The Nature of Bipolar Disorder

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The underlying pathophysiology of bipolar disorder is a continually evolving complexity of multilayer interacting and independent systems. The dearth of adequate preclinical or clinical models that incorporate the various features of the illness, i.e., acute and chronic, recurrent and episodic, and time-course and treatment-related variables, has made the consistency and interpretation of data difficult. Newer technologies and the availability of structurally and mechanistically distinct pharmacologic agents have expanded opportunities for experimental study. In addition to the well-known neurotransmitter systems that are disrupted in mood disorders, critical guanine nucleotide–binding protein (G protein)–coupled signaling pathways are implicated in modulating mood state. Regulation of gene expression and identification of factors regulating neuroplasticity and neurotrophic events in the central nervous system in bipolar disorder are 2 of the more recent approaches contributing to clarification of the pathophysiology and potential treatment options.

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Clinical investigations of both the underlying pathophysiology and the treatment of bipolar disorder have expanded over the last 4 decades to encompass a diverse array of biochemical and neuroendocrine approaches and are now accessing basic fundamentals of gene regulation and expression. Early experimental and therapeutic strategies focused on dysregulations of the hypothalamic-pituitary-adrenal (HPA) axis and the monoaminergic systems as pivotal components of the manic and depressive states; however, this chronic affective disorder, which is characterized by unique cycling phenomena including progressive, episodic, and frequently severe mood disruptions, is now widely recognized to involve more complex biological interactions.

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Space limitations necessitate our citing of review articles in many cases. A full reference list upon which this article was based is available upon request.

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The underlying pathophysiology of bipolar disorder, partially elucidated through years of study with lithium and subsequently pursued through studies with the newer applications of antiepileptics such as carbamazepine, valproate, lamotrigine, and topiramate, should be understood through 4 descriptive physiologic levels: molecular, cellular, systemic, and behavioral (Figure 1).[1–4] These levels show complex interactions within components of each level as well as exert influences between levels. It is important to appreciate that the biological processes that are risk factors for some clinical manifestations of bipolar disorder, such as mood cycling, may be operating distinct from the factors manifesting as clinical symptoms of mania or depression per se.[3,5,6] As genomic and molecular genetic studies of bipolar disorder progress, sophisticated experimental technology in analysis of gene expression and regulation is being applied to the neurobiology of bipolar disorder at the molecular levels; identification of specific gene sequences and associated factors that confer susceptibility or, equally importantly, exert a protective function in bipolar disorder will provide a starting point to follow the effects of the subsequent gene products through the different levels of physiologic outcome. The influences of these basic cellular and molecular mechanisms on critical neuronal circuitry and subsequent behavioral and clinical manifestations become dramatically complex as multiple signaling pathways in the limbic and limbic-associated regions of the brain contribute to the recurrent clinical affective symptomatology.[1]

Beyond the complexity of the numerous independent and associated biological pathways that are apparent in various aspects of the manic and depressive phases of the illness, concurrent physiologic feedback systems are undoubtedly activated in an attempt to compensate for the

Figure 1. The Pathophysiology of Bipolar Disorder: Levels of Analysis

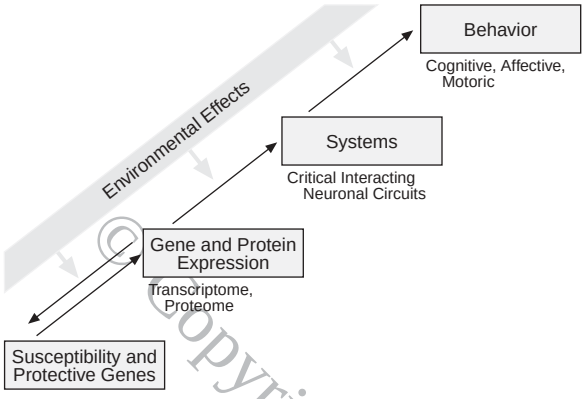
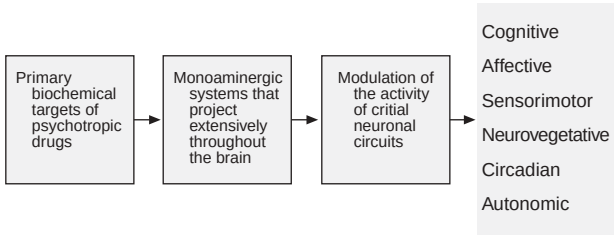


Figure 2. The Modulatory Role of Monoaminergic Systems in the Pathophysiology and Treatment of Mood Disorders



extreme fluctuations in normal pathways and to restore normal homeostasis. The array of molecular and cellular abnormalities and the compensatory mechanisms for these abnormalities may also develop longitudinally and will vary in conjunction with the stage and progression of illness. As a result of potential heterogeneity in these dysfunctional and response systems, individual patients may present with a unique collection of clinical symptoms consisting not only of mood disturbances but also of endocrine, circadian, and drug treatment–related patterns (Figure 2). Furthermore, the acute versus chronic nature of bipolar disorder, with frequently unpredictable recurrence and cycling between the phases, adds additional layers of difficulty in identifying consistent or comparable model systems or patient groups for study. However, while precise knowledge of the etiology and pathophysiology of this disorder that affects millions is still limited, it has become increasingly clear that elucidation of the signaling pathways affected in bipolar disorder will greatly facilitate the development of novel, improved therapeutics. Furthermore, advancing our knowledge of the underlying neurobiology of the illness and the neuronal circuits mediating disease and treatment manifestations will undoubtedly facilitate a much better understanding about the precise manner in which the susceptibility and protective genes interact with each other as well as with environmental factors to produce one of the most devastating and at the same time intriguing of all neuropsychiatric illnesses—bipolar disorder.

While the efficacy of lithium as a mood stabilizer has generated a large body of clinical and experimental findings in bipolar disorder, it has become increasingly appreciated that a substantial minority of patients fail to respond, or respond only partially, to lithium therapy. Several reports indicate that as many as 20% to 40% of patients either do not attain effective therapeutic remission with lithium or continue to incur significant morbidity. Thus, a critical task becomes the development of newer therapeutic agents that will have a quicker, more potent, and more specific mode(s) of action with fewer side effects, thereby enhancing compliance. Although the study of lithium has provided significant mechanistic information on physiologic and biochemical factors and systems associated with mood perturbation, the discovery and application of newer agents including carbamazepine, valproate, lamotrigine, topiramate, risperidone, clozapine, and olanzapine open additional avenues for clarifying the fundamentals of mood disorders at the molecular and cellular level. A review of the progress in elucidating the biochemical and genetic targets that may protect or predispose an individual to bipolar disorder also serves to reinforce the complexity of the multiple independent and overlapping cascades of neurotransmitter and signal transduction systems that may be responsible for the clinical symptoms of depression and mania. In this article, we have attempted to highlight some of the most relevant data over the years pertaining to the complex neurobiology involved in the pathophysiology of bipolar disorder. For the sake of brevity, we sometimes reference review articles.

NEUROTRANSMITTERS

The original "monoamine hypothesis" describing the relationship between excesses or deficiencies in monoamine neurotransmitter concentration in the central nervous system (CNS) and affective disorders (depression and mania) has been revised to include the role of the cellular receptors for the biogenic amines in these 2 states.[7–11] Not only have recent pharmacologic agents that were developed in the burgeoning era of the monoamine hypothesis been applied with definitive clinical effects on single episodes of mania or depression, but they have also provided research tools to further probe the biology of bipolar disorder. Additionally, the potential ability of antidepressants to trigger manic episodes and promote rapid cycling has led to insights into the pathophysiology of the illness.[12–14] Since the pathophysiologic pattern of bipolar disorder most likely involves dysfunction in the limbic

Figure 3. The Pathophysiology of Bipolar Disorder: Clues From the Molecular and Cellular Actions of Lithium

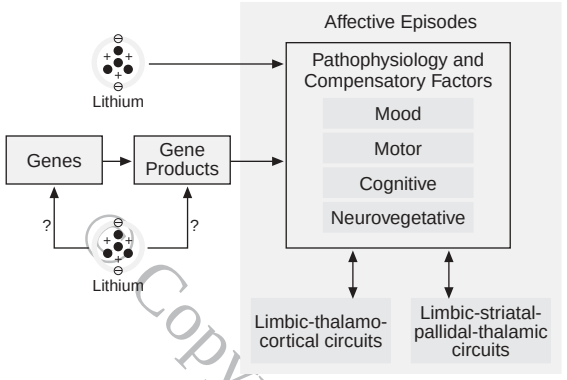


Table 1. The Pathophysiology of Mood Disorders: A Surfeit of Biochemical Findings[a]

Noradrenergic System
Urinary MHPG levels
Plasma norepinephrine levels
CSF norepinephrine, MHPG levels
Peripheral and central α_2-adrenergic receptors, β-adrenergic receptors
Neuroendocrine challenges
AMPT effects
Dopaminergic System
CSF HVA levels
Electroconvulsive therapy effects
AMPT challenges
Psychomotor retardation and activation
Cholinergic System
Serotonergic System
CSF 5-HIAA levels
Neuroendocrine challenges
Peripheral and central 5-HT_2 receptors
[^{3}H]imipramine, [^{3}H]paroxetine binding
PET findings on decreased 5-HT_{1A} binding
Tryptophan depletion effects
Corticotropin-Releasing Factor (CRF)
HPA axis hyperactivity
CRF in CSF
Pituitary and adrenal enlargement
Postmortem brain CRF receptors
Circadian Rhythms
Regional Fluorodeoxyglucose Uptake

[a]Abbreviations: AMPT = α-methylparatyrosine, CSF = cerebrospinal fluid, 5-HIAA = 5-hydroxyindoleacetic acid, HPA = hypothalamic-pituitary-adrenal, 5-HT = 5-hydroxytryptamine, HVA = homovanillic acid, MHPG = 3-methoxy-4-hydroxyphenylglycol, PET = positron emission tomography.

and limbic-related systems, which are extensively regulated by the biogenic amine neurotransmitter systems, these systems are prominently placed to mediate the complex behavioral manifestations of the disease and also serve as therapeutic targets (Figure 3). While these concepts seem straightforward, the multifunctional nature of neurotransmitter systems, the plethora of available neurotransmitter and neuropeptide candidates, and the surfeit of biochemical data complicate assignment of definitive meaning to the observed abnormalities (Table 1).

Noradrenergic System

The catecholamine hypothesis of mood disorders has been extensively investigated[7,8,15] but has proved difficult to study experimentally owing, at least in part, to the formidable methodological difficulties in assessing CNS noradrenergic function in humans, as noted above. Overall, however, the data in toto do suggest an abnormality of the noradrenergic system in bipolar disorder.[5,15,16] Studies of plasma norepinephrine suggest that, on average, bipolar depressed patients appear to have reduced-to-normal resting output of norepinephrine, with a highly exaggerated noradrenergic system response to standing. Plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) levels also tend to be lower in bipolar than in unipolar depressed patients and are higher in bipolar patients when they are manic than when they were depressed.[5,13]

For some years, measures of norepinephrine and its metabolites in cerebrospinal fluid (CSF) were thought to directly reflect the activity of brain noradrenergic systems. This assumption is, however, problematic, since high correlations have been found between indices of norepinephrine function in plasma and CSF.[17] Certain other methodological problems are unique to CSF measures. Standards for obtaining spinal fluid, such as elapsed time between needle insertion and sample collection, have not been established.[5,16] Therefore, subjects may not have the same degree of accommodation to the stress of the needle stick. Moreover, sampling at a single point in time may not reflect the biochemical process of depression or mania but rather a state-dependent fluctuation from a recent external or internal stress.[5,16]

Since the catecholamine hypothesis of mood disorders was proposed, attempts to characterize output of the noradrenergic system in depressed patients have focused on cumulative measurements of MHPG in urine more than any other single parameter. Detailed reviews of the literature differ in their conclusions, but overall, it appears that urinary MHPG levels are lower in bipolar depressed patients, and—similar to the plasma studies—longitudinal studies show that MHPG excretion is higher in the manic compared with the depressed state.[5,15,16,18]

Cerebrospinal fluid norepinephrine is reported to be higher in mania than in depression, and CSF MHPG appears to be lower in bipolar depression than in unipolar depression.[16] Additionally, CSF MHPG has also been reported to be elevated in manic patients compared with controls and to be reduced with lithium treatment, even when treatment was unsuccessful. Taken together, the CSF studies of norepinephrine and its metabolite MHPG suggest that norepinephrine output is higher in mania than in depression and that there may be relatively higher values in unipolar versus bipolar depression.

Over the years, a number of studies have also attempted to infer alterations in central adrenergic receptor function in mood disorders using neuroendocrine challenge paradigms as well as studies of adrenergic receptors on platelets and lymphocytes.[13,15] The caveats necessary when generating elaborate hypotheses about CNS adrenergic receptor dysfunction solely on the basis of studies of these tissues have already been articulated. In the aggregate, these studies *do* tend to suggest altered sensitivity of α_2- and β_2-adrenergic receptors in mood disorders. However, at present, a parsimonious interpretation is that they represent the sequelae, not the cause, of other, more primary perturbations.

Serotonergic System

As with the noradrenergic system, interest in the role of the serotonergic system in mood disorders derived from a long-standing tradition of research into the role of this indolamine in the mechanisms of action of effective treatments. A consistent body of data from CSF studies, neuroendocrine challenge studies, serotonin receptor and reuptake site binding studies, pharmacologic studies, and most recently, brain imaging studies support a role for alterations of serotonergic neurotransmission in depression.[5,19,20] The role of the serotonergic system in bipolar disorder, however, is less clear. Overall, investigators have reported reduced levels of 5-hydroxyindoleacetic acid (5-HIAA) in a subgroup of patients, especially those with impulsivity, aggression, and suicide attempts.[5,19]

A variety of studies using different methodological and technical approaches have devoted attention to the status of serotonin receptors in depression: decreased radioligand binding to the 5-hydroxytryptamine (5-HT) receptor in the platelets and midbrain of unipolar patients and increases in the density of serotonin-2 (5-HT_2) receptors in platelet and postmortem brain studies in depression.[19,20] Most recently, an intriguing preliminary positron emission tomography study[21] reported decreases in 5-HT_{1A} binding potential in several areas of brain in depressed patients, in particular in bipolar depressives and in unipolar patients who have bipolar relatives. Although the small sample size precludes a definitive interpretation of these findings, the use of similar receptor-specific and postreceptor-specific radioligands in future studies should greatly enhance our understanding of the pathophysiology of mood disorders. Distribution of the multiple, well-characterized 5-HT receptor subtypes is known to vary throughout the peripheral and central nervous system, and manipulation of the activity of these receptors in different disorders may have different primary and downstream outcomes. However, reasonable elucidation of these pathways may augment the basic understanding of the pathophysiology of mood disorders.[11] An emerging number of genetic studies are hinting at an association between polymorphisms in the serotonin transporter and tryptophan hydroxylase genes and features of mood disorders; however, these findings require extensive further validation.

Neurotransmitter depletion models, specifically in this case tryptophan depletion to lower serotonin levels, permit a more direct strategy to clarify the involvement of serotonergic systems in mood disorders. Tryptophan depletion results in reversal of the response to selective serotonin reuptake inhibitor (SSRI) (but not noradrenergic) antidepressant medications and recurrence of depression; however, depletion in healthy patients without evidence of mental illness and in nonmedicated patients with depression did not cause or intensify depression.[22] These studies again substantiate the underlying complexity of neurobiological systems inherent not only in depression but, by analogy, in bipolar illness. Two small studies[23,24] have investigated the effect of tryptophan depletion in recently manic, lithium-treated euthymic patients, with conflicting results.

Dopaminergic System

Several lines of evidence point to a role for dopamine in mood disorders. A relevant preclinical model derives from the crucial role of mesoaccumbens dopamine in the neural circuitry of reward and/or incentive motivational behavior.[25,26] Loss of motivation is one of the central features of depression, and indeed, anhedonia is one of the defining characteristics of melancholia; thus, a deficiency of dopamine systems stands out as a prime candidate for involvement in the pathophysiology of depression.[25,26] The strongest direct finding from clinical studies implicating dopamine in depression is reduced homovanillic acid (HVA, the major dopamine metabolite) in the CSF; indeed, this is one of the most consistent biochemical findings in depression.[5,16] There is also evidence for a decreased rate of CSF HVA accumulation in subgroups of depressed patients, including those with marked psychomotor retardation versus those with agitation.[26] Furthermore, depression occurs in up to 40% of patients with idiopathic Parkinson's disease and may precede motor symptoms.

The pharmacologic bridge also supports the notion that manipulation of the dopaminergic system is capable of modulating the illness. Thus, dopamine agonists appear to be effective antidepressants in some bipolar patients and are also able to precipitate mania in some bipolar patients.[5,13] Analyzing the effects of pharmacologic manipulations of the dopaminergic and noradrenergic systems, Goodwin and Sack[27] postulated that dopamine abnormalities are primarily involved in the hyperactivity and psychosis associated with the more severe stages of mania, whereas norepinephrine might be implicated in the euphoria-grandiosity more characteristic of hypomania. They heuristically envisioned a cascade in which the initial noradrenergic dysregulation of hypomania gives way to the hyperdopaminergic state of mania. Linkage of biochemical evidence and behavioral observation during measurement of psychomotor function and its relationship to catechol-

amine function in bipolar patients as evaluated by monitoring concentrations of catecholamines and their metabolites in the CSF and urine has shown that increased arousal and prominent psychomotor abnormalities are associated with the catecholamine system.[28] Investigators have utilized a catecholamine depletion strategy (most recently, via use of the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine [AMPT]) to elucidate the putative roles of dopamine and norepinephrine in mood disorders. In addition to inducing a recurrence of symptoms in depressed patients being treated with "noradrenergic antidepressants," AMPT may induce depressive symptoms in some euthymic, medication-free patients.[15,29] Most recently, investigators have examined the effects of AMPT administration on lithium-treated, euthymic bipolar disorder patients.[30] Intriguingly, they observed no mood-lowering effects of AMPT but observed a rebound hypomania in a significant percentage of the patients. Although preliminary, these results are compatible with a dysregulated signaling system wherein the compensatory adaptation to catecholamine depletion results in an "overshoot" due to impaired homeostatic mechanisms.

Cholinergic System

Much of the evidence supporting the involvement of the cholinergic system in some areas of mood disorders comes from neurochemical, behavioral, and physiologic studies in response to pharmacologic manipulations in investigations done in the early 1970s showing that the relative inferiority of noradrenergic compared with cholinergic tone was associated with depression, while conversely, the opposite was associated with mania.[9,10] Additional support is found from a study of the central cholinesterase inhibitor physostigmine (administered intravenously) in which transient modulation of symptoms in manic patients and induction of depression in euthymic bipolar patients stabilized with lithium were observed.[10] Further extensive studies on the effects of lithium to perhaps potentiate brain cholinergic systems have added to the evidence.[1] However, the therapeutic responses observed with antidepressant and antimanic pharmacologic agents do not correlate with their respective efficacy on the cholinergic system.

Hypothalamic-Pituitary-Adrenal Axis

A large body of data has documented abnormalities of the HPA axis in mood disorders.[31] Numerous reports document HPA axis hyperactivity in drug-free depressed and bipolar depressed patients. With respect to bipolar disorder, increased HPA activity has been associated with mixed-manic states, depression, and, less consistently, classic manic episodes.[5,20] Depression is associated with perturbation in HPA axis function, as observed through increased baseline HPA axis function (higher corticotropin-releasing factor [CRF] in CSF, increased plasma adrenocorticotropic hormone [ACTH] and cortisol concentrations) as well as failure to suppress plasma ACTH and cortisol during the dexamethasone suppression test (DST).[19] In patients diagnosed with severe depression, effective pharmacotherapy of the depression is linked to normalization of the hyperactive HPA axis and resultant mood elevation.[32] The DST has also been studied in patients with bipolar disorder; approximately 25% to 60% of depressed bipolar patients are reported to have abnormal DST results, with some (but not all) studies reporting a normalization of the results during the hypomanic or manic phases. The recent development of CRF antagonists for the treatment of depression will allow more direct clinical and experimental investigations into the role of CRF hypersecretion in the pathophysiology of depression.

The potential hyperactivation of the HPA axis in mood disorders has been revisited in recent years, in large part owing to the growing recognition of the brain atrophy that may be present in many patients (discussed later). It remains to be fully elucidated to what extent these findings represent the sequelae of the biochemical changes (for example, in glucocorticoid levels) accompanying repeated affective episodes per se. The suggestion that changes in glucocorticoid levels affect brain atrophy receives support from the observation that chronic stress or glucocorticoid administration has been demonstrated to produce atrophy and death of vulnerable hippocampal neurons in rodents and primates. Furthermore, magnetic resonance imaging (MRI) studies have also revealed reduced hippocampal volumes in patients with Cushing disease and posttraumatic stress disorder (other conditions associated with hypercortisolemia). Indeed, one of the most consistent effects of stress on cellular morphology is atrophy of hippocampal neurons.[33,34] This atrophy is observed in the CA3 pyramidal neurons, but not in other hippocampal cell groups (i.e., CA1 pyramidal and dentate gyrus granule neurons). Atrophy of CA3 pyramidal neurons also occurs upon exposure to high levels of glucocorticoids, suggesting that activation of the HPA axis very likely plays a major role in mediating the stress-induced atrophy. In addition to neuronal atrophy, longer-term exposure to stress (i.e., for several months) can result in true loss of hippocampal neurons in the CA3 pyramidal cell layer.[33] Furthermore, it is possible that recurrent mood disorders may lower the threshold for cell death/atrophy in response to a variety of other physiologic (e.g., normal aging) and pathologic (e.g., ischemia) events and thereby contribute to a variety of deleterious health-related effects.

SIGNAL TRANSDUCTION NETWORKS

Signal transduction networks, composed of membrane-bound and intracellular complexes of multiple protein, enzyme, and small molecule components, transmit extracellular signals to intracellular processing systems to direct the eventual cellular response to stimulation or inhibi-

tion.[35–37] These signaling networks mediate communications over a variety of time scales; thus, in addition to mediating instantaneous responses to receptor activation, signaling systems also participate in long-term neuroplastic events and regulate cellular memory. Cells that have experienced different stimuli may express a unique repertoire of signaling molecules and transcription factors, allowing for different responses to the identical stimuli. Signal transduction networks can amplify or redirect the initial stimuli and regulate both message propagation and feedback loops and thereby serve major integrative functions to form both intracellular and intercellular circuits. Given the important roles of signal transduction networks in regulating normal physiology as well as identifying signaling abnormalities in a number of disease states, their involvement in the pathophysiology and treatment of bipolar disorder would not be surprising. Pertinent for the present discussion is the observation that a variety of diseases arising from abnormalities in guanine nucleotide–binding protein (G protein)–coupled signal transduction pathways manifest relatively circumscribed symptomatology,[38,39] despite the widespread, often ubiquitous expression of the affected signaling proteins. Moreover, recent research has clearly identified signaling pathways as therapeutically relevant targets for the most effective pharmacologic treatments.[40–44] Indeed, the molecular and cellular targets underlying lithium's ability to stabilize an underlying dysregulation of limbic and limbic-associated functions strongly suggest that abnormalities in signaling pathways may also play a critical role in the pathophysiology of bipolar disorder.[45] We now turn to a discussion of the direct and indirect evidence supporting a role for abnormalities in signaling pathways in the pathophysiology of bipolar disorder.

The Cyclic Adenosine Monophosphate/ Protein Kinase A Signaling Pathway

The most commonly utilized strategy in patients with mood disorders has been to focus on receptor function in readily accessible blood elements, and much clinical research has focused on the activity of the cyclic adenosine monophosphate (cAMP) generating system in mood disorders. Overall, the preponderance of the evidence suggests altered receptor and/or postreceptor sensitivity of the cAMP generating system *in mood disorders in the absence of consistent alterations in the number of receptors themselves.*[46,47] A recent study[48,49] found higher levels of cAMP-stimulated phosphorylation of a ~22 kDa protein in platelets obtained from 10 treated euthymic bipolar disorder patients compared with healthy subjects; by contrast, no significant difference in basal phosphorylation was found between the groups. Follow-up studies identified the ~22 kDa protein as Rap1 and once again found higher cAMP-stimulated phosphorylation in the bipolar disorder patients.[48,49] Rahman and associates[50] have undertaken the most thorough series of studies investigating the cAMP/protein kinase A (PKA) system in postmortem human brain in bipolar disorder. They found that the levels of PKA regulatory subunits (as assessed by [^{3}H]cAMP binding) were significantly lower in cytosolic fractions of bipolar disorder frontal, temporal, occipital, and parietal cortex; cerebellum; and thalamus compared with matched controls. Furthermore, preliminary findings show that the reduction of regulatory subunits of PKA in the cytosolic fractions of bipolar disorder temporal cortex is accompanied by a higher basal kinase activity and significantly lower apparent activation constant for cAMP in the cytosolic fractions of bipolar disorder temporal cortex.[51] These observed changes in PKA provide additional important evidence for dysregulation in the $G\alpha_s$-mediated cAMP cascade in bipolar disorder.

Lithium and the cAMP Generating System

Lithium has been demonstrated to exert complex effects on the activity of adenylyl cyclase (AC), with the preponderance of the data demonstrating an elevation of basal AC activity while attenuating a variety of receptor-mediated responses.[46,52–54] Lithium in vitro inhibits the stimulation of AC by guanyl imidodiphosphate, or Gpp(NH)p (a poorly hydrolyzable analogue of guanosine triphosphate [GTP]), and calcium-calmodulin, both of which can be overcome by Mg^{2+}.[55–57] Lithium also competes with Mg^{2+} for membrane-binding sites, and the inhibition of solubilized catalytic unit of AC by lithium can also be overcome by Mg^{2+}. These findings suggest that lithium's inhibition of AC in vitro may be due to competition with Mg^{2+} on a site on the catalytic unit of AC.[55,57] However, the inhibitory effects of chronic lithium treatment on rat brain AC are not reversed by Mg^{2+}, and these inhibitory effects still persist after washing of the membranes but are reversed by increasing concentrations of GTP.[56] These results suggest that the physiologically relevant effects of lithium (i.e., those seen with chronic drug administration and not reversed immediately with drug discontinuation) may be exerted at the level of signal-transducing G proteins at a GTP-responsive step (discussed later). These 2 distinct actions of lithium on the AC system may explain the differing results obtained by investigators using rat membrane preparations or slice preparations.[43,44] These results have led to an investigation of the effects of lithium on the AC system in vivo, using microdialysis. These studies found that chronic lithium treatment produced a significant increase in basal and postreceptor-stimulated (cholera toxin or forskolin [FSK]) AC activity, while attenuating the β-adrenergic–mediated effect.[43,44,58] Interestingly, chronic lithium treatment resulted in an almost absent cAMP response to pertussis toxin, suggesting a lithium-induced attenuation of G_i function. It should be noted, however, that chronic lithium has also been found to increase not only cAMP levels,[59] but

also the levels of AC Type I and Type II mRNA and protein levels in frontal cortex,[60,61] suggesting that the complex effects of lithium on the system may represent the net effects of direct inhibition of AC, up-regulation of AC subtypes, and effects on the stimulatory and inhibitory G proteins. Most recently, the effects of lithium on the phosphorylation and activity of CREB (cAMP response element binding protein) have been examined in rodent brain and in cultured human neuroblastoma cells, with somewhat conflicting results.[62–64]

Another series of studies has examined the effects of lithium on AC in humans. In a longitudinal study of healthy volunteers, 2 weeks of lithium administration was found to significantly increase platelet basal and postreceptor-stimulated AC activity,[65] effects that are strikingly similar to those observed in rodent brain. Consistent with a lithium-induced increase in basal cAMP and AC levels, a more recent study[48] found that platelets obtained from lithium-treated euthymic bipolar patients enhanced basal and cAMP-stimulated phosphorylation into Rap1 (a PKA substrate) as well as into a 38 kDa phosphoprotein. Interestingly, these investigators did not find similar effects of lithium in healthy subjects.

Carbamazepine and the AC System

Considerable data suggest that carbamazepine (an atypical anticonvulsant) may be an effective alternative or adjunctive treatment to lithium, both for acute manic episodes as well as for long-term prophylaxis in bipolar disorder. Despite the widespread clinical use of carbamazepine, the cellular mechanism(s) underlying both its anticonvulsant and mood-stabilizing effects have not been fully elucidated.[66,67] In contrast to the effects observed with lithium and valproate described above, it has been demonstrated that carbamazepine has very modest effects on the protein kinase C (PKC) signaling pathway and G proteins (H.K.M., G. Chen, M.D., Ph.D., unpublished observations, October 1997). Carbamazepine has, however, been demonstrated to have many effects on the cAMP signaling pathway.

Carbamazepine has been demonstrated to decrease not only basal, but also norepinephrine-, adenosine-, veratridine-, and ouabain-stimulated cAMP levels in rodent brain.[68–74] Recent studies have also demonstrated that carbamazepine inhibits forskolin-induced c-*fos* gene expression in cultured pheochromocytoma (PC12) cells.[75] Thus, overall, considerable evidence indicates that carbamazepine inhibits cAMP formation. Studies have recently been undertaken to investigate the possible mechanisms by which carbamazepine inhibits the cAMP generating system. It was found that carbamazepine, at therapeutically relevant concentrations, inhibited both basal AC and FSK-stimulated cAMP accumulation in C6 glioma cells.[76] To further characterize the site at which carbamazepine exerts its inhibitory effects, ACs were purified from rat cerebral cortex using a FSK affinity purification column. Carbamazepine inhibited both basal and FSK-stimulated activity of purified AC.[76] Taken together, the data suggest that carbamazepine inhibits cAMP production by acting directly on AC and/or through factor(s) that are tightly associated with, and copurify with, AC. It is now well established that the antimanic effects of carbamazepine show a lag period of onset. In this context, it is noteworthy that Divish and colleagues[75] have demonstrated that carbamazepine attenuates FSK-induced c-*fos* (an immediate early gene) expression in PC12 cells. Since c-*fos* and other immediate-early genes are known to be involved in mediating a number of long-term neuronal responses,[77] these effects might be postulated to play a role in the delayed therapeutic effect of carbamazepine. Consistent with these observations, it was found that in C6 glioma cells, FSK enhances phosphorylation of CREB and that carbamazepine significantly inhibited FSK-induced phosphorylation of CREB. These results complement the results of Divish and associates[75] described above and suggest that the inhibitory effects of carbamazepine on AC have the potential to bring about long-term changes in gene expression and thereby regulate cellular function.

The Phosphoinositide/PKC Signaling Pathway

Peripheral cell studies and postmortem brain studies have generally revealed modest abnormalities in the phosphoinositide/PKC signaling system in bipolar disorder. Thus, one study[78] measured membrane phospholipids in platelets of 7 medication-free patients in the manic phase of bipolar disorder and 7 healthy comparison subjects. The relative percentage of platelet membrane phosphatidylinositol 4,5-bisphosphate (PIP_2) was significantly higher in the manic patients than in the comparison subjects. More recently in a single case study,[79] the same laboratory studied PIP_2 membrane values in a bipolar disorder patient in different mood states. They found that the relative percentage of PIP_2 in the platelet membranes increased with cycling from the euthymic into the manic state. After lithium treatment, PIP_2 decreased and was similar to the euthymic state. Consistent with these findings, van Calker and associates[80] have found increased sensitivity to agonist stimulation of the Ca^{2+} response in neutrophils of manic depressive patients, effects that were normalized by lithium treatment. Investigators have also attempted to determine if the altered phosphoinositide signaling found in peripheral cells from bipolar disorder patients is present in the CNS. In this context, Mathews and associates[81] found increased $G\alpha_{q/11}$ immunoreactivity in postmortem occipital cortex from patients with bipolar disorder. However, these elevated levels of $G\alpha_{q/11}$ were accompanied by *reduced* agonist-induced phosphoinositide turnover,[82] although the potential effects of long-term lithium treatment remain to be fully delineated. To date, few studies have directly examined PKC in bipolar disorder.

Friedman and associates[83] investigated PKC activity and translocation in response to serotonin in platelets obtained from bipolar disorder subjects before and during lithium treatment. They found that the ratios of membrane-bound to cytosolic PKC activities were elevated in platelets obtained from the manic subjects, compared with patients with schizophrenia and healthy controls. In addition, serotonin-stimulated platelet PKC translocation was found to be enhanced in manic subjects. Moreover, lithium treatment for up to 2 weeks resulted in a reduction in cytosolic and membrane-associated PKC activities and in an attenuated PKC translocation in response to serotonin. These preliminary results suggest that alteration in platelet PKC is associated with the manic phase of bipolar illness. More recently, the same laboratory has measured PKC isozyme levels, activity, and translocation in postmortem brain tissue from bipolar disorder patients; they have found increased PKC activity and translocation as well as elevated levels of cytosolic α- and membrane-associated PKC γ and PKC ζ isozymes in brains obtained at postmortem from bipolar disorder patients compared with controls.[84,85] These results may be specific to bipolar disorder, since another laboratory has recently found significantly *decreased* phorbol dibutyrute ([^{3}H]PDBU) binding sites in both membranous and cytosolic postmortem brain samples (Brodmann areas 8 and 9) obtained from teenage suicide victims compared with control subjects.[86]

Table 2. Direct and Indirect Evidence Supporting a Role for Protein Kinase C (PKC) in the Pathophysiology and Treatment of Bipolar Disorder[a]

Kindling produces dramatic increases in membrane-associated PKC in hippocampus and amygdala
Amphetamine produces increases in PKC activity and GAP-43 phosphorylation (implicated in neurotransmitter release)
PKC inhibitors block the biochemical and behavioral responses to amphetamine and cocaine and also block cocaine-induced sensitization
Dexamethasone administration increases PKC activity and the levels of PKC α and PKC ε in rat frontal cortex and hippocampus
Membrane/cytosol PKC partitioning in platelets is increased in manic subjects; normalizes with lithium treatment
Bipolar patients' brains show increased PKC activity and translocation compared with controls
Lithium and valproate regulate PKC activity, PKC α, PKC ε, and MARCKS
Preliminary data suggest that PKC inhibitors may have efficacy in the treatment of acute mania
Typical and atypical antipsychotics regulate the levels of PKC α and ε in areas of rat brain

[a]Abbreviations: GAP = growth cone–associated protein, MARCKS = myristoylated alanine-rich C kinase substrate.

Lithium and PKC

The "inositol depletion hypothesis" posited that lithium, as an uncompetitive inhibitor of inositol-1-phosphatase (IMPase), produced its therapeutic effects via a depletion of neuronal *myo*-inositol levels. Although this hypothesis has been of great heuristic value, numerous studies have examined the effects of lithium on receptor-mediated phosphoinositide responses, and although some report a reduction in agonist-stimulated PIP_2 hydrolysis in rat brain slices following acute or chronic lithium administration, these findings have often been small, inconsistent, and subject to numerous methodological differences.[42,87] Most recently, a proton magnetic resonance spectroscopy (MRS) study[88] has demonstrated that lithium-induced *myo*-inositol reductions are observed in the frontal cortex of bipolar disorder patients after only 5 days of lithium administration, *at a time when the patients' clinical state is completely unchanged*. Consequently, these and other studies suggest that while inhibition of IMPase may represent an initiating lithium effect, reducing *myo*-inositol levels per se is not associated with therapeutic response. This finding has led to the working hypothesis that some of the initial actions of lithium may occur with a relative reduction of *myo*-inositol; this reduction of *myo*-inositol initiates a cascade of secondary changes in the PKC signaling pathway and gene expression in the CNS, effects that are ultimately responsible for lithium's therapeutic efficacy.

Indeed, evidence accumulating from various laboratories has clearly demonstrated that lithium, at therapeutically relevant concentrations, exerts major effects on the PKC signaling cascade.[40–42,85,89] PKC is highly enriched in brain and plays a major role in regulating presynaptic and postsynaptic aspects of neurotransmission. PKC is one of the major intracellular mediators of signals generated upon external stimulation of cells via a variety of neurotransmitter receptor subtypes, which induce the hydrolysis of membrane phospholipids. PKC is now known to exist as a family of closely related subspecies, has a heterogeneous distribution in brain (with particularly high levels in presynaptic nerve terminals), and plays a major role in the regulation of neuronal excitability, neurotransmitter release, and long-term alterations in gene expression and plasticity (Table 2).

The preponderance of the currently available data suggests that acute lithium may activate PKC, whereas chronic lithium results in an attenuation of phorbol ester–mediated responses accompanied by a down-regulation of PKC isozymes in the brain. Using quantitative autoradiographic techniques, it was demonstrated that chronic (5-week) lithium administration results in a significant decrease in membrane-associated PKC in several hippocampal structures, most notably the subiculum and the CA1 region, in the absence of any significant changes in the various other cortical and subcortical structures examined.[90,91] Furthermore, immunoblotting using monoclonal anti-PKC antibodies has revealed isozyme-specific decreases in PKC α and PKC ε (which have been particularly implicated in facilitating neurotransmitter release), in the absence of significant alterations in PKC β, PKC γ, PKC δ, or PKC ζ. It is also noteworthy that exposure of immortalized hippocampal cells,[37] neuroblastoma cells, or

PC12 cells[92] to lithium (1.0 mM) in vitro produces isozyme-selective decreases in PKC α and (in the case of PC12 cells) PKC ε. In the absence of suitable animal models for the major psychiatric disorders, a major problem inherent in neuropharmacologic research is the difficulty in precisely ascribing therapeutic relevance to any observed biochemical finding. One approach that has been utilized is the identification of common biochemical targets that are modified by drugs belonging to the same therapeutic class (e.g., antimanic agents) but possessing distinct chemical structures (i.e., lithium, valproate, and olanzapine, the only 3 drugs approved by the Food and Drug Administration for the treatment of bipolar disorder) when administered in a "therapeutically relevant" paradigm. Although they most likely do not work by precisely the same mechanisms, identifying the biochemical targets that are regulated in concert by these 2 chemically distinct agents may provide important clues about molecular mechanisms underlying mood stabilization in the brain.

In view of the significant effects of lithium on PKC, outlined above, the effects of valproate on various aspects of PKC functioning have also been investigated. It has been found that the structurally highly dissimilar agent valproate produces effects on the PKC signaling pathway that are strikingly similar to lithium's effects.[42,93] Interestingly, chronic lithium and valproate appear to regulate PKC isozymes by distinct mechanisms, with valproate's effects appearing to be largely independent of *myo*-inositol. This biochemical observation is consistent with the clinical observations that some patients show preferential response to one of the agents and that one often observes additive therapeutic effects in patients when the 2 agents are coadministered. In view of the pivotal role of the PKC signaling pathway in the regulation of neuronal excitability, neurotransmitter release, and long-term synaptic events, we postulated that the attenuation of PKC activity may play a role in the antimanic effects of lithium and valproate. In a pilot study,[94] it was found that tamoxifen (a nonsteroidal antiestrogen known to be a PKC inhibitor at higher concentrations) may, indeed, possess antimanic efficacy. While awaiting further replication, these data further support the potential role of the PKC pathway in mediating the pathophysiology of bipolar disorder.

A major strategy that has been utilized to investigate the downstream consequences of lithium-induced alteration in PKC isozymes is the examination of the effects of chronic lithium on endogenous PKC substrates in brain. The most prominent substrate for PKC in brain is an acidic protein, myristoylated alanine-rich C kinase substrate (MARCKS), which has been implicated in regulating long-term neuroplastic events. Lenox and associates[95] demonstrated that chronic lithium administration dramatically reduced MARCKS expression in hippocampus, an effect that was not immediately reversed following lithium discontinuation. Subsequent studies carried out in immortalized hippocampal cells have demonstrated that this action of chronic lithium on MARCKS regulation is dependent on both the inositol concentration and the level of receptor-mediated activation of phosphoinositide hydrolysis.[96] Recent studies provide evidence for the regulation of transcription as a major site for the action of chronic lithium on MARCKS expression in brain.[97] Since MARCKS may offer a specific target for pharmacologic agents to aim for in mood-stabilizing drugs, the effects of the anticonvulsant valproate as well as lithium were studied in immortalized hippocampal cells and were shown to induce a time- and concentration-dependent reduction in MARCKS protein expression. The activity of valproate was observed within a concentration range and time course considered consistent with clinical studies of patients with bipolar disorder. Additionally, therapeutic concentrations of combined lithium and valproate induced an additive reduction in MARCKS, consistent with experimental findings that the 2 drugs work through different mechanisms on the PKC system and with the clinical observation of the additivity of the 2 drugs in treatment responses.[4]

Table 3. Signal Transduction Abnormalities in Bipolar Disorder: Preliminary Postmortem Findings[a]

Increased $G\alpha_s$ immunoreactivity (but unchanged mRNA levels)
Increased GTPγS and forskolin-stimulated adenylyl cyclase activity
Increased receptor/G protein coupling
Increased $G\alpha_{q/11}$ levels and PKC β levels
Reduced receptor-mediated phosphoinositide turnover
Increased levels of selected PKC isozymes
Reduced [^{3}H]cAMP binding (possible reduction in binding to regulatory subunits of PKA)
Increased PKA activity

[a]Abbreviations: cAMP = cyclic AMP, G protein = guanine nucleotide–binding protein, GTP = guanosine triphosphate, mRNA = messenger ribonucleic acid, PKA = protein kinase A, PKC = protein kinase C.

G Proteins

Several independent laboratories have examined G proteins in patients with mood disorders (Table 3).[46,47,81,98] Young and associates[46,99] were the first to report increased levels of $G\alpha_s$ in bipolar disorder patients in 2 separate studies. Compared with controls matched with respect to age, postmortem interval, and brain pH, they found increased levels of $G\alpha_s$ in frontal, temporal, and occipital cortices but not in hippocampus, thalamus, or cerebellum in postmortem brain tissue from patients with bipolar disorder.[46,99] As discussed, this group also found increases in FSK-stimulated adenylate cyclase activity in postmortem brain, compatible with a postreceptor abnormality in bipolar disorder. The findings of elevated $G\alpha_s$ levels and/or function are also supported by the recent findings of Friedman and Wang,[100] who found increased agonist-activated [^{35}S]GTPγS binding to G protein α subunits in frontal cortical membrane preparations from bipolar disorder postmortem brain. Garcia-Sevilla and colleagues[101] have reported increased levels of $G\alpha_{i1/2}$ in prefrontal cortical

samples obtained at postmortem from depressed patients who committed suicide, effects that were apparently attenuated by antemortem antidepressant treatment. Overall, the findings in postmortem brain tissue in unipolar depression have been less consistent (see Warsh et al.[47] for a summary of postmortem findings). In keeping with the G protein abnormalities in brain tissue obtained at postmortem from bipolar disorder patients, Schreiber and colleagues[102] reported "hyperfunctional" G protein function in leukocytes of untreated manic patients by demonstrating that agonist-stimulated binding of [^{3}H]Gpp(NH)pm (a stable, nonhydrolyzable analog of GTP) was enhanced in leukocyte membranes of untreated manic patients compared with controls. These findings suggest the presence of increased levels of G_s proteins and/or enhanced receptor-mediated activation of G proteins in leukocytes from untreated manic subjects. More recently, investigators have reported significantly higher levels of $G\alpha_s$ in mononuclear leukocytes from depressed bipolar but not unipolar patients.[103] Another study[52,53] quantitated the levels of the major G protein α subunits in both platelet and leukocyte membranes from untreated (predominantly manic) and lithium-treated, euthymic bipolar disorder patients and observed higher levels of the 45 kDa form of $G\alpha_s$ in the combined group of bipolar disorder patients (treated and untreated) compared with controls. A recent study[104] has found elevated levels of $G\alpha_s$ mRNA in granulocytes obtained from bipolar but not unipolar patients. This study also found nonsignificant elevations in the levels of $G\alpha_{i2}$ in unmedicated bipolar patients, which, intriguingly, were modulated by lithium in bipolar but not unipolar patients. Similar to what has been observed in the CNS, one recent study[105] has evaluated the role of platelet G proteins as "signal coincidence detectors" and has found this function to be impaired in depressed patients. Overall, the most consistent finding to emerge is that in both peripheral cells and postmortem brain tissue from bipolar patients, elevations are observed in the predominant subspecies of $G\alpha_s$ present in the tissues examined. Since $G\alpha_s$ is a ubiquitously expressed protein, it may appear counterintuitive that an abnormality in this protein may play a role in the pathophysiology of bipolar disorder. However, there is already precedence for clinical disorders arising from abnormalities in the levels of $G\alpha_s$, which present with limited clinical manifestations, despite the ubiquitous expression of the protein.[39] These heterogeneous clinical effects and tissue-specific manifestations have been postulated to arise from differences in receptor, G protein, and effector stoichiometries in different tissues and from tissue-specific differences in the ability of different cells to compensate for the abnormality. It should be emphasized, however, that there is at present *no evidence* to suggest that the alterations in the levels of $G\alpha_s$ are due to a mutation in the $G\alpha_s$ gene itself.[106] Indeed, there are numerous transcriptional and posttranscriptional mechanisms that regulate the levels of G protein α subunits, and the elevated levels of $G\alpha_s$ could potentially represent the sequelae of alterations in any one of these other biochemical pathways. Thus, at this point, considerable caution is clearly required in the interpretation of the data, since they derive primarily from peripheral cell models and may not adequately reflect CNS pathology. The possibility of abnormalities in the biochemical pathways that regulate $G\alpha_s$ levels in bipolar disorder is currently undergoing further study.[47]

Lithium and G proteins. Considerable evidence now indicates that chronic lithium administration affects G protein function[3,40,41,63]; however, the preponderance of the data suggests that lithium, at therapeutically relevant concentrations, has no direct effects on G proteins and does not consistently alter G protein subunit levels.[1,107] Although some studies have reported modest changes in the levels of G protein subunits, the preponderance of the data suggests that the effects of chronic lithium on signal-transducing properties occur in the absence of changes in the levels of G protein subunits per se.[1] Chronic in vivo lithium treatment has been shown to produce a significant increase in pertussis toxin–catalyzed [^{32}P] adenosine diphosphate (ADP)-ribosylation in rat frontal cortex and human platelets. Since pertussis toxin selectively ADP-ribosylates the undissociated, inactive $\alpha\beta\gamma$ heterotrimeric form of G_i, these results suggest that lithium attenuates G_i function via a stabilization of the inactive conformation. These results suggest that the removal of the "inhibitory tone" by lithium may be responsible for the elevations in basal AC and the responses to agents activating the stimulatory pathway *distal to the receptor*.[43,58] Consistent with this hypothesis, lithium has been shown to potentiate the hyperactivity induced by intra-accumbens cholera toxin administration (which activates the stimulatory G proteins, G_s and G_{olf}).[108] These long-term effects of chronic lithium on G protein are most likely 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. In this context, it is noteworthy that investigators have demonstrated that lithium alters the levels of endogenous ADP-ribosylation in C6 glioma cells[109] and in rat brain,[110] suggesting another mechanism by which chronic lithium may indirectly regulate the activity of these critical signaling proteins.

Valproate and G proteins. Recent studies have examined the effects of valproate on components of the β-adrenergic receptor–coupled cAMP generating system.[111] Chronic valproate has been shown to produce a significant alteration of the β-adrenergic receptor–coupled cAMP generating system in cultured cells in vitro; these effects were observed at concentrations of valproate similar to those attained in the plasma in the clinical treatment of neuropsychiatric disorders. In contrast to what has been observed with chronic lithium treatment (discussed above), it was found that chronic valproate produced a sig-

nificant reduction in the density of β-adrenergic receptors. Interestingly, the decrease in numbers of β-adrenergic receptors (approximately 30%) was accompanied by an even greater decrease in receptor- and postreceptor-mediated cAMP accumulation, suggesting that chronic valproate also exerts effects at the β-adrenergic receptor/G_s interaction or at postreceptor sites (e.g., G_s, AC). Consistent with such a contention, it was indeed found that chronic, but not acute, valproate incubation induced a marked decrease in the levels of $G\alpha_s$ 45 but no other G protein α subunits examined ($G\alpha_s$ 52, $G\alpha_{i1/2}$, $G\alpha_o$, or $G\alpha_{q/11}$). In view of the suggested involvement of G_s in the pathophysiology of manic depressive illness (vide supra), as well as the effects of lithium on the β-adrenergic receptor/G_s/AC system, these effects may play a role in valproate's therapeutic effects and are worthy of further study.

Abnormalities of Calcium Signaling in Bipolar Disorder

Acting via intracellular proteins such as MARCKS and calmodulin, and enzymes such as PKC, AC, and CaM kinase, calcium ions have been shown to regulate the synthesis and release of neurotransmitters, neuronal excitability, cytoskeletal remodeling, and long-term neuroplastic events. Thus, it is not surprising that a large number of studies have investigated intracellular Ca^{2+} in peripheral cells in bipolar disorder.[46,112,113] In view of the caveats associated with studies of peripheral circulating cells, the remarkable consistency of the findings is surprising indeed. Studies have consistently revealed elevations in both resting and stimulated intracellular Ca^{2+} levels in platelets, lymphocytes, and neutrophils of patients with bipolar disorder. The calcium abnormalities have been postulated to represent state-dependent findings,[112] but recent studies using transformed lymphoblasts from bipolar disorder patients have revealed similar abnormalities, suggesting that they may be trait dependent.[113] The regulation of free intracellular Ca^{2+} is a complex, multifaceted process involving extracellular entry, release from intracellular stores following receptor-stimulated phosphoinositide hydrolysis, uptake into specific organelles, and binding to specific proteins. Thus, the abnormalities observed in bipolar disorder could arise from abnormalities at a variety of levels, and recent studies suggest that the abnormality lies beyond the receptor.[114] Interestingly, recent studies have demonstrated that the Rap1 phosphorylation state is related to platelet intracellular calcium signaling,[115] suggesting a possible relationship between these 2 documented abnormalities. Since PKC is also known to regulate calcium signaling at multiple levels, more recent studies have investigated the putative role of PKC in mediating calcium abnormalities in bipolar disorder. Preliminary analysis suggests that alterations in tonic PKC activity may play an important role in mediating the abnormal intracellular calcium responses observed in bipolar disorder (H.K.M., R. M. Post, M.D., unpublished observations, October 1996).

Do Animal Models Support the Involvement of Signaling Pathways in the Pathophysiology of Bipolar Disorder?

The use of animal models of disease and in vitro experiments to clarify complex neuropsychiatric disorders in humans has been reviewed, and caution must be exercised when applying results from these restricted models to the human environment.[116,117] Two current models that have had reasonable heuristic value in the study of mood disorders have been kindling and behavioral sensitization.[116,117] The increased, sensitized behavioral response, measured through behavioral or electrophysiologic changes to repetition of the same stimulus, whether a pharmacologic agent or an electrical challenge, can persist for months or years after discontinuation of the stimuli. The cellular mechanisms responsible for these long-lasting behavioral responses in kindled or stimulant-sensitized animals are actively under study, including both G protein–mediated pathways and PKC signaling. Briefly, evidence that regulation of G proteins is a component of the biochemical alterations inherent in the effects of chronic stimulation comes from the significant enhancement of psychostimulant-induced motor activity and release of dopamine in the nucleus accumbens reported after treatment with pertussis toxin, an inhibitor of specific G proteins (G_i and G_o).[118] Additionally, specific decreases in G protein subunit concentrations in the ventral tegmental area and nucleus accumbens of rats have been reported in response to chronic cocaine administration.[119]

Alterations in PKC function have been implicated through experiments with chronic and acute stimulant administration leading to sensitization and kindling as well as alterations in neurotransmitter release during amphetamine administration in animal models. Substantial increases in membrane-associated but not cytosolic PKC have been documented in the bilateral hippocampus for up to 4 weeks and in the amygdala/pyriform cortex at 4 weeks as well as after the last seizure in hippocampal-kindled rats.[120] Studies of perfused rat striatial slices treated with PKC stimulators or inhibitors also show that amphetamine-mediated dopamine release through the plasmalemmal dopamine transporter is dependent on PKC function.[121] Since drug treatment with either lithium or valproate also targets components of the PKC pathway with a diametrically opposite effect in critical limbic structures in behaviorally sensitized and kindled rats, one has greater confidence in extrapolating these results to humans.[42] Extensive data also suggest that long-term alterations in midbrain dopaminergic neurotransmission are involved in the establishment of behavioral sensitization, and recent data implicate a cascade of changes in gene expression, including neurotropic and apoptotic factors.[116]

Therefore, induced behaviors established in animal models now provide experimental systems in which to explore the contributions of various signal transduction pathways and gene control elements.

CIRCADIAN RHYTHMS

Mood disorders are intimately associated with disruptions in circadian rhythms; this may be particularly true for bipolar disorder, which is characterized by an episodic pattern, hallmark recurrence, and fluctuations in symptoms and severity, course of illness, and responses to treatments. Depressed patients frequently evidence shortened rapid eye movement latency, early wakening, and advances in hormonal and temperature rhythms, implying dysregulation of internal oscillators with respect to other internal clock systems or external time-dependent stimuli.[5,122,123] A circadian clock located in the hypothalamic suprachiasmatic nucleus regulates the circadian phases of mammalian locomotor activity, which during synchronization can cause time-consistent circadian rhythms of very distinct amplitudes. Studies on model systems in *Drosophila* mutants have located circadian clock genes that are strongly suspected, through additional studies with mouse genes, to be highly evolutionarily conserved.[124] Since chronic (not acute) lithium treatment affects, although with variabilities in advancing or delaying the time of highest amplitude, all biological systems in an evolutionarily conserved manner, genes controlling the circadian clock might be awry in mood disorders and be the ultimate targets of lithium.[125] Without question, these studies are in their infancy and must be further supported with additional investigations.[111,126]

CELL DEATH AND ATROPHY IN MOOD DISORDERS

Studies have recently demonstrated that although mood disorders have traditionally been conceptualized as neurochemical disorders, reductions in regional CNS volume and cell numbers (both neurons and glia) are observed in many patients. One line of evidence comes from structural imaging studies, which have recently begun to provide important clues about the neuroanatomical basis of mood disorders. Although the findings are not as consistent as those reported in schizophrenia, in toto, the volumetric neuroimaging studies have demonstrated an enlargement of third and lateral ventricles, as well as reduced gray matter volumes in parts of the orbital and medial prefrontal cortices, the ventral striatum, and the mesiotemporal cortex.[21,127] Lending support to the structural neuroimaging literature are multiple functional brain imaging studies that have shown abnormalities in metabolic rate and blood flow in these same areas in major depression.[21] In addition to the accumulating neuroimaging evidence, several postmortem brain studies are now providing direct evidence for reductions in regional CNS volume and cell number.[42,43,128] Together, the preponderance of the data from the neuroimaging studies and the growing body of postmortem evidence presents a convincing case that there is indeed a reduction in regional CNS volume, accompanied by a reduction in cell numbers in at least a subset of patients with mood disorders. It is thus noteworthy that recent studies have led to a completely unexpected target for the actions of chronic lithium and valproate in the frontal cortex—the cytoprotective protein bcl-2.[2,129]

Chronic treatment of rodents with "therapeutic" doses of lithium and valproate was found to produce a *doubling* of bcl-2 levels in frontal cortex, an effect that was primarily due to a marked increase in the number of bcl-2 immunoreactive cells in layers II and III of frontal cortex. Interestingly, the importance of neurons in layers II–IV of the frontal cortex in mood disorders has recently been emphasized, since primate studies have indicated that these are important sites for connections with other cortical regions and major targets for subcortical input.[130] Furthermore, these are the very same brain areas where the greatest neuronal or glial diminution has been observed in postmortem studies of major depression subjects.[128,129] Chronic lithium also markedly increased the number of bcl-2 immunoreactive cells in the dentate gyrus and striatum.[2] The lithium-induced increase in bcl-2 levels has also been convincingly replicated in rat cerebellar granule cells in recent studies.[130] Consistent with the known cytoprotective effects of bcl-2, lithium, at therapeutically relevant concentrations, has been shown to exert neuroprotective effects in a variety of paradigms. Thus, in vitro, lithium has been demonstrated to protect against the deleterious effects of glutamate, *N*-methyl-D-aspartate receptor activation, aging, serum/nerve growth factor deprivation, ouabain, and thapsigargin (which mobilizes intracellular 1-methyl-4-phenypyradinium ion [MPP^+], Ca^{2+}, and β-amyloid).[2,43,44]

Cytoprotective effects of lithium have also been demonstrated in rodent brain in vivo. Thus, lithium treatment has been shown to attenuate the biochemical deficits produced by kainic acid infusion, ibotenic acid infusion, and forebrain cholinergic system lesions and to also attenuate the behavioral deficits produced by forebrain cholinergic system lesions.[131,132] Recent studies by Chuang and associates have shown that lithium exerts dramatic protective effects against middle cerebral artery occlusion, reducing not only the infarct size (56%) but also the neurologic deficits (abnormal posture and hemiplegia).[133] Most recently, the same research group has demonstrated that in vivo lithium treatment robustly protects neurons in the striatum from quinolinic acid–induced toxicity in a putative model of Huntington's disease.[134] It is indeed striking that this seemingly simple monovalent cation that has been used clinically for decades has been shown to exert neuroprotective effects in these preclinical models.

In addition to its effects on bcl-2, the effects of lithium on other signaling pathways and transcription factors[40,41,52,53] may also contribute to its neuroprotective effects. Perhaps foremost among these is glycogen synthase kinase-3β (GSK-3β). GSK-3β is an evolutionarily highly conserved enzyme known to play a critical role in development as well as in the mature CNS by regulating various cytoskeletal events and gene expression. It is now clear that lithium, at concentrations similar to those attained clinically, is an inhibitor of GSK-3β.[135] Since GSK-3β has been demonstrated to contribute to apoptotic cell death[136] and appears to play a role in the hyperphosphorylation of *tau* observed in Alzheimer's disease, there has been considerable recent excitement about the possibility of developing novel GSK-3β inhibitors not only for the treatment of recurrent major depression, but potentially also for more classic neurodegenerative diseases.[40,41,43]

Does Lithium Affect Neurogenesis?

The demonstration that neurogenesis occurs into senescence in the human brain has generated considerable excitement about the possibility of pharmacologically treating disease-related neuronal atrophy or loss.[137,138] The localization of pluripotent progenitor cells and neurogenesis occurs in restricted brain regions. The greatest density of new cell birth is observed in the subventricular zone and the subgranular layer of the hippocampus. Cells born in the subventricular zone migrate largely to the olfactory bulb, and those born in the subgranular zone migrate into the granule cell layer. A large number of the newborn daughter cells are known to die rapidly, likely via apoptosis.[138] Thus, increasing bcl-2 levels could enhance the survival of the newborn cells, allowing them to differentiate into neurons. Additionally, bcl-2 has been shown to have robust effects on the regeneration of CNS axons.[139] In view of bcl-2's major neuroprotective and neurotrophic role, a study[91] was undertaken to determine if lithium, administered at therapeutically relevant concentrations, affects neurogenesis in the adult rodent brain. (Kempermann and Gage[138] have suggested the use of *neurogenesis* to refer to a series of events [including proliferation of a neuronal precursor or stem cell and survival of the daughter cells] that result in the appearance of a new neuron.) To investigate the effects of chronic lithium administration on neurogenesis, mice were treated with "therapeutic" lithium (mean ± SD plasma levels = 0.97 ± 0.20 mM) for ~4 weeks. After treatment with lithium for 14 days, the mice were administered single doses of BrdU (bromodeoxyuridine, a thymidine analog that is incorporated into the DNA of dividing cells) for 12 consecutive days. Lithium treatment continued throughout the duration of the BrdU administration. Following BrdU immunohistochemistry, 3-dimensional cell counting was performed using a computer-assisted image analysis system. This system is based on the optical disector method and estimates the number of cells, independent of section thickness and cell shape. It was found that chronic lithium administration does, indeed, result in an increase in the number of BrdU-positive cells in the dentate gyrus.[91] Moreover, approximately two thirds of the BrdU-positive cells also double-stained with the neuronal marker NeuN, confirming their neuronal identity. Double-staining of BrdU and bcl-2 was also observed, and studies using bcl-2 transgenic animals are currently under way to delineate the role of bcl-2 overexpression in mediating the effects of lithium on hippocampal neurogenesis.

Can Lithium's Neurotrophic and Neuroprotective Effects be Demonstrated Longitudinally in the Human Brain in Vivo?

Although a considerable body of preclinical data now demonstrates neurotrophic and neuroprotective effects of lithium in preclinical models, it has not been clear if lithium can actually exert similar neurotrophic effects in the *human brain* in vivo. A longitudinal clinical study[140] was undertaken, using proton MRS to quantitate *N*-acetylaspartate (NAA) levels. NAA has been proposed to represent a putative marker of neuronal viability and has been utilized to follow the course of neurodegenerative disorders. It was found that chronic lithium administration at therapeutic doses increased NAA concentration in the human brain in vivo. Furthermore, a striking correlation (~0.97) was observed between lithium-induced NAA increases and regional voxel gray matter content. A follow-up study[141] was undertaken to determine if lithium-induced increases in bcl-2 would also lead to neuronal increases, and thus to increased brain gray matter volume in patients with manic depressive illness. Brain tissue volumes were determined using high-resolution 3-dimensional MRI at baseline (medication-free, after a minimum 14-day washout), and then repeated after 4 weeks of lithium at therapeutic doses. This study showed that chronic lithium significantly increases total gray matter content in the human brain of patients with manic depressive illness. No significant changes were observed in brain white matter volume or in quantitative measures of regional cerebral water content, thereby providing strong evidence that the observed increases in gray matter content are likely owing to neurotrophic effects as opposed to any possible cell swelling or osmotic effects associated with lithium treatment.

CONCLUSION

As we have discussed, the complexity of the biological and behavioral features of mania and depression has resulted in a vast, diverse compendium of corresponding data that have contributed to the understanding of the pathophysiology of bipolar disorder at different biochemical and neurobiological levels. Although preclinical models and clinical studies are challenging to design so that

they mirror the variability of the illness in terms of its acute and chronic nature, episodic and cycling clinical status, and associated morbidity, fairly robust results have been obtained for a variety of systems. Neurotransmitter dysfunction has been confirmed in biochemical, physiologic, and neuroanatomical studies in patients and animal models and has become a target for pharmacologic intervention. Abnormalities in signal transduction cascades are also strongly supported in the underlying neurobiology of the disorder because of their pivotal biological role in areas of the brain associated with the disease process, their similarity with known diseases with etiologic aberrant signaling function, and their experimental findings during drug modulation. More recently, application of new investigational approaches to study the temporal and spatial pattern of gene expression changes has revealed a potential role for neurotrophic and neuroprotective factors both in the pathophysiology of disease progression and in optimal long-term treatment by mood stabilizers.[40,44,142]

Drug names: carbamazepine (Tegretol and others), clozapine (Clozaril and others), lamotrigine (Lamictal), olanzapine (Zyprexa), risperidone (Risperdal), tamoxifen (Nolvadex), topiramate (Topamax).

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