Clinical studies over the past 40 years have attempted to uncover the biological factors mediating the pathophysiology of bipolar disorder (BD) utilizing a variety of biochemical and neuroendocrine strategies. These studies have for the most part rested upon the conceptual foundation that monoamine signaling and hypothalamic–pituitary–adrenal axis disruption are integral to the pathophysiology of both depression and mania (Bowden 1997; Goodwin and Jamison 1990). Although such investigations have been heuristic over the years, they have been of limited value in elucidating the unique biology of this affective disorder, which must include an understanding of the underlying basis for the predilection to episodic and often profound mood disturbance that can become progressive over time. Kindling and progressive sensitization to seizures, taken from the epilepsy literature, has been very useful as a model for focusing attention to the long-term course of the illness, but thus far has been less useful in explicating the biological processes leading to instability in the regulation of mood; however, recent research characterizing the contributory roles of signaling pathways in various facets of kindling and sensitization holds promise in this regard. More recent research strategies designed to uncover the molecular mechanisms underlying our pharmacologic treatments coupled with more advanced brain imaging studies remain promising approaches as we enter the next millennium.

We should also keep in mind that a true understanding of the pathophysiology of BD must address its neurobiology at different physiologic levels (i.e., molecular, cellular, systems, and behavioral; Figure 1). Abnormalities in gene expression undoubtedly underlie the neurobiology of the disorder at the molecular level, and this will become evident as we identify the susceptibility and protective genes for BD in the coming years. Once this has been accomplished, however, the even more difficult work of examining the impact of the faulty expression of these gene products (proteins) on integrated cell function must begin. It is at these levels that we have identified some protein candidates using the psychopharmacologic strategies noted above and that will be more fully elucidated below. The precise manner in which these candidate molecular and cellular targets may or may not relate to the faulty expression of susceptibility gene products is yet to be determined. The task becomes even more daunting when one considers the possibility that a major component of the pathophysiology of BD may stem from discordant
biological rhythms ranging from ultradian to infradian that ultimately drive the periodic recurrent nature of the disorder (Bunney and Bunney 2000; Ehlers et al. 1988; Ikonomovic and Manji 1999; Lenox and Manji 1998; Mandell et al. 1984; Wehr and Goodwin 1983). The subsequent challenge for the basic and clinical neuroscientist will be the integration of these molecular/cellular changes to the systems and ultimately to the behavioral level, wherein the clinical expression of BD becomes fully elaborated.

Before critically reviewing the data relevant to the pathophysiology of BD, it is necessary to consider the complexity of our task (Table 1). To begin with, we would suggest that altered expression of critical proteins resulting from a series of susceptibility genes predisposes to a dysregulation of signaling in regions of the brain predisposing to periodic loss of homeostasis and clinical manifestation of affective symptomatology (i.e., mania and/or depression; Depue et al. 1987; Goodwin and Jamison 1990). Thus the biological processes underlying the risk for mood cycling may be very distinct from the biology driving the clinical symptoms of mania or depression (Goodwin and Jamison 1990). In this regard it should be noted that there is increasing evidence for a shared genetic risk for both bipolar and unipolar disorders (Berrettini 2000), suggesting that the underlying pathophysiologic processes predisposing to recurrent mood disturbance may share common features. Furthermore, the clinical picture and system response are the result of a complex dynamic interaction between the dysregulated signaling systems and activation of existing physiologic feedback mechanisms designed to compensate for extreme changes (Post and Weiss 1999). In this way, the constellation of symptoms including not only mood but also autonomic, endo-

Table 1. The Pathophysiology of Bipolar Disorder: Constraints for Experimental Design

- Complex disease with diagnostic heterogeneity
- Episodic nature of symptoms and distinct symptom clusters
- The biology underlying recurrence/cyclicity may be distinct from that responsible for specific symptom clusters
- Disease progression dictates changes over the course of the illness
- Dynamic interaction between compensatory/adaptive changes in the brain and primary neurobiology of the disorder
- Effect of treatment and treatment withdrawal on measures
- Potential circadian rhythm abnormalities suggest that single time-point studies may be inadequate
- Relative inaccessibility of target organ and dependence on peripheral models
- Lack of suitable animal models
- The characterization of “mood” as a quantitative trait (quantitative trait locus analysis) has not yet been accomplished

Figure 1. The pathophysiology of bipolar disorder (BD). This figure highlights the fact that a complete understanding of the pathophysiology of BD must address its neurobiology at different physiologic levels (i.e., molecular, cellular, systems, and behavioral). PKC, protein kinase C; MARCKS, myristoylated alanine-rich C kinase substrate; GSK-3, glycogen synthase kinase-3; MAP kinase, mitogen-activated protein kinase; Bcl-2, B-cell leukemia/lymphoma; proteome, the population of cellular protein species and their expression level; transcriptome, the population of cellular messenger RNA species and their expression level.
crine, sleep/wake, and circadian activity determinants will reflect not only the stage and progression of illness, but also the unique individual characteristics conferring heterogeneity in clinical presentation and diagnosis. In light of this complexity and the dynamic properties of the system, research strategies examining biochemical and endocrine variables would be expected to be subject to a high degree of inherent variability not only when using cross-sectional analyses between patients, but also when utilizing longitudinal designs over time within individual patients. Furthermore, the use of peripheral sources of tissue and postmortem brains to address biochemical/neuroendocrine activity within patients’ brains introduces another set of variables inherent in the experimental design and most often conferring significant constraints on data interpretation.

Classical Monoaminergic Neurotransmitter and Neuroendocrine Systems

The stimulus for the study of the biogenic amines in patients with BD was provided by the discovery of effective pharmacologic treatments for depression and mania (Goodwin and Jamison 1990). Although these pharmacologic agents have prominent effects on single episodes of depression and mania, even more pertinent to our discussion are the major effects that these agents exert on the long-term course of the illness (Goodwin and Jamison 1990; Manji and Potter 1997). Antidepressants in general, and tricyclics in particular, may increase the frequency of cycles and worsen long-term outcome (Wehr and Goodwin 1987). In addition to this compelling pharmacologic data, the biogenic amine neurotransmitter systems are distributed extensively in the limbic system, which is implicated in the regulation of sleep, appetite, arousal, sexual function, endocrine function, and emotional states such as fear and rage. The clinical picture of BD involves disruption of behavior, circadian rhythms, neurophysiology of sleep, and neuroendocrine and biochemical regulation within the brain (for reviews, see Goodwin and Jamison 1990; Holsboer 1995). Thus it is not surprising that clinical research strategies over the years have identified dysregulation of noradrenergic, dopaminergic, serotonergic, and cholinergic systems as well as the hypothalamic–pituitary axis in patients with BD. The behavioral and physiologic manifestations of the illness are complex and undoubtedly mediated by a network of interconnected neurotransmitter pathways (Lenox 1987; Manji 1992); the monoamine neurotransmitter systems are ideally placed to mediate such complex behavioral effects, and thus have represented attractive candidate systems underlying the pathophysiology of BD. The multiple functions of central neurotransmitter/neuroendo-

Table 2. Bipolar Disorder: A Putative Role for Signaling Pathways

- Regulate the functional balance and multiple neurotransmitter systems
- Dynamic regulation of complex signaling networks forms the basis for higher order brain function mediating mood and cognition
- Critical role in fine-tuning of signals (amplifiers, attenuators, and integrators of multiple signals)
- Critical role in maintaining cellular “memory” and long-term neuroplastic events
- Major targets for the actions of hormones, including gonadal steroids, thyroid hormones, and glucocorticoids
- Abnormalities are compatible with life (e.g., a variety of human diseases are from defects in G protein–coupled signal transduction pathways)
- Brain regional dysregulation and circumscribed symptomatology are indeed possible despite the ubiquitous expression of signaling proteins
- Signaling proteins have been identified as targets for medications that are most effective in the treatment of mood disorders

Signaling Networks: The Cellular Machinery Underlying Information Processing and Long-Term Neuroplastic Events

It is hardly surprising that abnormalities in multiple neurotransmitter systems and physiologic processes have been found in a disorder as complex as BD. Signal transduction pathways are in a pivotal position in the central nervous system (CNS), able to affect the functional balance between multiple neurotransmitter systems, and may therefore play a role in mediating the more “downstream” abnormalities in multiple neurotransmitter systems and physiologic processes. Moreover, recent research has clearly identified signaling pathways as therapeutically relevant targets for our most effective pharmacologic treatments (Table 2). Indeed, the molecular and cellular targets underlying lithium’s ability to stabilize an underlying dysregulation of limbic and limbic-associated function strongly suggest that abnormalities in signaling pathways may also play a critical role in the pathophysiology of BD.

Multicomponent, cellular signaling pathways interact at various levels, thereby forming complex signaling networks that allow the cell to receive, process, and respond to information (Bhalla and Iyengar 1999; Bourne and Nicoll 1993). These networks facilitate the integration of
signals across multiple time scales and the generation of distinct outputs depending on input strength and duration, and regulate intricate feed-forward and feedback loops (Weng et al 1999). These properties of signaling networks suggest that they play critical roles in cellular memory; thus, cells with different histories, and therefore expressing different repertoires of signaling molecules and transcription factors, interacting at different levels, may respond quite differently to the same signal over time. Given their widespread and crucial role in the integration, amplification, and fine-tuning of physiologic processes, it is not surprising that abnormalities in signaling pathways have now been identified in a variety of human diseases (Milligan and Wakelam 1992; Spiegel 1998; Weintraub 1995). Pertinent to the present discussion is the observation that a variety of diseases manifest relatively circumscribed symptomatology, despite the widespread, often ubiquitous expression of the affected signaling proteins.

Although complex signaling networks are likely present in all eukaryotic cells and control various metabolic, humoral, and developmental functions, they may be especially important in the CNS, where they serve the critical roles of first amplifying and “weighting” numerous extracellularly generated neuronal signals and then transmitting these integrated signals to effectors, thereby forming the basis for a complex information processing network (Bhalla and Iyengar 1999; Bourne and Nicoll 1993; Manji 1992). The high degree of complexity generated by these signaling networks may be one mechanism by which neurons acquire the flexibility for generating the wide range of responses observed in the nervous system (Table 2). These pathways are thus undoubtedly involved in regulating such diverse vegetative functions as mood, appetite, and wakefulness and are therefore likely to be involved in the pathophysiology of BD. We now turn to a discussion of the direct and indirect evidence supporting a role for abnormalities in signaling pathways in the pathophysiology of BD.

Alterations of Transmembrane Cellular Signaling Pathways in Animal Models of BD

The need to use caution in the appropriate application of animal models to an understanding of complex neuropsychiatric disorders has been well articulated (Einat et al, in press; Post and Weiss 1999), and in fact it is unlikely we will ever develop animal models that display the full range of symptomatology as clinically expressed in man. Two current models that have had reasonable heuristic value in the study of mood disorders are kindling and behavioral sensitization (Einat et al, in press; Post and Weiss 1999). In both of these paradigms, there is increased responsivity (either behavioral or electrophysiologic) to repeated “low dose” stimulation (pharmacologic or electric) over time. Indeed, the sensitized behavioral response has been reported to persist for months and years after discontinuation of drug administration. Considerable evidence implicates long-term alterations in midbrain dopaminergic transmission in the development of behavioral sensitization, but the cellular mechanism(s) underlying the long-term changes in excitability observed in kindled or stimulant-sensitized animals have not been fully elucidated. In recent years, an appreciation of the major role of signal transduction pathways in the regulation of neuronal excitability has led to extensive research into their involvement in kindling and behavioral sensitization, and a growing body of evidence implicates alterations in both protein kinase C (PKC; Table 3) and certain G proteins (especially G_i and G_o). Studies have demonstrated that pertussis toxin (which inactivates G_i and G_o) produces a significant augmentation of psychostimulant-induced motor activity, and dopamine release in the nucleus accumbens (Chen et al 1999; Steketee and Kalivas 1991). In keeping with these studies, specific decreases in G\textsubscript{o} levels have been observed in response to chronic cocaine in the ventral tegmental area and nucleus accumbens (Nestler et al 1990), once again consistent with the hypothesis that regulation of G proteins represents part of the biochemical changes that underlie the effects of chronic psychostimulants. Similarly, injection of pertussis toxin into the amygdala of kindled rats markedly modifies kindled seizures, effects that appear to be mediated via an alteration in the after-discharge threshold.

Table 3. Protein Kinase C: Pathophysiology and Treatment of Bipolar Disorder

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>KINDLING</td>
<td>Produces dramatic increases in membrane-associated PKC in the hippocampus and amygdala</td>
</tr>
<tr>
<td>AMPHETAMINE</td>
<td>Produces increases in PKC activity, and GAP-43 phosphorylation (implicated in neurotransmitter release)</td>
</tr>
<tr>
<td>PKC INHIBITORS</td>
<td>Block the biochemical and behavioral responses to amphetamine and cocaine and also block cocaine-induced sensitization</td>
</tr>
<tr>
<td>DEXAMETHASONE ADMINISTRATION</td>
<td>Increases PKC activity, and increases the levels of PKCα and PKCɛ in the rat frontal cortex and hippocampus</td>
</tr>
<tr>
<td>INCREASED MEMBRANE/ CYTOSOL PKC PARTITIONING</td>
<td>Platelets from manic subjects; normalized with lithium treatment</td>
</tr>
<tr>
<td>INCREASED PKC ACTIVITY AND TRANSLATION</td>
<td>Bipolar disorder brains, as compared with control subjects</td>
</tr>
<tr>
<td>LITHIUM AND VALPROATE</td>
<td>Regulate PKC activity, PKCα, PKCɛ, and MARCKS</td>
</tr>
<tr>
<td>PRELIMINARY DATA</td>
<td>Suggest that PKC inhibitors may have efficacy in the treatment of acute mania</td>
</tr>
<tr>
<td>TYPICAL AND ATYPICAL ANTI-PsYCHOTICS</td>
<td>Regulate the levels of PKCα and PKCɛ in areas of the rat brain</td>
</tr>
</tbody>
</table>

PKC, protein kinase C; GAP, growth cone–associated protein; MARCKS, myristoylated alanine-rich C kinase substrate.
With respect to the PKC signaling system, dramatic increases in membrane-associated PKC have been observed in the bilateral hippocampus up to 4 weeks after the last kindled seizure and in the amygdala/pyriform cortex at 4 weeks after (Daigen et al 1991). Studies have also implicated alterations in PKC activity as mediators of long-term alterations in neuronal excitability in the brain following chronic stimulant use. Thus, both acute and chronic amphetamine produce an alteration in PKC activity, its cytosol relative to membrane distribution, and the phosphorylation of a major PKC substrate, growth cone–associated protein 43 (GAP-43), which has been implicated in long-term alterations of neurotransmitter release (Kantor and Gnegy 1998). Furthermore, PKC inhibitors have been shown to block the acute responses to both amphetamine and cocaine (as assessed by both behavioral and in vivo microdialysis studies) as well as cocaine-induced sensitization (Kantor and Gnegy 1998).

It is indeed striking that behavioral sensitization and kindling produce robust alterations in the PKC signaling pathway in critical limbic structures, since lithium and valproate (VPA) also target the very same biochemical targets (see below). Thus, although considerable caution clearly needs to be employed when extrapolating from the findings observed in these two models, the fact that they are associated with effects on PKC signaling that are the opposite of those observed with chronic lithium or VPA is compelling indeed.

Are G Protein–Coupled Signal Transduction Pathways Targets for Mood-Stabilizing Agents?

The cAMP/Protein Kinase A Signaling Pathway as a Target for the Actions of Mood Stabilizers and Antidepressants

As discussed above, dysregulation of cellular signaling networks in the brain likely underlies the oscillations in behavioral states observed in mood disorders. Thus, it is not surprising that increasingly recent research on the cellular mechanisms underlying lithium’s therapeutic effects has focused on the transmembrane signaling pathways in the brain (for reviews, see Lenox and Manji 1998; Mork et al 1992; Post et al 2000).

Lithium and the AC System

It has been demonstrated that lithium exerts complex effects on the activity of adenylyl cyclase (AC), with the preponderance of the data demonstrating an elevation of basal AC activity but an attenuation of variety of receptor-mediated responses (Jope 1999a, 1999b; Manji et al 2000; Wang et al 1997). Lithium in vitro inhibits the stimulation of AC both by Gpp(NH)p (a poorly hydrolyzable analog of guanosine triphosphate [GTP]) and by Ca2+ /calmodulin, suggesting that lithium in vitro is directly able to inhibit the catalytic unit of AC (Mork et al 1992; Newman and Belmaker 1987). Since these inhibitory effects of lithium in vitro can be overcome by Mg2+, it has been postulated that they are mediated (at least in part) by a direct competition with magnesium (whose hydrated ionic radius is similar to that of lithium) for a binding site on the catalytic unit of AC (Mork and Geisler 1989; Mork et al 1992; Newman and Belmaker 1987); however, the inhibitory effects of chronic lithium treatment on rat brain AC are not reversed by Mg2+ and still persist after washing of the membranes, but are reversed by increasing concentrations of GTP (Mork and Geisler 1989; Mork et al 1992; Newman and Belmaker 1987). These results suggest that the effects of chronic lithium (those that are more likely to be therapeutically relevant) may be exerted at the level of signal-transducing G proteins at a GTP-responsive step (discussed below). These two distinct actions of lithium on the AC system may explain the differences in results that have been obtained by investigators using rat membrane preparations and those obtained with the use of rat slice preparations (Lenox and Manji 1998). This has led to an investigation of lithium’s effects on the AC system in vivo, using microdialysis. These studies found that chronic lithium treatment produced a significant increase in basal and post–receptor stimulated (cholera toxin or forskolin) AC activity, while attenuating the β-adrenergic–mediated effect (Manji et al 2000; Masana et al 1992). Interestingly, chronic lithium treatment resulted in an almost absent cyclic adenosine monophosphate (cAMP) response to pertussis toxin, suggesting a lithium-induced attenuation of G1 function. It should be noted, however, that chronic lithium has been found to increase not only cAMP levels (Wiborg et al 1999), but also the levels of AC type I and type II messenger RNA (mRNA) and protein levels in the frontal cortex (Colin et al 1991; Jensen et al 2000), suggesting that lithium’s complex effects on the system may represent the net effects of direct inhibition of AC, upregulation of AC subtypes, and effects on the stimulatory and inhibitory G proteins. Most recently, lithium’s effects on the phosphorylation and activity of cyclic AMP response element–binding protein (CREB) have been examined in the rodent brain and in cultured human neuroblastoma cells, with somewhat conflicting results (Ozaki and Chuang 1997; Wang et al 1999).

A series of studies have also examined lithium’s effects on AC in humans. In a longitudinal study of healthy volunteers (utilized to overcome the potentially confounding, significant effects of alterations in mood state–dependent biochemical and neuroendocrine parameters), 2 weeks of lithium administration was found to significantly
increase platelet basal and post-receptor stimulated AC activity (Risby et al 1991), effects that are strikingly similar to those observed in the rodent brain. Consistent with a lithium-induced increase in basal cAMP and AC levels, a more recent study found that platelets obtained from lithium-treated euthymic bipolar patients exhibited enhanced basal and cAMP-stimulated phosphorylation of Rap1 (a protein kinase A [PKA] substrate), as well as another unidentified 38-kd phosphoprotein (Perez et al 2000a). Somewhat surprisingly, these investigators did not find similar effects of lithium in healthy subjects.

**Carbamazepine and the AC System**

The effects of carbamazepine (CBZ) on the AC system have been less extensively studied. It is noteworthy, however, that CBZ has been reported to decrease not only basal and stimulated cAMP production in rodents, but also cerebrospinal fluid levels of cAMP in manic patients (Post et al 2000). Recent studies have found that CBZ, at therapeutically relevant concentrations, inhibits both basal AC and FSK-stimulated cAMP accumulation in C6 glioma cells (Chen et al 1996). Furthermore, CBZ also inhibits both basal and FSK-stimulated activity of AC purified from the rat cortex, suggesting that CBZ inhibits cAMP production by acting directly on AC and/or through factor(s) that are tightly associated with and copurify with AC (Chen et al 1996). Carbamazepine’s effects on cAMP-mediated transcriptional events have also been investigated, and CBZ has been found to inhibit forskolin-stimulated phosphorylation of CREB (Chen et al 1996) and c-fos expression (Divish et al 1991). Together, the results suggest that CBZ brings about a dampening of the AC system; these effects may play a role in both its antiepileptic and its antimanic effects, but such a contention requires extensive further validation.

**Antidepressants and the AC System**

The chronic administration of antidepressants (ADs) is known to bring about a variety of biochemical effects, including an enhanced coupling between Go, and the catalytic unit of AC (Rasenick et al 2000). Several studies have also demonstrated that the postreceptor components of the cAMP system are regulated by long-term AD treatments, including cAMP-dependent protein kinase enzyme activity (Nestler et al 1989; Popoli et al 2000). Taken together, these results suggest that ADs, via their complex effects on G proteins and the AC signaling system, may attenuate β-adrenergic–mediated activation of Gi, while enhancing the effects of agents operating by pathways independent of the β-adrenergic receptor (ßAR). Consistent with these results, ADs have been demonstrated to activate cAMP-dependent and calcium/calmodulin-dependent protein kinases, effects that are accompanied by increases in the endogenous phosphorylation of selected substrates (microtubule-associated protein 2 and synaptotagmin; Popoli et al 2000). Furthermore, recent data from Duman and associates (1997) have demonstrated that the chronic treatment of rats with a variety of ADs increases the levels of CREB mRNA, CREB, and CRE DNA binding activity in the hippocampus. The same laboratory has demonstrated that chronic AD treatment also increases the expression of two important genes known to be regulated by CREB—namely, brain-derived neurotrophic factor and its receptor trkB (Duman et al 1997). Preliminary postmortem human brain studies have also revealed increased CREB levels in patients treated with ADs, providing indirect support for the rodent and cell culture studies (Dowlatshahi et al 1998).

**Lithium and G Proteins**

Abundant experimental evidence has shown that lithium attenuates receptor-mediated second messenger generation in the absence of consistent changes in the density of the receptors themselves (Lenox and Manji 1998; Mork et al 1992). There is now considerable evidence that chronic lithium administration affects G protein function (Jope 1999a, 1999b; Lenox and Manji 1998; Wang and Friedman 1999); however, the preponderance of data suggests that lithium, at therapeutically relevant concentrations, does not have any direct effects on G proteins and does not consistently alter G protein subunit levels (Ellis and Lenox 1991; Lenox and Manji 1998). Although some studies have reported modest changes in the levels of G protein α subunits, the preponderance of the data suggests that the effects of chronic lithium on signaling pathways occur in the absence of changes in the levels of G protein subunits per se (Lenox and Manji 1998); however, chronic in vivo lithium treatment has been shown to produce a significant increase in pertussis toxin–catalyzed [32P]adenosine diphosphate ([32P]ADP) ribosylation in the rat frontal cortex. Since pertussis toxin selectively adenylates the undissociated, inactive αβγ heterotrimeric form of Gi, these results suggest that lithium attenuates Gi function via a stabilization of the inactive conformation. These observations 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 (Jope 1999b). Consistent with this hypothesis, lithium has been shown to potentiate the behavioral hyperactivity induced by intra-accumbens choleratoxin administration (which activates the stimulatory G proteins, Gi and Go; Kofman et al 1998).

Recent studies have also examined the effects of chronic lithium on G protein function in humans and have
generally observed reduced receptor/G protein coupling (Lenox and Manji 1998; Risby et al 1991). The effects of 2 weeks of lithium administration on G protein measures has also been examined in healthy volunteers. Similar to the findings in the rat brain, lithium did not affect the levels of platelet G protein α subunits, but produced a significant increase in pertussis toxin–catalyzed [32P]ADP ribosylation, once again suggesting a stabilization of the inactive dissociated αβγ heterotrimeric form of Gi. These long-term effects of chronic lithium on G protein are 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 (Young and Woods 1996) and in the rat brain (Nestler et al 1995), suggesting another novel mechanism by which chronic lithium may indirectly regulate the activity of these critical signaling proteins.

The Protein Kinase C Signaling Cascade

Over the last decade there have been major advances in our understanding of the critical role of the PKC signaling pathway as a therapeutically relevant target for the long-term actions of mood stabilizers. The “inositol depletion hypothesis” posited that lithium, as an uncompetitive inhibitor of inositol-1-phosphatase, produced its therapeutic effects via a depletion of neuronal myo-inositol (mI) levels. Although this hypothesis has been of great heuristic value, numerous studies have examined the effects of lithium on receptor-mediated phosphoinositide (PI) responses, and although some report a reduction in agonist-stimulated phosphatidylinositol 4,5-bisphosphate (PIP2) hydrolysis in rat brain slices following acute or chronic lithium, these findings have often been small and inconsistent, and subject to numerous methodological differences (Jope and Williams 1994; Lenox and Manji 1998). Most recently, a magnetic resonance spectroscopy study has demonstrated that lithium-induced mI reductions are observed in the frontal cortex of BD patients after only 5 days of lithium administration, at a time when the patients’ clinical state is completely unchanged (Moore et al 1999). Consequently, these and other studies suggest that whereas inhibition of inositol monophosphatase may represent an initiating lithium effect, reducing mI levels per se is not associated with therapeutic response. This led to our working hypothesis that some of the initial actions of lithium may occur with a relative reduction of mI—this reduction of mI 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 (Table 3). Currently available data suggest that acute lithium exposure facilitates a number of PKC-mediated responses, whereas longer exposure results in an attenuation of phorbol ester–mediated responses, which is accompanied by a downregulation of specific PKC isoforms (discussed in Manji and Lenox 1999). Studies in rodents have demonstrated that chronic (but not acute) lithium produces an isozyme-selective reduction in PKC α and ε in the frontal cortex and hippocampus, in the absence of significant alterations in the β, γ, δ, or ζ isoforms. Concomitant studies carried out in immortalized hippocampal cells in culture exposed to chronic lithium show a similar reduction in the expression of both the PKC α and ε isoforms in the cell as determined by immunoblot (Chen et al, in press; Manji and Lenox 1999).

A major strategy that has been utilized to investigate the downstream consequences of lithium-induced alteration in PKC isoforms is the examination of the effects of chronic lithium on endogenous PKC substrates in the brain. The most prominent substrate for PKC in the 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 (1992) provided the first evidence for the effects of chronic administration of lithium on the regulation of MARCKS in the rat brain. They demonstrated that chronic lithium administration dramatically reduced MARCKS expression in the hippocampus, effects that were 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 upon both the inositol concentration and the level of receptor-mediated activation of PI hydrolysis (Watson and Lenox 1996). Although these effects of lithium on PKC isoforms and MARCKS are striking, a major problem inherent in neuropharmacologic research is the difficulty in attributing therapeutic relevance to any observed biochemical finding. It is thus noteworthy that the structurally dissimilar antimanic agent VPA produces effects strikingly similar to those of lithium on PKC isoforms and on the expression of the MARCKS protein (Manji and Lenox 1999; Watson et al 1998).

Are There Abnormalities in Signaling Pathways in BD?

The cAMP-Generating System

The data reviewed above strongly suggest that regulation of cellular signaling transduction pathways play a major
role in the therapeutic effects of lithium and VPA. Do these clinically effective agents, in fact, correct an underlying abnormality in signaling pathways in BD? The future development of selective receptor and second messenger ligands for positron emission tomography studies may permit the direct assessment of CNS receptor and postreceptor sensitivity in humans. To date, however, studies of receptor and postreceptor function in mood disorders have been limited to indirect research strategies. The most commonly utilized strategy has been to characterize receptor function in readily accessible blood elements, and much clinical research has focused on the activity of the 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 the absence of consistent alterations in the number of receptors themselves (for a review, see Wang et al. 1997). A consistently observed decrease in leukocyte βAR function in depression could reflect an inherited abnormality of the βAR/Gs/AC complex, as suggested by the findings utilizing immortalized (Epstein–Barr virus–transformed) lymphocytes (Wright et al. 1984). These findings need to be replicated, however, and a number of additional confounding factors need to be considered. In this context, twin studies suggest that variations in isoproterenol-stimulated cAMP production are most likely due to “environmental” effects on the number or sensitivity of βARs (Ebstein et al. 1986). A recent study found higher levels of cAMP-stimulated phosphorylation of a ~22-kd protein in platelets obtained from 10 treated euthymic BD patients, as compared with healthy subjects; by contrast, there was no significant difference in the basal phosphorylation between the groups (Perez et al. 2000a, 2000b). Follow-up studies identified the ~22-kd protein as Rap1, and once again found higher cAMP-stimulated phosphorylation in the BD patients (Perez et al. 2000a, 2000b).

Warsh and associates have undertaken the most thorough series of studies investigating the cAMP/PKA system in postmortem brains of BD patients. Due to the instability of cAMP in postmortem brain tissue, this research group has investigated downstream targets of cAMP. They found that the levels of PKA regulatory subunits (as assessed by [3H]cAMP binding) were significantly lower in cytosolic fractions of BD frontal, temporal, occipital, and parietal cortices; cerebellum; and thalamus, as compared with matched control subjects (Rahman et al. 1997). Furthermore, preliminary findings show that the reduction of regulatory subunits of PKA in the cytosolic fractions of the BD temporal cortex is accompanied by a higher basal kinase activity and significantly lower apparent activation constant for cAMP in the cytosolic fractions of the BD temporal cortex (Fields et al. 1999). Whether such changes result in altered endogenous cAMP-stimulated phosphorylation in the brain, similar to what has been reported in platelets from euthymic BD patients (Perez et al. 2000a, 2000b), remains to be demonstrated. Nevertheless, these observed changes in PKA provide additional important evidence for a potential dysregulation of the Goαs-mediated cAMP signaling pathway in BD.

G Proteins in Mood Disorders

In view of the indirect evidence for abnormalities at postreceptor sites described above, it is not surprising that several independent laboratories have examined G proteins in patients with mood disorders (Chen et al. 1999; Mathews et al. 1997; Wang et al. 1997; Warsh et al. 2000). Young and associates (1993) were the first to report increased levels of Goαs in BD. Compared with control subjects matched with respect to age, postmortem interval, and brain pH, they found increased levels of Goαs in the frontal, temporal, and occipital cortices (but not in the hippocampus, thalamus, or cerebellum) in postmortem brain tissue from patients with BD. This group also found increases in forskolin-stimulated AC activity in postmortem brains of BD patients, compatible with enhanced Gs/AC signaling in BD (Warsh et al. 2000). The findings of elevated Goαs levels and/or function are also supported by the recent observations of Friedman and Wang (1996), who found increased agonist-activated [35S]GTPγS binding to G protein α subunits in frontal cortical membrane preparations from BD patients. Several studies have also examined G proteins in peripheral circulating cells and have found elevated levels of Goαs protein (Chen et al. 1999; Wang et al. 1997; Warsh et al. 2000) and mRNA levels (Spleiss et al. 1998), although the dependency on clinical state remains unclear. Similar to what has been observed in the CNS, one recent study has evaluated the role of platelet G proteins as “signal coincidence detectors,” and has found this function to be impaired in depressed patients (Mooney et al. 1998). Overall, the most consistent finding to emerge is that in both peripheral cells and postmortem brain tissue from BD patients elevations are observed in the predominant subspecies of Goαs present in the tissues examined. It should be emphasized, however, that there is at present no evidence to suggest that the alterations in the levels of Goαs are due to a mutation in the Goαs gene itself. Indeed, there are numerous transcriptional and posttranscriptional mechanisms that regulate the levels of G protein α subunits (Warsh et al. 2000), and the elevated levels of Goαs could potentially represent the indirect sequelae of alterations in any one of these other biochemical pathways. Thus, at this point considerable caution is required in the interpretation of the data, since
they derive primarily from peripheral cell models and postmortem studies involving a small number of patients.

**Abnormalities of Calcium Signaling in BD**

Acting via intracellular proteins such as MARCKS and calmodulin, and enzymes such as PKC, AC, and Ca\(^{2+}\)/calmodulin-dependent 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 BD (discussed in Dubovsky et al 1992; Emamghoreishi et al 1997; Wang et al 1997). 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 BD. The calcium abnormalities have been postulated to represent state-dependent findings (Dubovsky et al 1992), but recent studies using transformed lymphoblasts from BD patients have revealed similar abnormalities, suggesting that they may be trait dependent (Emamghoreishi et al 1997). The regulation of free intracellular Ca\(^{2+}\) is a complex, multifaceted process involving extracellular entry, release from intracellular stores following receptor-stimulated PI hydrolysis, uptake into specific organelles, and binding to specific proteins. Thus, the abnormalities observed in BD could arise from abnormalities at a variety of levels, and recent studies suggest that the abnormality lies beyond the receptor (Hough et al 1999). Interestingly, recent studies have demonstrated that Rap1 phosphorylation state is related to platelet intracellular calcium signaling (Magnier et al 1995), suggesting a possible relationship between these two documented abnormalities. Since PKC is also known to regulate calcium signaling at multiple levels (Ozaki et al 1997; Shibata et al 1996; Si-Tahar et al 1996), more recent studies have investigated the putative role of PKC in mediating the calcium abnormalities in BD; preliminary analysis suggests that alterations in tonic PKC activity may play an important role in mediating the abnormal intracellular calcium responses observed in BD (H.K. Manji and R.M. Post, unpublished observations, June 1998).

**Are There Abnormalities of PKC Signaling in BD?**

To date, there have only been a limited number of studies directly examining PKC in BD. Friedman and associates (1993) investigated PKC activity and PKC translocation in response to serotonin in platelets obtained from BD subjects before and during lithium treatment. They reported that the ratios of platelets membrane-bound to cytosolic PKC activities were elevated in the manic subjects. In addition, serotonin-elicited platelet PKC translocation was found to be enhanced in those subjects. With respect to brain tissue, Wang and Friedman (1996) measured PKC isozyme levels, activity, and translocation in postmortem brain tissue from BD patients; they reported increased PKC activity and translocation in BD brains, as compared with control subjects, effects that were accompanied by elevated levels of selected PKC isozymes in cortices of BD subjects.

In view of the pivotal role of the PKC signaling pathway in the regulation of neuronal excitability, neurotransmitter release, and long-term synaptic events, it was postulated that the attenuation of PKC activity may play a role in the antimanic effects of lithium and VPA. In a pilot study it was found that tamoxifen (a nonsteroidal antiestrogen known to be a PKC inhibitor at higher concentrations) may, indeed, possess antimanic efficacy (Bebchuk et al 2000). These data further support the potential role of the PKC pathway in mediating the pathophysiology of BD. The data implicating the PKC signaling system in the pathophysiology of BD are particularly compelling in view of the growing body of data suggesting that this signaling pathway represents a therapeutically relevant target for both lithium and VPA (see above). The complex effects of lithium on the PKC signaling pathway represent an attractive and heuristic mechanism by which the expression of various proteins involved in long-term neuronal plasticity and cellular response is modulated, thereby compensating for as yet genetically undefined physiologic abnormalities in critical regions of the brain. It is our strong conviction that it is at the cellular and molecular level that some of the most exciting advances in our understanding of the pathophysiology of BD will take place in the coming years. Current studies of the long-term lithium-induced changes of the PKC signaling pathway (including PKC isozyme regulation, posttranslational modification of key phosphoproteins, and PKC-mediated alterations in gene and protein expression) are a most promising avenue for future investigation.

**Concluding Remarks**

As we have demonstrated, there is a considerable body of evidence both conceptually and experimentally in support of abnormalities in the regulation of signaling as integral to the underlying neurobiology of BD. The pathophysiology of this illness must account for not
only the profound changes in mood but also a constellation of neurovegetative features derived from dysfunction in limbic-related regions such as the hippocampus, hypothalamus, and brain stem. The highly integrated monoamine and prominent neuropeptide pathways are known to originate and project heavily within these regions of the brain, and it is thus not surprising that abnormalities have been noted in their function across clinical studies. In fact, the contribution of these pathways to the pathophysiology of BD must be reasonably robust, given the variability that might be expected in assessing such dynamic systems under the constraints in experimental design imposed upon such research. Whereas in the past much of the research effort has been focused upon identifying which of these systems might be etiologic in nature, we suggest that we might be better served by addressing their relative contributions in the system response to the underlying neurobiology of the disease process. This will become particularly important as we begin to identify the susceptibility genes for BD in the coming years. More recently, investigators have begun to identify components of signal transduction pathways in the brain such as PKC that may not only play a role in the pathophysiology of the disease but also represent targets for the action of our most efficacious treatments (i.e., lithium and VPA). This experimental strategy may prove to be most promising, since it provides data derived from the physiologic response of the system in affected individuals and addresses the critical dynamic intereraction with pharmacologic agents that effectively modify the clinical expression of the pathophysiology. It is also noteworthy that a growing body of data is demonstrating significant reductions in regional CNS volume and cell numbers (both neurons and glia) in BD (Drevets et al 1997; Manji et al, in press; Rajkowska et al 1999). Furthermore, it has been increasingly recognized that for many patients there is a progressive decline in overall functioning; the potential role of cell death/atrophy in determining long-term outcome remains to be elucidated. Nevertheless, it is noteworthy that psychopharmacologic research strategies have most recently identified long-term actions of these mood stabilizers in the regulation of expression of genes implicated in processes involved in neuroplasticity, neuroprotection, and even neurogenesis (Chen et al, in press; Lenox and Watson 1994; Manji et al 1999, in press; Moore et al, in press). To what extent these long-term processes in the brain are critical to the ability of these drugs to compensate for the pathophysiology of BD as well as stabilize the clinical course and progression of BD has yet to be defined (Manji et al 1999).

**References**


Wang HY, Friedman E (1999): Effects of lithium on receptor-


