the rat brain and platelet preparations, which is consistent with an uncoupling of the tonic inhibitory influence of the G protein. These data are also consistent with the studies demonstrating that effects of chronic lithium exposure may be exerted at a GTP-responsive step and may result in an alteration in the conformational state (active/inactive) of the G protein. Such a contention is supported by the recent observation that chronic in vivo lithium administration reduces the subsequent in vitro sensitivity of rat cortical membranes to guanine nucleotide-induced reductions in pertussis toxin catalyzed [32P]ADP-ribosylation. This is also consistent with the results of Mork and Geisler, who demonstrated that the addition of exogenous guanine nucleotides (but not Mg++) was able to overcome the effects of lithium on rat brain AC activity. Most recently, it has been shown that lithium promotes calpain-mediated proteolytic cleavage of Go, effects which have been postulated to occur via a stabilization of the G protein in its αβγ heterotrimeric conformation; moreover, these investigators also found that the addition of GDPβS was able to overcome the effects of lithium. Thus, these findings suggest that chronic lithium may lead to the GTP-dependent stabilization of Gi (and Go) in the undissociated αβγ conformation.

At present, the possible effects of chronic lithium on the absolute levels of Gαi1 and Gαi2 remain unclear; two independent laboratories have not observed any alterations, whereas another laboratory has reported small but significant decreases in the levels of the Gαi, Gαs, and Gαq in rat frontal cortex. However, chronic lithium administration appears to reduce the mRNA levels of a number of G proteins in rat brain, including α1, αq, and αi1. This is of interest in light of studies implicating PKC in the down-regulation of G protein mRNAs, and its posited role in the action of chronic lithium (see below). In summary, although there is evidence that competition with magnesium accounts for some of the in vitro effects of lithium on G proteins and speculation that an interaction with GTP binding might be relevant to the chronic effects of lithium, a direct effect of lithium on guanine nucleotide activation of G protein remains unsubstantiated. Most recently, investigators have demonstrated that lithium alters the levels of endogenous ADP-ribosylation in C6 glioma cells and in rat brain, suggesting another mechanism through which chronic lithium may indirectly regulate the activity of these critical signaling proteins. Thus, overall the data suggest that the long-term effects of chronic lithium on G protein may more likely be attributable to an indirect posttranslational modification of the G protein(s) and a relative change in the dynamic equilibrium of the active/inactive states of protein conformation, potentially resulting in modulation of receptor-mediated signaling in critical regions of the brain.

Protein Kinase C and Phosphoprotein Substrates

As noted above, recent evidence accumulating from various laboratories points to a role for PKC in mediating the action of lithium in a number of cell systems and the
brain (see reviews in references 10 and 93). PKC is a family of at least 12 phosphorylating isozymes with differing tissue, intracellular and regional distribution within the brain, second messenger activators, and substrate affinities, indicating distinct cellular functions.129–132 Posttranslational phosphorylation of selective PKC protein substrates within the cell are responsible for regulation of processes critical to cell secretion, membrane trafficking, transcription, ion transport, receptor signaling, and transformation. Recent studies have demonstrated that chronic lithium administration in rats results in a significant decrease in the membrane-associated PKC α and ε isoforms in the hippocampus.133,134 It is noteworthy that exposure of neuroblastoma cells135 or PC12 cells136 to 1 mM of lithium in vitro produces isozyme-selective decreases in PKCα and (in the case of PC12 cells) PKCε. Recent studies have also investigated the effects of valproate (an anticonvulsant with demonstrated antimanic properties) on PKC isoforms and substrates. Chronic valproate has been found to produce strikingly similar reductions in the levels of PKC α and ε.137 These findings are of particular interest and have led to a pilot clinical investigation showing efficacy of the use of tamoxifen, an effective PKC inhibitor, in the treatment of acute mania.121 The precise mechanism(s) by which chronic lithium treatment produces these isozyme-specific effects is presently unclear; however, PKC subspecies are known to exhibit subtle differences in their biochemical characteristics and cellular localization and also differ in their susceptibility to degradation after activation.138–140 In addition, the down-regulation of the ε PKC isozyme after chronic lithium exposure can be reversed by the coadministration of myo-inositol in vivo,141 a finding that is consistent with the action of chronic lithium resulting in an inositol depletion, accumulation of DAG, (diacylglycerol) and subsequent activation of PKC isoforms as described above. While we as yet do not fully understand the precise profile of PKC isozymes activated by lithium, we have begun to investigate critical protein substrates for PKC that may provide further insight into the mechanism of long-term action of lithium in the brain.

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 myristoylated alanine-rich C-kinase substrate (MARCKS). Direct activation of PKC by phorbol esters in immortalized hippocampal cells will effectively down-regulate the MARCKS protein.141 Chronic lithium administered at therapeutically relevant concentration (1 mEq/kg in brain) to rats over a 4-week period has been shown to result in a marked reduction in MARCKS in the hippocampus, which is not observed after acute treatment and persists beyond treatment discontinuation.142 The down-regulation of MARCKS expression in brain has also been observed at concentrations as low as 0.7 mM.134 The lithium-induced reduction in MARCKS has recently been replicated in immortalized hippocampal cells and shown to be prevented and reversible in the presence of elevated inositol concentrations.95 Furthermore, activation of muscarinic receptor-coupled PI signaling significantly potentiates the down-regulation of MARCKS protein induced in the presence of 1 mM of lithium, which supports the role of the PI signaling pathway and PKC isozymes in this long-term action of lithium.95 MARCKS binds calmodulin in a calcium-dependent fashion and crosslinks actin at the plasma membrane; both these events are inhibited by PKC-mediated phosphorylation that serves to translocate MARCKS from the membrane to the cytosol.143,144 MARCKS has been implicated in cellular processes associated with cytoskeletal restructuring and signaling that may be related to long-term neuroplastic changes in processes associated with receptor signal transduction and neurotransmitter release. Recent studies have indicated that this action of chronic lithium on MARCKS protein expression is not shared by psychotropic drugs in general, but is a property of valproate at therapeutic concentrations relevant to the treatment of acute mania.145,146 Thus MARCKS may represent a clinically relevant target for the mood-stabilizing action of chronic lithium, which serves to regulate aberrant signaling in the brain of patients suffering from manic-depressive illness.

LITHIUM AND GENE EXPRESSION

The prophylactic efficacy of lithium generally requires a number of weeks to develop,12,94,98 suggesting long-term neuroplastic alterations potentially mediated at the genomic level. Indeed, increasing evidence suggests that lithium affects gene expression, possibly via PKC-induced alterations in nuclear transcription regulatory factors responsible for modulating the expression of specific genes.147,148 Several recent studies have demonstrated that lithium alters the expression of the immediate early gene c-fos in different cell systems including the brain.149,150,151 These lithium-induced effects on the expression of c-fos mRNA, generally thought to represent a “master switch” to turn on a “second wave” of specific neuronal genes of functional importance, offer a mechanism for affecting long-term events in the brain. Chronic in vivo administration of lithium resulting in clinically relevant levels alters the expression of a number of genes in rat brain, several of which are known neuromodulatory peptide hormones (prodynorphin, preprotachykinin) and their receptors (glucocorticoid type II), and are known to contain PKC-responsive elements.12,73,153–156 Recent studies have also demonstrated that the effects of both lithium and valproate on the regulation of AP-1 DNA binding activity in cultured cells157,158 may be mediated at least in part by PKC. The demonstration of the long-term modulation of the genetic expression of critical proteins involved offers new strategies for unraveling the complex physiologic effects of chronic lithium in the prophylaxis of recurrent epi-
sodes of affective illness in patients with manic-depressive illness.

**NEUROANATOMICAL SITE OF ACTION**

While data related to neuroanatomical localization of lesions in brains of patients who have manic-depressive illness are now being accumulated by using structural and functional neuroimaging strategies, alterations in right hemisphere regions related to limbic and frontal association areas have been of particular interest (see review in reference 11). The premise that lithium exerts its therapeutic actions by acting at such specific neuroanatomical sites and/or their cells of projection is supported by several lines of evidence. First, atomic absorption spectrophotometric, radiographic dielectric track registration, and nuclear magnetic resonance studies indicate that lithium does not distribute evenly throughout the brain after either acute or chronic administration, but preferentially accumulates in forebrain diencephalon, e.g., hypothalamus, and telencephalon structures, e.g., caudate and hippocampus.\(^{159-170}\) Second, as noted previously, preclinical studies of the effects of lithium on neurotransmitter systems have revealed changes, particularly in the 5-HT system, that are specific to certain brain regions. Third, the relative regional and cellular distribution of overactive ligand-gated ion channels in the brains of patients who have manic-depressive illness may be important in dictating relative rates of lithium transport.\(^{27}\) Fourth, studies assessing PI turnover after lithium administration indicate regional differences in inositol depletion and agonist-stimulated \([\text{H}]\text{IP}\) accumulation, primarily between forebrain structures and hindbrain structures.\(^{115,169,171}\) Moreover, the effects of lithium will be most apparent in cells not only where inositol is limiting, but also those undergoing the greatest activation of receptor-mediated PI hydrolysis.\(^{176-178}\) Finally, regional brain distribution of PKC isozymes and alterations in MARCKS expression after chronic lithium, e.g., hippocampus, may confer even further specificity of action.\(^{133,142}\) Collectively, these studies indicate that the long-term therapeutic action of lithium may indeed possess cellular distribution of overactive ligand-gated ion channels in determining relative specificity in cell/regional transport in brain that may reflect aberrant signaling underlying the pathophysiology of manic-depressive illness. Moreover, such dysregulation of signaling may also serve to dictate cell/regional extent of inositol depletion in the presence of chronic lithium that triggers PKC-mediated downstream changes in expression of proteins such as MARCKS and neuroplasticity that restabilize a dysregulated pattern of signaling in critical regions of the brain. To what extent chronic lithium alters the patterns of gene and protein expression through PKC dependent versus independent mechanisms is yet to be discovered. However, future studies should begin addressing the relevance of such changes to the therapeutic efficacy of this psychotropic agent. Experimental designs should incorporate the critical clinical variables that are known regarding the use of lithium in the treatment of manic-depressive illness. As we gain understanding of the susceptibility genes underlying manic-depressive illness and the development of appropriate mutant mouse models, such clinically relevant studies of the mechanism of action of lithium will be facilitated in the future.

**Drug names:** amphetamine (Benzadrine), haloperidol (Haldol and others), naloxone (Narcan), tamoxifen (Nolvadex).

**REFERENCES**

3. Ure A. Researches on gout. Medical Times 1844/1845;11:1145
4. Aulde J. The use of lithium bromide in the treatment of manic-depressive illness. Experimental Designs should incorporate the critical clinical variables that are known regarding the use of lithium in the treatment of manic-depressive illness. As we gain understanding of the susceptibility genes underlying manic-depressive illness and the development of appropriate mutant mouse models, such clinically relevant studies of the mechanism of action of lithium will be facilitated in the future.

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4. Aulde J. The use of lithium bromide in the treatment of manic-depressive illness. Experimental Designs should incorporate the critical clinical variables that are known regarding the use of lithium in the treatment of manic-depressive illness. As we gain understanding of the susceptibility genes underlying manic-depressive illness and the development of appropriate mutant mouse models, such clinically relevant studies of the mechanism of action of lithium will be facilitated in the future.

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110. Tseng FY, Pasquali D, Field JB. Effects of lithium on stimulated metabolic parameters in dog thyroid slices. Acta Endocrinology (Copenhagen) 1989;121:615–620
114. Godfrey PF, McClue SJ, White AM, et al. Subacute and chronic in vivo lithium treatment inhibits agonist- and sodium fluoride-stimulated inosi-


