Lithium and the Brain: A Psychopharmacological Strategy to a Molecular Basis for Manic Depressive Illness

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Lithium, an effective treatment for mania and the prevention of recurrent episodes of both mania and depression in patients with manic depressive illness, exerts multiple biochemical effects. However, any clinically relevant site of action of lithium must occur at therapeutic concentrations attained in the brain of patients and must account for the lag period accompanying onset of action as well as effects persisting beyond discontinuation of treatment. This monovalent cation acts as a potent uncompetitive inhibitor in the receptor-coupled breakdown of inositol phospholipids, resulting in a relative depletion of inositol and an alteration in the generation of diacylglycerol, an endogenous activator of protein kinase C. In our laboratory, we are examining the action of chronically administered lithium on posttranslational modification of specific phosphoproteins involved in regulating signal transduction in the brain. We have found that chronic, but not acute, administration of lithium in rats markedly reduces a major phosphoprotein substrate of protein kinase C in the hippocampus, an effect that persists beyond the cessation of lithium treatment. This protein, myristoylated alanine-rich C kinase substrate ("MARCKS"), is implicated in synaptic neurotransmission, calcium regulation, and cytoskeletal restructuring. These findings have relevance for the long-term action of lithium in stabilizing an underlying dysregulation in the brain and may move us closer to formulating a molecular basis of manic depressive illness.

Indexing Terms: protein kinase C/rat models/phosphoproteins/inositol phospholipids/mood disorders

Historical Perspective

Lithium was discovered as an element in 1817 and has been used to treat a multitude of somatopsychic disorders over the years (1). In particular, because lithium forms the most soluble salt known with uric acid, it was proposed as early as the mid-19th century as a treatment for gout or "uric acid diathesis," which was described as including mood-related symptoms ranging from "gouty mania" to depression. By the late 19th century, physicians such as John Aulde in America and Carl Lange in Denmark reported clinical observations related to the prophylactic efficacy of lithium in the treatment of recurrent symptoms of depression (2,3). The clinical use of lithium in conventional medicine fell into disrepute in this country in the early and middle 20th century, in light of exaggerated claims for its therapeutic efficacy as well as its potential for toxicity.

Widespread and unregulated marketing of "lithia salts" in potions and mineral waters, often at negligible concentrations, for a variety of ills contributed to the general disillusionment of the traditional medical establishment. The use of lithium salt as a substitute for sodium to treat patients with chronic cardiac and renal diatheses resulted in an increased incidence of morbidity and mortality related directly to lithium toxicity.

Not until John Cade reported his observations in 1949 was lithium considered once again a potential pharmacotherapeutic agent for treating mania in patients with manic depressive illness (4). In a series of studies of the uric acid diathesis of mania, Cade injected urine from manic patients into guinea pigs. To solubilize the uric acid, he used lithium, and subsequently reported that within 2 h of administration of lithium the animals became lethargic and unresponsive but thereafter showed full recovery. This seminal observation resulted in his administering lithium to a series of 10 manic patients and reporting a rather startling reduction of psychotic excitement in all of them. Later, in a series of now-famous clinical investigations, Mogens Schou established lithium as an effective antimanic treatment and a prophylactic therapy for recurrent affective episodes in patients with manic depressive illness (5). Because of its past history, however, the introduction of lithium into the clinical practice of psychiatry as a psychopharmacological treatment for manic depressive illness in this country was delayed almost a decade, to the mid-1960s.

Clinical Efficacy

Lithium, one of the most clinically efficacious pharmacological agents now available for treating a major psychiatric disorder, has been used clinically with a variety of behavioral disorders. Clinical indications for the treatment of affective illness include:

1. Long-term treatment to reduce the frequency and severity of recurrent episodes of mania and depression in bipolar illness.

2. Acute treatment of mania in which the antimanic effect is more specific than that of neuroleptics but has a longer latency of action (5–10 days).

3. Long-term prophylaxis for recurrent episodes of depression in unipolar illness.

4. Adjunctive treatment with antidepressants for refractory depressive episodes.

Lithium is most effective and selective for the long-term prophylactic treatment of recurrent episodes of both mania and depression in patients diagnosed with bipolar disorder (6). The unique clinical action of lithium in the treatment of the profound mood cycling seen in this

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disorder requires a lag period for onset and is not immediately reversed upon discontinuation of treatment. In properly screened patient populations, published reports support its efficacy in >80% of patients studied (6, 7). Lithium is also effective in the treatment of acute mania in this same patient population. Because its onset of action is delayed for between 5 and 10 days, lithium is often initially combined with other medications, e.g., benzodiazepines, to achieve adequate clinical management of the patient (8). To a lesser but clinically relevant extent, lithium is also used to prevent recurrent episodes of depression in patients with unipolar disorder. This clinical treatment profile of lithium clearly underscores its long-term properties as a mood stabilizer.

Several other characteristics of chronic treatment with lithium are also important in understanding its mechanism of action. If lithium is abruptly withdrawn from a patient with bipolar disorder after long-term treatment, affective symptomatology does not immediately reappear. However, there is now clear evidence that as many as 50% of such patients will experience a recurrence of either a manic or depressive episode within 5 months, with a clearly higher risk for a manic episode early in the withdrawal period (9, 10). Lithium also has a rather narrow therapeutic index, clinical toxicity being evident in some patients at plasma concentrations 50% greater than the therapeutic range; thus, its therapeutic action may be on a continuum with some of its toxic manifestations. Our laboratory has been interested in addressing the biological targets responsible for the long-term therapeutic action of lithium in the brain.

Mechanism of Action

The underlying biological processes responsible for the episodic clinical manifestation of mania and depression may be related to faulty homeostatic regulation in the brain (6). Patients with this disorder may lack the necessary system flexibility to adaptively respond to the periodic fluctuations in the internal and external state, but instead experience sudden oscillations beyond immediate compensatory control (11, 12). This system failure results in the clinical manifestations of disruption of behavior and profound changes in mood, circadian rhythm, and neurophysiology of sleep, as well as significant alterations in neuroendocrine regulation, all consistent with a dysregulation within the limbic system and associated regions of the brainstem and prefrontal cortex. A better understanding of the molecular mechanisms by which lithium achieves its mood stabilization properties in these regions of the brain might not only lead to the development of even better psychopharmacological treatment approaches but also bring us closer to a molecular basis of the disorder itself. This research strategy has the advantage of focusing the investigator within the complex array of neurochemical systems within the brain, but has limitations in terms of extrapolation to the pathogenesis of the disorder, as will be discussed below.

Signal Transduction

Regulation of signal transduction within limbic and limbic-related regions of the brain, altering the balance of activity of the complex network of neurotransmitter pathways, remains an attractive site for the therapeutic action of a drug like lithium. Over the past several years, emerging data have confirmed that the phosphoinositide second-messenger system is an important site of action of lithium in the brain (see Fig. 1) (13, 14). Lithium, at therapeutically relevant concentrations, is a potent inhibitor of the intracellular enzyme inositol monophosphatase (K1, 0.8 mmol/L), which results in an accumulation of inositol 1-monophosphate and a reduction in the generation of free inositol (15–17). Because the brain has limited access to inositol other than that derived from recycling of the inositol polyphosphates, the ability of neurons to maintain sufficient supplies of myo-inositol can be crucial to the resynthesis of the phosphoinositides and thus maintenance and efficiency of signaling (18). Furthermore, because the mode of enzyme inhibition is uncompetitive, lithium’s effects have been postulated to be most pronounced in systems undergoing the highest rate of hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2), wherein the physiological consequence of lithium’s action in brain is derived through the relative depletion of free inositol.2 Thus, the selective action of lithium has to be attributed to its preferential action in brain, which results in suppressing the hydrolysis of phosphatidylinositol (PII) in the most overactive receptor-mediated neuronal pathways, and thereby modulating their relative activity.

2 Nonstandard abbreviations: PKC, protein kinase C; MARCKS, myristoylated alanine-rich C kinase substrate; PI, phosphatidylinositol; PIP2, phosphatidylinositol 4,5-bisphosphate; CMP-PA, cytidine monophosphate-phosphatidate; DAG, diacylglycerol; and PA, phosphatidate.

Fig. 1. Agonist occupancy of the receptor binding site results in G protein-mediated coupling and activation of phospholipase C (PLC), which induces the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) and the generation of two second-messengers, i.e., diacylglycerol (DAG) and myo-inositol 1,4,5-trisphosphate (Ins 1,4,5 P3). Ins 1,4,5 P3 binds to receptors in the endoplasmic reticulum (ER) and releases intracellular calcium. DAG is an endogenous activator of protein kinase C (PKC). Lithium inhibits the enzyme inositol monophosphatase, which prevents the recycling of inositol and increases accumulation of metabolites in the DAG pathway, potentially reducing critical pools of PIP2, required for signal transduction. PIP, phosphatidylinositol 4-phosphate; G, a GTP-binding protein, e.g., Gp, Go; R, receptor; A, a receptor-specific agonist; Ins 1P, myo-inositol 1-phosphate; Ins 3P, myo-inositol 3-phosphate; Ins 4P, myo-inositol 4-phosphate.
Because several subtypes of adrenergic (e.g., α₁), cholinergic (e.g., m₁, m₃, m₅), serotoninergic (e.g., 5HT₂, 5HT₁c), and dopaminergic (e.g., D₁) receptors are coupled to PIP₂ turnover in the central nervous system and mediate both excitatory and inhibitory pathways (14, 20–22), such a hypothesis offers a plausible explanation for the therapeutic efficacy of lithium in treating both poles of manic depressive illness by the compensatory stabilization of an inherent biogenic amine imbalance in critical regions of the brain (23).

Phosphoinositide Signaling

The evidence discussed above suggests that at least some of lithium’s critical effects are mediated via long-term processes set in motion during exposure of the brain to lithium. Although it is still difficult to demonstrate that the effect of chronic lithium on inositol can be a meaningful reduction of a phosphoinositide (e.g., PIP₂) pool associated with signal transduction, there is evidence that it may mediate long-term events via post-translational changes in the phosphorylation of selective protein substrates. As a consequence of lithium-induced inositol depletion in the presence of continued receptor-mediated hydrolysis of phosphoinositides (PIP₂), dicoclyglycerol (DAG) and its related metabolites, phosphatidate (PA) and cytidine monophosphate-phosphatidate (CMP-PA), accumulate in a variety of cell types, including the brain, presumably because of a reduction in the recycling of inositol into critical PIP₂ pools (see Fig. 1). These findings are further supported by data demonstrating that this increase can be reversed by the addition of inositol (24–28). Studies in our own laboratory have replicated these observations in a CHO-K1 cell line transfected with the human m₁ muscarinic receptor subtype, which is known to couple to PI response (29, 30). As shown in Fig. 2, time-course analysis determined that, in the presence of lithium, the CMP-PA response to carbachol under these conditions was very rapid (significant accumulation as early as 2 min after the addition of carbachol) and could be significantly reduced by the addition of inositol. We have also observed that CMP-PA formation in cells grown and assayed in standard Ham’s F12 medium supplemented with fetal bovine serum (100 mL/L) was markedly less than in cells grown in inositol-depleted media.

Protein Kinase C (PKC)

Inasmuch as DAG is a known endogenous activator of PKC, these findings are consistent with our earlier observations that the action of chronic lithium may stem initially from its potent effects in inhibiting the recycling of inositol through the receptor-mediated hydrolysis of PIP₂ and its indirect action in accumulating DAG (23, 31) with subsequent changes in the activation of PKC isoenzymes, altering the relative “constitutive phosphorylation” of key phosphoprotein substrates (32). Data from several laboratories, including our own, provide convincing evidence for a role for PKC in mediating the effects of lithium exposure in various cell systems including the brain (23, 33–38). PKC-mediated post-translational modification of proteins involved in receptor-mediated signal transduction, neurotransmitter release, ion-channel activity, and transcriptional regulation have major physiological implications for neuronal function throughout the brain (39).

Because alterations in endogenous PKC substrates might reveal both subtle and selective effects of lithium on PKC activity, we examined the phosphoprotein substrates for PKC in the soluble and membrane fractions from hippocampi of control animals and animals given chronic lithium treatment (4 weeks), as well as from rats treated acutely with lithium (2 h) and rats withdrawn from chronic lithium administration for 40 h. We found a significant reduction (45%) in the in vitro phosphorylation of a prominent protein with an apparent molecular mass of 83 kDa in the soluble fraction of hippocampi from animals subjected to chronic lithium treatment (37). Other phosphoproteins, including other PKC substrates, were not changed by the chronic lithium treatment. Animals acutely achieving similar therapeutic concentrations of lithium in the brain did not demonstrate a significant change in phosphorylation of these proteins. Animals withdrawn from lithium showed a partial reversal of the effects on the 83-kDa protein seen during chronic lithium treatment. The phosphoprotein appeared to correspond to a major myristoylated alanine-rich C kinase substrate (MARCKS) in brain. This was subsequently confirmed by using an antibody raised to highly purified MARCKS that recognizes both phosphorylated and nonphosphorylated forms of the protein. Western blot analyses provided evidence for a marked reduction in the amount of MARCKS in both the soluble (57%) and membrane (71%) fraction from hippocampus of rats receiving chronic lithium treatment (Table 1). However, there was no such evidence for a reduction of MARCKS in rats receiving acute doses of lithium, and the reduction observed in the hippocampus of rats receiving repeated doses of lithium was still present at 40 h after abrupt discontinuation of the lithium diet. These findings ap-
MARCKS is a well-known acidic protein that is one of the major substrates for PKC in brain; it belongs to a highly homologous family of proteins that have widespread tissue and subcellular distribution (40, 41). The protein has been sequenced and cloned from animal (42, 43) and, recently, human brain (44). MARCKS proteins are phosphorylated reversibly by several pharmacological agents and growth factors, including muscarinic agonists, vasopressin, bradykinin, and phorbol ester, all of which are known to activate PKC (45–52). The protein undergoes cotranslational myristoylation and possesses a highly conserved lysine-rich region for PKC phosphorylation and the binding of calmodulin. Phosphorylation appears to regulate calmodulin binding to MARCKS, making this protein, among others, potentially instrumental in coordinating PKC and calcium activation through redistribution of calmodulin for appropriate regulation of intracellular signaling (53–55). MARCKS is found in both the soluble and membrane fraction of the cell (56). It has been suggested that the relative state of fatty acylation vs phosphorylation may determine the subcellular distribution of the protein at a given time (42) and may be associated with the regulation of physiological events such as signal transduction and neurotransmitter release in brain (40, 47). More recently, in other cell systems, Thelen et al. (57) suggested that MARCKS may represent a “critical effector molecule in the transduction pathway” of agonist-dependent responses in cells, with additional data supporting a prominent role for this protein in plastic processes involving membrane-cytoskeletal elements of the cell (58, 59). MARCKS is a protein cross-linking filamentous actin, where regulation of its cross-bridging between actin and the plasma membrane occurs by both PKC-mediated phosphorylation and calcium–calmodulin binding (60). The ability of MARCKS to bind both calmodulin and actin has implicated MARCKS in several cellular processes, including secretion and membrane trafficking, cell motility, cell cycle regulation, and cellular transformation (61).

**Strategy for Drug Development and Pathogenesis of Manic Depressive Illness**

The plasticity of synaptic processes responsible for regulating neurotransmission within the limbic system must play a significant role in mediating homeostasis of behavioral–vegetative–emotional responses over time. Cytoskeletal restructuring and membrane trafficking are integral to the function of processes such as neurotransmitter release and up/down regulation of receptor-response systems, so necessary for appropriate physiological adaptation of the organism to both internal and external events. In the presence of normal compensatory systems and fine-tuning, aberrant signals generated by faulty neurotransmitter systems might be amplified and degenerate into inappropriate oscillations beyond immediate physiological control. An interaction of chronic lithium with proteins critical to such long-term molecular events may represent an attractive target for the therapeutic action of this psychotropic agent in achieving clinical stability in patients with manic depressive illness.

However, although these proteins may represent an optimal target for the clinical action of a mood stabilizer such as lithium, they are not necessarily directly linked to the pathogenesis of the disorder. The ultimate gene product(s) underlying the dysregulation in the brain fundamental to the clinical manifestations of manic depressive illness may be at a level of neuronal expression quite distant from the adaptive processes responsible for regulating the balance of neurotransmitter activity and signal transduction. For example, in the case of diabetes, endocrinologists already have identified a molecular target for their pharmacotherapeutic strategy. Preventing the clinical manifestations of diabetes by closely monitoring and controlling insulin concentrations would appear to be separate from understanding the genetic predisposition associated with the various subtypes of type 1 and type 2 diabetes. Yet it is through our understanding of the genetics of this disorder as well as the evolving knowledge of the insulin receptor-response complex and regulation of glucose transport that we will eventually discover the etiological processes underlying the disease. Similarly, with the identification of the molecular target(s) directly associated with the long-term clinical efficacy of lithium, we will be in a position to better design future strategies for drug development and, in concert with molecular genetic approaches, to address the ultimate pathogenesis of manic depressive illness.

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