# 79

# MECHANISM OF ACTION OF ANTIDEPRESSANTS AND MOOD STABILIZERS

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Bipolar disorder (BPD), the province of mood stabilizers, has long been considered a recurrent disorder. For more than 50 years, lithium, the prototypal mood stabilizer, has been known to be effective not only in acute mania but also in the prophylaxis of recurrent episodes of mania and depression. By contrast, the preponderance of past research in depression has focused on the major depressive episode and its acute treatment. It is only relatively recently that investigators have begun to address the recurrent nature of unipolar disorder (UPD) and the prophylactic use of longterm antidepressant treatment. Thus, it is timely that we address in a single chapter the most promising research relevant to the pharmacodynamics of both mood stabilizers and antidepressants.

As we have outlined in Fig. 79.1, it is possible to characterize both the course and treatment of bipolar and unipolar disorder in a similar manner. Effective treatments exist for the acute phases of both disorders; maintaining both types of patients on such drugs on a long-term basis decreases the likelihood and intensity of recurrences. Further, because the drugs are given long-term, they produce a cascade of pharmacologic effects over time that are "triggered" by their acute effects. Both classes of psychotropic drugs incur a lag period for therapeutic onset of action, even in the acute phase; therefore, studies during the past two decades have focused on the delayed (subchronic) temporal effects of these drugs over days and weeks. Consequently, it is widely thought that the delayed pharmacologic effects of these drugs are relevant for either the initiation of behavioral improvement or the progression of improvement beyond that initiated by acute pharmacologic actions. The early realization that lithium is effective prophylactically in BPD and

the more recent understanding that antidepressants share this property in UPD have focused research on long-term events, such as alterations in gene expression and neuroplasticity, that may play a significant role in stabilizing the clinical course of an illness. In our view, behavioral improvement and stabilization stem from the acute pharmacologic effects of antidepressants and mood stabilizers; thus, both the acute and longer-term pharmacologic effects of both classes of drugs are emphasized in this chapter.

# **MOOD STABILIZERS**

The term mood stabilizer within the clinical setting is commonly used to refer to a class of drugs that treat BPD. However, for the purpose of our discussion, it is important to differentiate the three clinical phases of BPD-acute mania, acute depression, and long-term prophylactic treatment for recurrent affective episodes. Although a variety of drugs are used to treat BPD (i.e., lithium, anticonvulsants, antidepressants, benzodiazepines, neuroleptics), we suggest that only a drug with properties of prophylaxis should be referred to as a mood stabilizer and included in this chapter. Significant evidence supports a therapeutic action for lithium, both in acute mania and prophylactically in a major subset of patients with BPD 1. However, the data for longterm prophylaxis with anticonvulsants (i.e., valproate, carbamazepine), although supported in part in clinical practice, remains less well established scientifically (see Chapter 77). In the absence of a suitable animal model, an experimental approach, used to ascribe therapeutic relevance to any observed biochemical finding, is the identification of shared biochemical targets that are modified by drugs belonging to the same therapeutic class (e.g., antimanic agents) but possessing distinct chemical structures (e.g., lithium and valproate). Although unlikely to act via identical mechanisms, such common targets may provide important clues

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**FIGURE 79.1.** Mood stabilizers and antidepressant actions: short-term and long-term events. Lithium and antidepressants have acute effects on synaptic signaling that serve to trigger progressively longer-term events in signal transduction; these in turn lead to changes in gene expression and plastic changes in brain. The acute affects in critical regions of the brain result in changes in certain behavioral and physiologic symptomatology (e.g., activation, sleep, appetite) that facilitate the acute clinical management of mania or depression. Subchronic effects lead to amelioration of symptoms related directly to mood, whereas it is thought that the longer-term (chronic) effects underlie the prophylactic properties of these drugs to prevent recurrent affective episodes in both unipolar and bipolar disorders.

about molecular mechanisms underlying mood stabilization in the brain. Thus, in our discussion, we use studies of lithium as a prototypal mood stabilizer and cross-reference evidence for the anticonvulsants when the data are available. Furthermore, it is important to note that drugs that are useful in the treatment of acute mania or depression may not necessarily have prophylactic properties (1) and, as in the case of antidepressants that are effective in treating BPD, may actually serve to destabilize the illness. Although it is likely that the targets for lithium action early in treatment trigger its long-term properties of mood stabilization, to what extent the biological mechanisms underlying longterm lithium prophylaxis contribute to the efficacy of lithium in acute mania remain to be demonstrated.

Studies through the years have proposed multiple sites for the action of lithium in the brain, and such research has paralleled advances in the field of neuroscience and the experimental strategies developed during the past half-century. For the most part, proper interpretation of these data has at times been limited by experimental design, which has often ignored not only the clinically relevant therapeutic range of concentrations and onset of action of lithium, but also critical control studies defining its specificity of action in comparison with other monovalent cations and classes of psychopharmacologic agents. While the targets for the action of lithium have shifted from ion transport and presynaptic neurotransmitter-regulated release to postsynaptic receptor regulation, to signal transduction cascades, to gene expression and neuroplastic changes in the neuropil, the research strategy has evolved from a focus on a class of neurotransmitter to the ability of the monovalent cation to alter the pattern of signaling in critical regions of the brain in a unique manner. It is in this context that we highlight the most current thinking regarding putative sites for the therapeutic action of lithium in the brain, which is heuristic and sets the stage for future research directions.

#### Ion Transport

Ion-gated channels, which are driven by either adenosine triphosphate (ATP) or the net free energy of transmembrane concentration gradients, regulate the distribution of lithium across the cell membrane. These transport systems are critical for the regulation of resting lithium in the bulk cytoplasm in that they regulate steady-state intracellular ion concentrations that set the threshold for depolarization in excitable cells. Lithium exchanges readily with sodium; however, by virtue of its high energy of hydration, it can also substitute for the divalent cations calcium and magnesium, which may account for some of its major biochemical sites of action. Much of the anticonvulsant properties of valproate, carbamazepine, and lamotrigine have been attributed to their ability to inhibit sustained repetitive firing by prolonging the recovery of voltage-gated sodium channels from inactivation (2). However, it is important to note that anticonvulsant activity appears to be neither necessary nor sufficient for mood stabilization because lithium has proconvulsant properties outside its narrow therapeutic range.

Although some membrane transport systems specifically recognize lithium and regulate its transmembrane concentration (e.g., a gradient-dependent Na-Li exchange process) (3,4), it is likely that the primary regulation of lithium is affected by transport systems that accept the lithium ion as a substitute for their normal ionic substrates. The Na, K-ATPase pump has been extensively studied in relation to the membrane transport of lithium and the therapeutic effect of lithium (see refs. 5 and 6 for review). Based on measurements of lithium in peripheral neurons and synaptosomal membrane fractions from brain, long-term lithium treatment was found to decrease Na, K-ATPase activity, particularly in hippocampus (7). Various groups have studied Na, K-ATPase activity in patients with mood disorders and have reported alterations in the erythrocyte-to-plasma ratio of lithium in patients with BPD as a function of clinical state and genetic loading. Despite the fact that clinical studies through the years have been constrained by relatively small and often variable findings, evidence has been found that Na, K-ATPase activity may be reduced, especially in the depressed phase of both UPD and BPD, and is associated with an increase in sodium retention (see refs. 1,6 for review). Furthermore, long-term lithium treatment has been observed to result in an increased accumulation of lithium and activity of Na, K-ATPase in erythrocyte membranes, with concomitant reduction of sodium and calcium within erythrocytes in patients with BPD. Because the concentration of free calcium ion tends to parallel the concentration of free sodium ion, this finding may account for observations that intracellular calcium is increased in patients with BPD (8). Interestingly, when patients with BPD were treated with lithium, Na, K-ATPase activity was found to be increased, consistent with observations of reduced Ca<sup>2+</sup> after treatment. However, such evidence from blood cells must be interpreted with caution; more recent data support the evolution of specific gene products for Na, K-ATPase expressed and uniquely regulated after translation, not only in neurons but in brain regions (9,10).

Although a balance of resting lithium conductance and net transport/efflux mechanisms regulates lithium homeostasis, the ligand gating of ion channels on the time scale of channel activity may play a more significant role in the regulation of intracellular lithium concentration within regulatory sites of an excitable cell such as the neuron. In the local environment of a dendritic spine, the surface area-tovolume ratio becomes relatively large, such that the lithium component of a synaptic current may result in significant (as much as fivefold to 10-fold) increases in intracellular lithium concentration following a train of synaptic stimuli (11). Such an activity-dependent mechanism for creating focal, albeit transient, increases of intracellular lithium at sites of high synaptic activity may play a role in the therapeutic specificity of lithium and its ability to regulate synaptic function in the brain.

# Neurotransmitter Signaling/Circadian Rhythm

In search of a link between the mechanism of action of lithium and neurotransmission, the effect of lithium has been extensively studied in virtually every neurotransmitter system. Earlier studies focused on the modulation of presynaptic components, including the synthesis, release, turnover, and reuptake of neurotransmitters. In recent years, the focus has shifted to postsynaptic events, such as the regulation of signal transduction mechanisms (see refs. 12-14 for review). Despite the fact that some of the results of the presynaptic and postsynaptic investigations are not in full agreement, at present the evidence supports the action of lithium at multiple sites that modulate neurotransmission. Lithium appears to reduce presynaptic dopaminergic activity and acts postsynaptically to prevent the development of receptor up-regulation and supersensitivity. In the cholinergic system, lithium enhances receptor-mediated responses at neurochemical, electrophysiologic, and behavioral levels. Long-term lithium treatment increases GABAergic inhibition and has been shown to reduce excitatory glutamatergic neurotransmission. It is of interest that valproate has been shown to enhance  $\gamma$ -aminobutyric acid (GABA) signaling, and the anticonvulsant lamotrigine has been shown to reduce glutamatergic neurotransmission. (2). It is currently thought that the effect of lithium on the spectrum of neurotransmitter systems may be mediated through its action at intracellular sites, with the net effect of long-term lithium attributed to its ability to alter the balance among neurotransmitter/neuropeptide signaling pathways.

One of the unique and most robust properties of lithium is its ability to lengthen the circadian period across species-unicellular organisms, plants, invertebrates, and vertebrates (including primates)-so that a phase delay in the circadian cycle often results (see refs. 15,16 for review). These effects are noted following long-term but not acute exposure and occur within the range of concentrations used in humans to treat BPD (0.6 to 1.2 mM). It has long been recognized that a dysregulation of circadian rhythms is associated with the clinical manifestation of recurrent mood disorders in patient populations (see refs. 17,18 for review). In fact, it appears to be the interaction between the circadian pacemaker and the sleep-wake cycle that determines variations in sleepiness, alertness, cognitive performance, and mood (19-22). The early morning awakening, shortened latency in rapid-eye-movement (REM) sleep, and advances in hormonal and temperature regulation of many depressed patients, including those with BPD, are thought by some investigators to indicate a phase advance of the central pacemaker within the suprachiasmatic nucleus of the hypothalamus relative to other internal oscillators or external zeitgebers (23-27). Lithium may achieve its therapeutic and prophylactic effects by altering the balance of neurotransmitter signaling in critical regions of the brain, such as the hypothalamus, and resynchronizing the physiologic systems underlying recurrent affective illness (1,28–30).

# **Signal Transduction**

#### Phosphoinositide Cycle

Since it was discovered that lithium is a potent inhibitor of the intracellular enzyme inositol monophosphatase (IMPase) ( $K_i = 0.8 \text{ mM}$ ), which converts inositol monophosphate to inositol (31,32), receptor G protein-coupled phosphoinositide (PI) hydrolysis has been extensively investigated as a site for the action of lithium as a mood stabilizer (see ref. 33 for review) (Fig. 79.2). The "inositol-depletion hypothesis" posited that lithium produces its therapeutic effects via a depletion of neuronal *myo*-inositol levels. Furthermore, because the mode of enzyme inhibition of IMPase is uncompetitive, likely through interaction with  $Mg^{2+}$  binding sites (34), the preferential site of action for lithium was proposed to be on the most overactive receptor-mediated neuronal pathways undergoing the highest rate of phosphatidylinositol 4,5 bisphosphate (PIP<sub>2</sub>) hydrolysis (35,36). It is also of interest that a number of structurally similar phosphomonoesterases that require magnesium have also been found to be inhibited by lithium at  $K_i$  values below 1 mM (37,38).

In cell systems and in cerebral cortical slices of chronically



**FIGURE 79.2.** Molecular targets for lithium in phosphoinositide (PI) signaling. Pathways depicted within the figure are three major sites for an inhibitory action of lithium: inositol 1-monophosphatase (IMPase); inositol polyphosphate 1-phosphatase (IPPase); and glycogen synthase kinase 3 $\beta$  (GSK-3 $\beta$ ). Inhibition of IMPase and IPPase can result in a reduction of *myo*-inositol (*myo*-Ins) and subsequent changes in the kinetics of receptor-activated phospholipase C (PLC) breakdown of phosphoinositide-4,5-bisphosphate to diacylglycerol (DAG) and inositol-1,4,5-trisphosphate. Alteration in the distribution of inositol phosphates can affect mechanisms mediating presynaptic release. DAG directly activates protein kinase C (PKC), and this activation results in downstream post-translational changes in proteins that affect receptor complexes and ion channel activity and in transcription factors that alter gene expression of proteins such as MARCKS (myristoylated alanine-rich C-kinase substrate), which are integral to long-term neuroplastic changes in cell function. Inhibition of GSK-3 $\beta$  within the wnt-receptor (wnt-R) pathway alters gene transcription and neuroplastic events through an increased expression of downstream proteins such as  $\beta$ -catenin. In addition, this inhibition can indirectly affect phosphoinositide 3 kinase pathways and intermediate factors (e.g., Bcl-2 and MAP kinases), which are thought to mediate cell growth and survival.

treated rats, the effects of lithium on receptor-coupled PI signaling (39-42) and the down-regulation of myristoylated alanine-rich C-kinase substrate (MARCKS) protein (discussed below) can be prevented or reversed by a high concentration of myo-inositol. Recent genetic data from Drosophila indicate a role for the upstream inositol polyphosphatase (IPPase) as an additional target for lithium (43) (Fig. 79.2). Drosophila harboring a null mutation for the IPPase gene demonstrate aberrant firing of the neuromuscular junction, an effect that is mimicked by the treatment of wild-type flies with lithium. Although studies during the past several years have provided evidence that myo-inositol clearly plays a role in the action of lithium, it is evident that lithium-induced myo-inositol reduction may depend on cell type (39) and that sites other than PI signaling may be lithium targets, depending on the physiologic system under investigation. In studies examining the in vivo physiologic effects of lithium, such as polyuria or enhancement of cholinergically induced seizures, the addition of myo-inositol reduced but did not fully reverse the lithium-induced effects (44,45). Furthermore, the effect of long-term lithium on developmental polarity in the Xenopus embryo is rescued in the presence of *myo*-inositol (46), but this effect may not be totally attributable to a direct effect of lithium on IMPase (47) (see below).

Although the lithium-induced reduction in agonist-stimulated PIP<sub>2</sub> hydrolysis in rat brain slices has often been small and inconsistent, probably secondary to the size of the signaling-dependent PIP<sub>2</sub> pool (33,48), a recent study of patients with BPD in which proton magnetic resonance spectroscopy was used has demonstrated a significant lithium-induced reduction in myo-inositol levels in the right frontal lobe (49). However, the reduction in myo-inositol preceded the improvement in mood symptoms, indicating a temporal dissociation between changes in myo-inositol and clinical improvement. Consequently, these and other studies suggest that although inhibition of IMPase may represent an initial effect of lithium, reducing myo-inositol levels per se may be more important in the specificity of the cellular site of action for lithium than in the actual therapeutic response, which may be mediated by a cascade of downstream changes in signal transduction and gene expression (see below).

## Adenylyl Cyclase

The other major receptor-coupled second-messenger system in which lithium has been shown to have significant effects is the adenylyl cyclase system. The cyclic AMP (cAMP) generating system plays a major role in the regulation of neuronal excitability and has been implicated in the pathophysiology of seizure disorders (50–52) and BPD (53). Studies in a variety of cell systems and in human brain have demonstrated that lithium attenuates receptor-coupled activation of the cAMP pathway at concentration that inhibits 50% (IC<sub>50</sub>) values ranging from 1 to 5mM (see ref. 1 for review). Lithium *in vitro* inhibits adenylyl cyclase activity stimulated by guanosine triphosphate (GTP) or calcium/ calmodulin, both of which interact directly with adenylyl cyclase (54–56). These inhibitory effects of lithium are antagonized by Mg<sup>2+</sup>, which suggest that the action of lithium on the adenylyl cyclase system is mediated by direct competition with Mg<sup>2+</sup> (55). However, attenuation of adenylyl cyclase activity following long-term lithium treatment in rat cortical membranes was not antagonized by Mg<sup>2+</sup> alone but was reversed by increasing concentrations of GTP, which implies that the effect of long-term lithium treatment may be mediated at the level of G proteins (54,56).

Recent studies have examined the effects of valproate on components of the  $\beta$ - adrenergic receptor ( $\beta$ AR)-coupled cAMP generating system (57). Long-term valproate at a clinically relevant concentration has been shown to produce a significant alteration of the BAR-coupled cAMP generating system in cultured cells in vitro. In contrast to long-term lithium (discussed above), long-term valproate was found to produce a significant reduction in the density of  $\beta$ ARs. Data generated during the past two decades reveal that carbamazepine inhibits basal and forskolin-stimulated activity of purified adenylyl cyclase and also basal and stimulated adenylyl cyclase in rodent brain and neural cells in culture (57-60). In addition, carbamazepine has been reported to reduce elevated cAMP in the cerebrospinal fluid (CSF) of manic patients (61). It appears that carbamazepine inhibits cAMP production by acting directly on adenylyl cyclase or through factor(s) that co-purify with adenylyl cyclase.

Lithium may have dual effects on the intracellular generation of cAMP. Whereas lithium decreases receptor-coupled stimulation of adenylyl cyclase, lithium increases basal levels of cAMP formation in rat brain (62,63). In addition, longterm lithium has been found to increase not only cAMP levels (64) but also levels of adenylyl cyclase type I and type II messenger RNA (mRNA) and protein levels in frontal cortex (65,66), which suggests that the net effect of lithium may derive from a direct inhibition of adenylyl cyclase, upregulation of adenylyl cyclase subtypes, and effects on G proteins. Thus, it has been suggested that the action of lithium on the adenylyl cyclase system depends on state of activation; under basal conditions, in which tonic inhibition of cAMP formation through  $G_{\alpha i}$  is predominant, levels of cAMP are increased, whereas during receptor activation of adenylyl cyclase mediated by  $G_{\alpha s}$ , cAMP formation is attenuated. It has been suggested that such a "bimodal model" for the mechanism of action of lithium may account for its therapeutic efficacy in both depression and mania (12). Although this would appear to be overly simplistic, it may bear clinical relevance to side effects of lithium, such as nephrogenic diabetes insipidus and subclinical hypothyroidism, which have generally been attributed to inhibition of vasopressin or thyrotropin-sensitive adenylyl cyclase.

### G Proteins

As noted above, considerable evidence indicates that lithium attenuates receptor-mediated second-messenger generation in the absence of consistent changes in receptor density (see refs. 1,67 for review). Although lithium has been reported to reduce PI signaling via alteration in G-protein function in cell preparations (68-70), these data have not been replicated in rat or monkey brain (71,72). Although it appears that long-term lithium administration affects G-protein function (12,73), the preponderance of data suggest that lithium, at therapeutically relevant concentrations, does not have any direct effects on G proteins (1,74). A number of studies have reported modest changes in the levels of Gprotein subunits; however, the effects of long-term lithium on signal transducing properties occur in the absence of changes in the levels of G-protein subunits per se (1,63,65, 75). At the mRNA level, some evidence suggests that  $G_{\alpha s}$ ,  $G_{\alpha i1}$ , and  $G_{\alpha i2}$  may be down-regulated in rat cerebral cortex following long-term lithium (65,75,76). Again, however, these effects are small, and their physiologic significance is still unclear. Interestingly, the valproate-induced reduction in the density of  $\beta$ ARs (noted above) was accompanied by an even greater decrease in receptor- and post-receptor-mediated cAMP accumulation, which suggests that long-term valproate may exert effects at the  $\beta$ AR/G<sub>s</sub> interaction, or at post-receptor sites (e.g., G<sub>s</sub>, adenylyl cyclase). A subsequent study has reported a reduction in the levels of  $G_{\alpha s}$ -45 but not in the levels of any of the other G-protein  $\alpha$  subunits examined ( $G_{\alpha s}$ -52,  $G_{\alpha i1/2}$ ,  $G_{\alpha o}$ ,  $G_{\alpha q/11}$ ) following longterm exposure to valproate (77).

Long-term lithium treatment has been shown to produce a significant increase in pertussis toxin-catalyzed [<sup>32</sup>P]adenosine diphosphate (ADP)-ribosylation in rat frontal cortex and human platelets (1). Because pertussis toxin selectively ADP-ribosylates the undissociated, inactive  $G\alpha\beta\gamma$  heterotrimeric form of G<sub>i</sub>, these data are consistent with a stabilization of G<sub>i</sub> in the inactive conformation and an elevation in basal adenylyl cyclase activity. In this context, it is noteworthy that lithium appeared to increase the levels of endogenous ADP-ribosylation in C6 glioma cells and rat brain, whereas anticonvulsants either reduced ADP-ribosylation or had no effect (78,79). Currently, it is thought that the effects of long-term lithium may in part be mediated through post-translational modifications of G proteins that in turn may alter its coupling to receptor and second-messenger systems (1). However, given the relative abundance of G proteins, the physiologic impact of the level of posttranslational changes induced by therapeutic levels of lithium on the balance of receptor-mediated signaling in brain is yet to be determined.

# Protein Kinases and Protein Kinase Substrates

Based on the action of lithium in the PI signaling pathway, as discussed earlier, it was hypothesized that long-term prophylactic effects of lithium might be mediated via the diacylglycerol (DAG) arm of the PIP<sub>2</sub> hydrolytic pathway consequent to relative depletion of myo-inositol and subsequent DAG-mediated action on the regulation of protein kinase C (PKC) and specific phosphoprotein substrates (80,81) (Fig. 79.2). Studies during the past several years have provided evidence that PKC plays a crucial role in mediating the action of long-term lithium in a variety of cell systems, including primary and immortalized neurons in culture, and in rat brain (see refs. 12,33,81,82 for review). PKC represents a large family of at least 12 isozymes that are closely related in structure but differ in several ways-intracellular and regional distribution in the brain, second-messenger activators, specificity of association with the RACK (receptor for activated C-kinase) proteins, and substrate affinities-all of which suggest distinct cellular functions for these isozymes. PKC plays a major role in the regulation of neuronal excitability, neurotransmitter release, and longterm alterations in gene expression and plasticity. In fact, PKC activity has been implicated in processes underlying amygdala kindling and behavioral sensitization, putative animal models for BPD (83,84). PKC isozymes are highly expressed in the brain, with the  $\gamma$  isoform expressed exclusively, and are localized both presynaptically and postsynaptically. PKC is located in the cytoplasmic and membrane compartments of cells, and its activation requires translocation from the cytosol to RACK proteins within the membrane. Translocation from the cytosol to the membrane is most often associated with phosphorylation and activation of the enzyme, which is followed by autocatalysis and downregulation of the enzyme on prolonged activation.

Studies of long-term lithium administration in the rat have demonstrated a reduction in membrane-associated PKC- $\alpha$  and PKC- $\epsilon$  in the subiculum and in CA1 regions of the hippocampus (85,86). In brain slices from lithiumtreated rats exposed to phorbol ester, a known activator of PKC, a marked reduction was noted in the translocation of PKC activity from the cytoplasm to the membrane, and this was accompanied by a reduction in phorbol esterinduced serotonin release (87). Studies of long-term lithium in both C6 glioma cells and immortalized hippocampal cells in culture also demonstrate a reduction in the expression of these same PKC isozymes (see ref. 88 for review). This is interesting in light of data demonstrating an enhancement of PKC activity in platelets of patients during a manic episode (88). Moreover, administration of myo-inositol to rats was reported to reverse the down-regulation of PKC- $\epsilon$  in brain following long-term lithium, consistent with a role of myo-inositol in the downstream action of lithium on regulation of PKC by DAG. It is of note that valproate produces effects on the PKC signaling pathway similar to that reported for lithium (33,89). Long-term lithium and valproate appear to regulate PKC isozymes by distinct mechanisms, however, with the effects of valproate appearing to be largely independent of myo-inositol. These studies have

led to a pilot clinical study of the use of tamoxifen, a drug known to inhibit PKC *in vitro*, in the treatment of acute mania (90,91). Although the preliminary results appear consistent with the hypothesis, the sample size was small, and it is not known whether this drug *in vivo* inhibits PKC isozymes or whether its other properties (i.e., anti-estrogenic) play a role.

The activation of PKC results in the phosphorylation of a number of membrane-associated phosphoprotein substrates, the most prominent of which in brain is the MARCKS protein. Direct activation of PKC by phorbol esters in immortalized hippocampal cells effectively downregulates the MARCKS protein (92). Long-term lithium administered to rats during a period of 4 weeks in clinically relevant concentrations dramatically reduces the expression of MARCKS protein in the hippocampus, and these findings have been replicated and extended in immortalized hippocampal cells in culture (39,93,94). Studies in hippocampal cells have demonstrated that the extent of downregulation of MARCKS expression after long-term lithium exposure (1 mM) depends on both the inositol concentration and activation of receptor-coupled PI signaling, consistent with the hypothesis as stated above. Recent studies provide evidence for the regulation of transcription as a major site for the action of long-term lithium on MARCKS expression in brain (95). Moreover, this action of lithium in the brain and hippocampal cells is apparent only after long-term administration and persists beyond abrupt discontinuation of the drug for an extended period of time, paralleling the clinical time course of the therapeutic effects of lithium during initial treatment and discontinuation. Subsequent studies have discovered that this property of reducing the expression of MARCKS in hippocampal cells is shared by the anticonvulsant valproic acid, but not by other classes of psychotropic agents (96). Additionally, therapeutic concentrations of combined lithium and valproate have induced an additive reduction in MARCKS, also consistent with experimental findings that the two drugs work through different mechanisms on the PKC system and the clinical observation of the additivity of the two drugs in treatment responses (96). The altered expression of MARCKS further supports the role of PI signaling and PKC in the action of long-term lithium in the brain and may serve to provide insight regarding a role for neuroplasticity in the long-term treatment of BPD, as discussed below.

A crucial component of cAMP signaling is protein kinase A (PKA), which is a principal mediator of cAMP action in the central nervous system. Long-term lithium treatment has been shown to increase the regulatory and catalytic subunits of PKA in rat brains, an effect that appears to result in increased cAMP binding (97). Consistent with a lithiuminduced increase in basal cAMP and adenylyl cyclase levels, a more recent study has reported that platelets from lithiumtreated euthymic patients with BPD demonstrated an enhanced basal and the cAMP-stimulated phosphorylation of Rap1 (a PKA substrate) and a 38-kilodalton phosphoprotein not observed in healthy controls (98). The effects of lithium on the phosphorylation and activity of cAMP response element binding (CREB) protein, however, have been examined in rodent brain and in cultured human neuroblastoma cells, with somewhat conflicting results (99, 100). Postmortem studies of brains of patients with BPD have shown changes in cAMP binding and in PKA activity in temporal cortex (101,102). These findings suggest that alterations in PKA activity may be associated with the action of lithium. It is of interest in this regard that carbamazepine attenuates forskolin-induced phosphorylation of CREB in C6 glioma cells (57).

It is well-known that lithium ion can have a significant effect on the development of a variety of organisms (103). In Xenopus, lithium significantly alters the ventral-dorsal axis of the developing embryo (104). One hypothesis regarding this action of lithium was based on its inhibition of IMPase and alteration in the dorsal-ventral balance of PI signaling in the embryo (105,106). Support for this hypothesis was derived from the observation that exposure to high concentrations of myo-inositol can reverse the effect of lithium (107). However, lithium ion has been shown to inhibit the activity of glycogen synthase kinase 3B (GSK-3 $\beta$ ) ( $K_i = 2.1 \text{ mM}$ ) directly, thereby antagonizing the wnt signaling pathway, known to be instrumental in normal dorsal-ventral axis development in the Xenopus embryo (108-111). Furthermore, studies in which an embryo expressing a dominant negative form of GSK-3 was used have demonstrated that myo-inositol can reverse the resulting aberrant axis development in Xenopus, which suggests that myo-inositol reversal of dorsalization of the embryonic axis by lithium may be mediated, at least in part, by events independent of IMPase inhibition (47). Substrates for GSK- $3\beta$  in cells include not only glycogen synthase but also  $\beta$ catenin and microtubule-associated proteins (MAPs), both of which have been implicated in cytoskeletal restructuring; further,  $\beta$ -catenin is known to play a role in the expression of transcription factors [e.g., lymphoid enhancer factor (LEF) and T cell factor (TCF)]. Recent studies in human neuroblastoma cells have demonstrated that valproic acid also inhibits GSK-3 $\beta$ , after which levels of  $\beta$ -catenin increase (112). Thus, GSK-3 $\beta$  may contribute to our understanding of an action for long-term lithium observed in events associated with apoptosis and neuroplasticity, as discussed below.

#### **Gene Expression**

The clinical data indicating that onset of the therapeutic effect of lithium requires days to weeks of lag time and that reversal of the therapeutic effect on discontinuation occurs during a period of weeks to months suggest that the therapeutically relevant action of lithium in the brain involves long-term neuroplastic changes mediated by gene regulation. Evidence has accumulated that lithium can regulate gene expression via nuclear transcriptional factors. One of the immediate early genes, *c-fos*, works as a master switch of gene regulation through interactions with *cis*-acting elements and other transcriptional factors. Lithium has been shown to alter the expression of *c-fos* in various cell systems (113) and in the brain (114–116); however, its effects have varied depending on brain region, cell type, and time course examined. (112,117–119).

It is known that *c-fos* interacts with *jun* family members to form activator protein 1 (AP-1), which binds to a common DNA site. Studies in both cell culture and rat brain following long-term lithium exposure in vivo demonstrate an enhancement of AP-1 DNA binding activity (99,120). Subsequent studies in cells with an AP-1-coupled reporter gene have confirmed a time- and concentration-dependent increase in transcriptional activity in the presence of lithium (121,122). These studies have also noted increases in the protein levels of c-fos, c-jun, and phosphorylated CREB. It is of interest that phosphorylation of c-jun inhibits DNA binding, whereas phosphorylation of CREB activates gene expression; both are substrates for GSK-3 $\beta$  activity, which is inhibited by lithium. However, when AP-1 binding activity was measured following receptor activation, lithium treatment attenuated the induced AP-1 DNA binding activity (123,124). These seemingly contradictory findings suggest that the effect of lithium on gene transcription may depend on the activity level of the neurons. It has been suggested that by increasing AP-1 binding activity at the basal level, but decreasing it during stimulation, lithium can constrain the overall magnitude of fluctuations of gene expression as a function of neuronal activity (125). Valproic acid has been shown to have similar effects on the activity of AP-1 (120,122,126), which lends support to the possibility that gene regulation through AP-1 may represent a target for mood stabilizers. In addition, carbamazepine has been shown to inhibit forskolin-induced c-fos gene expression in cultured pheochromocytoma (PC12) cells (127). It must be kept in mind, however, that AP-1 binding activity is responsive to a multitude of signals and is unlikely to define the specific action underlying the therapeutic effect of lithium in BPD. Future studies may fruitfully examine a potential role for lithium in the regulation of newly discovered candidate genes linked to BPD (128), in addition to those implicated in its pathophysiology (129).

Lithium-induced alterations in gene expression may also account for recent findings of a neuroprotective effect in some cell systems. A number of groups have demonstrated a neuroprotective effect of lithium in systems both *in vivo* and *in vitro* against a variety of insults, including glutamateinduced excitatory apoptosis (130–132). It is well established that neuronal survival during apoptosis or programmed cell death depends on the relative expression of "executioner" proteins and "protector" proteins and the presence of neurotrophic factors. The B-cell lymphoma/ leukemia 2 gene (*bcl2*), abundantly present in mammalian

neurons, encodes one of the protector proteins that inhibits apoptosis and cell death under variety of circumstances. Recent studies in rat brain have demonstrated that long-term exposure to lithium or valproate increases the expression of the polyomavirus enhancer-binding protein  $2\beta$  gene (PEBP2B), a regulator of bcl2 expression (133). Subsequent studies in rat brain have demonstrated an increase in cells immunoreactive for Bcl-2 in layers II and III of frontal cortex, dentate gyrus, and striatum after long-term lithium (134). In cultured cerebellar granule cells, long-term treatment with lithium induces a concentration-dependent decrease in p53 and bax (apoptotic genes) mRNA and protein, with a concomitant increase in *bcl2* at both the mRNA and protein levels (135). It is of interest that these actions of lithium have been attributed to an enhancement of the PI<sub>3</sub> kinase pathway, in which GSK-3ß plays a prominent role (136) (Fig. 79.2). To what extent this neuroprotective effect may be related to the long-term prophylactic effect of lithium in stabilizing the course of BPD and the putative role of cellular loss in the pathophysiology of affective disorders remains to be demonstrated (137).

# Neuroplasticity and Cytoskeletal Remodeling

Recent studies in a number of laboratories have provided evidence that long-term lithium treatment may alter molecular substrates underlying neuroplastic changes in brain that mediate alterations in interneuronal connectivity. As noted above, developmental studies in the Xenopus embryo have recently provided evidence that lithium can act as an inhibitor of GSK-3 $\beta$ , a component of the wnt signaling pathway, at concentrations that may be relevant to clinical treatment (110). Several groups have reported that inhibition of GSK- $3\beta$  by lithium reduces phosphorylation of tau protein in different cell systems, the effect of which is to enhance the binding of tau to microtubules and promote microtubule assembly (110,138–140). Lithium treatment also decreases phosphorylation of MAP-1B, a microtubule-associated protein involved in microtubule dynamics within the growth cone and axonal outgrowth (141). Lithium-induced dephosphorylation of MAP-1B reduces its ability to bind to microtubules; in cerebellar granule neurons, this effect was accompanied by axonal spreading and increases in growth cone area and perimeter (142,143). Thus, it is possible under the appropriate conditions that inhibition of GSK-3β by lithium can induce significant changes in microtubule assembly that result in changes in the association dynamics among cytoskeletal proteins mediating neuroplastic changes in regions of the brain.

The significance of actin-membrane remodeling in the long-term action of lithium is also supported by a series of studies demonstrating that long-term lithium down-regulates the expression of the PKC substrate MARCKS in brain, as noted previously. MARCKS is a complex protein that binds calmodulin in a calcium-dependent manner; it also binds and cross-links filamentous actin, thereby conferring focal rigidity to the plasma membrane. Following phosphorylation of its phosphorylation site domain in the presence of activated PKC, MARCKS translocates from the plasma membrane and neither binds calmodulin nor crosslinks actin. Thus, this protein is in a key position to transduce extracellular signals to alterations in the conformation of the actin cytoskeleton, which are critical to cellular processes underlying development and signaling, including morphogenesis and secretion. MARCKS is enriched in neuronal growth cones, developmentally regulated, and necessary for normal brain development (144-146). MARCKS expression remains elevated in specific regions of the hippocampus and limbic-related structures, which retain the potential for plasticity in the adult rat (147,148) and human brain (149), and its expression is induced in the mature central nervous system during axonal regeneration (150). Recent studies support a role for MARCKS in plastic events associated with learning and memory. Induction of longterm potentiation, thought to be a physiologic component of learning and memory, elevates MARCKS phosphorylation in hippocampus (151). Moreover, adult mutant mice expressing MARCKS at 50% exhibit significant spatial learning deficits that are reversed in the presence of a MARCKS transgene (144). These data reveal that MARCKS plays an important role in the mediation of neuroplastic processes in the developing and mature central nervous system. Thus, by virtue of its action in signaling pathways utilizing PI/PKC and GSK-3B cascades (Fig. 79.2), long-term lithium administration may alter presynaptic and postsynaptic membrane structure to stabilize aberrant neuronal activity in critical regions of the brain involved in the regulation of mood (92).

## ANTIDEPRESSANTS

#### **Neurotransmitter Signaling**

Antidepressants are usually classified according to structure [e.g., tricyclic antidepressants (TCAs)] or function [e.g., monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs)]. However, it may be more useful to classify them according to the acute pharmacologic effects that are presumed to trigger behavioral improvement. If this is done, the antidepressants can be grouped in four categories (Table 79.1). First are the drugs that selectively block the reuptake of norepinephrine (NE). These include certain TCAs and TCA-like compounds (maprotiline). Another drug that falls into this category is reboxetine, although it is distinct structurally from the TCAs and TCAlike compounds (152). It is currently available as an antidepressant in European and South American countries but is not yet marketed in the United States. Second are the SSRIs, which, as their class name implies, selectively block the reuptake of serotonin [5-hydroxytryptimine (5-HT)] in vivo.

Third are the drugs that act nonselectively on noradrenergic and serotoninergic neurons with a resultant enhancement of synaptic transmission. Some TCAs are in this category, as are the MAOIs. Some novel drugs are also in this category. One of these is venlafaxine, discussed in more detail later. Another is mirtazapine. Mirtazapine is not a potent inhibitor of the reuptake of either NE or 5-HT (153). It is a relatively potent antagonist, though, of inhibitory  $\alpha_2$  autoreceptors on noradrenergic nerves. By blocking such autoreceptors, mirtazapine removes their inhibitory influence on noradrenergic transmission. Thus, even though it is not a reuptake inhibitor, mirtazapine can directly enhance NE-mediated transmission (154–156). In this respect, then, it might be appropriate to place mirtazapine in the first

Category	Mechanism	Examples	Current Classification (If Any)
I	Selective blockade of NE reuptake (SNRIs)	DMI, NT amoxapine, maprotiline reboxetine	TCAs TCA-like
II	Selective blockade of 5-HT reuptake (SSRIs)	Citalopram, fluoxetine, paroxetine, sertraline	SSRIs
111	Nonselective enhancement of NE and 5-HT transmission	IMI, AMI phenelzine, tranylcypromine venlafaxine mirtazapine	TCAs MAOIs (sometimes with SSRIs)
IV	Unknown potent stimulatory effects on NE or 5-HT	trimipramine bupropion nefazodone, trazodone	TCA

TABLE 79.1. MECHANISM-BASED CLASSIFICATION FOR ANTIDEPRESSANTS

5-HT, 5-hydroxytryptamine (serotonin); AMI, amitriptyline; DMI, desipramine; IMI, imipramine; MAOI, monoamine oxidase inhibitor; NE, norepinephrine; NT, nortriptyline; SNRI, selective norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant.

category. However, mirtazapine may also enhance serotoninergic transmission, albeit *indirectly* (157–159). This enhancement is caused in part by NE activation of  $\alpha_1$  noradrenergic receptors located on serotoninergic soma and dendrites to increase cell firing and the release of 5-HT (160,161). Mirtazapine may also block inhibitory  $\alpha_2$  adrenoceptors located on serotoninergic terminals (i.e., heteroceptors) (154,162). However, some recent data call into question the likelihood that mirtazapine enhances serotoninergic transmission (163). Whether mirtazapine increases serotoninergic transmission may depend on the state of activation of the central noradrenergic system when the drug is administered. Further research is needed to clarify this point. At this time, we have placed mirtazapine in the third category.

In the fourth and final heterogeneous group are drugs without known potent, acute pharmacologic effects that result in enhancement of noradrenergic or serotoninergic transmission. In other words, their mechanisms of action are unknown. Drugs in this category include the TCA trimipramine and also bupropion, nefazodone, and trazodone. It has been speculated that bupropion acts through dopaminergic mechanisms because it is the only antidepressant that more potently blocks the reuptake of dopamine than that of either NE or 5-HT (164). However, in reality, bupropion and its metabolites are very weak inhibitors of the reuptake of all three biogenic amines, with potencies in the micromolar range (164). Perhaps this is why the data regarding whether bupropion inhibits dopamine reuptake in patients at clinically relevant doses are at best conflicting (165). Some data indicate an as yet ill-defined effect of bupropion or its hydroxylated metabolite on noradrenergic function (164), but the efficacy of bupropion cannot at this time be attributed to effects on noradrenergic transmission.

The most potent acute effect of nefazodone and trazodone on serotoninergic or noradrenergic systems is their antagonism of 5-HT<sub>2A</sub> receptors (166). They are very weak inhibitors of NE reuptake and relatively weak inhibitors of 5-HT reuptake (167). If enhancement of serotoninergic transmission is a mechanism that ultimately leads to clinical efficacy, it is not clear how antagonism of the 5-HT<sub>2A</sub> receptor produces such enhancement. Some data indicate that 5-HT<sub>2</sub>-receptor antagonism enhances 5-HT<sub>1A</sub>-receptor responsivity (168,169), or that 5-HT<sub>2</sub>-receptor antagonists share discriminative stimulus properties with 5-HT<sub>1A</sub>receptor antagonists (170). However, not everyone finds such effects (171), and whether such an effect would enhance endogenous serotoninergic transmission is uncertain. Thus, acute pharmacologic properties that contribute to the efficacy of the drugs in the fourth category remain unknown.

Originally, brain tissue from rats was used to measure the potencies of drugs *in vitro* to block the reuptake of <sup>3</sup>H-NE or <sup>3</sup>H-5-HT. Subsequently, radioligand binding techniques were developed such that the potencies of antidepressants to displace the specific binding of ligands to the

norepinephrine transporter (NET) or serotonin transporter (SERT) could be measured. These studies were also carried out in brain tissue, usually from rats. The potencies of drugs to produce such effects were thought to be reflective of their potencies at blocking NE or 5-HT uptake clinically. The cloning of the SERT and NET in the early 1990s enabled many types of studies not possible heretofore (172). Among these are studies in which the human NET (hNET) or human SERT (hSERT) is transfected, often stably, into cells that normally do not have any NET or SERT. These cells can be maintained in cell culture systems and used to measure the uptake of <sup>3</sup>H-NE and <sup>3</sup>H-5-HT by the *b*NET and *b*SERT, respectively, and the binding of radioligands to the hNET and hSERT. Further, such cells can be used to measure the potencies of antidepressants to block such effects. The advantage of such systems, obviously, is that potencies are measured directly on human transporters. The disadvantages of such systems are equally obvious-namely, they are artificial, and a variety of factors can influence results (173). As Kenakin (173) has written, "Transfecting the cDNA of a receptor protein into a foreign cell and expecting a physiologic system can be likened to placing the Danish King Hamlet on the moon and expecting Shakespeare to emerge."

It might be illustrative to compare potencies of antidepressants obtained with the different preparations and approaches. This is done in Tables 79.2 and 79.3. Irrespective of the noradrenergic parameter chosen (Table 79.2), the orders of potency are almost identical, especially for the most potent compounds (i.e., desipramine > nortriptyline > amitriptyline = imipramine > paroxetine). Also, citalopram is the least potent drug on all measures. Perhaps the value that most stands out quantitatively from the others is that for <sup>3</sup>H-NE uptake by *h*NET. In general, these values tend to be sixfold to 10-fold higher (i.e., potencies are less) than those found to inhibit such uptake into rat brain synaptosomes. An interesting specific difference is seen with venlafaxine; its potency to inhibit <sup>3</sup>H-NE uptake by rat brain is five to eight times greater than its potency on the other noradrenergic parameters. For serotoninergic parameters also, the rank order of potencies appears reasonably similar irrespective of the specific parameter-namely, paroxetine > sertraline  $\geq$  citalopram  $\geq$  fluoxetine  $\geq$  imipramine  $\geq$ venlafaxine  $\geq$  amitriptyline > nortriptyline  $\geq$  desipramine  $\geq$  nefazodone. However, the potencies found for most of the drugs to inhibit *b*SERT binding are greater than those to inhibit <sup>3</sup>H-5-HT uptake by the *h*SERT, oftentimes eightfold to 20-fold greater (Table 79.3).

It is important to recognize that potencies obtained *in vitro* for any pharmacologic effect give only some indication of whether the pharmacologic effect in question could occur clinically. High potency *in vitro* (e.g.,  $\leq 10 \text{ n}M$ ) certainly increases the likelihood that an effect will occur clinically, and low potency (e.g.,  $\geq 500 \text{ n}M$ ) decreases the probability. However, as emphasized by others (179), whether or not a

Drug	<sup>3</sup> H-NE Uptake (Rat)	<i>r</i> NET Binding	<sup>3</sup> H-NE Uptake (Human)	<i>h</i> NET Binding
Amitriptyline	14	9	102	27
Citalopram	>3,000ª	>3,000	>30,000	>5,500
Desipramine	0.6	0.3	3.5	0.7
Fluoxetine	143	473	2186	508
Imipramine	14	11	142	28
Nefazodone	570	555	713	489
Nortriptyline	2	1	21	3
Paroxetine	33	59	328	62
Sertraline	220	1597	1716	618
Venlafaxine	210	1067	1644	1664

TABLE 79.2. VALUES	5 (nM) OF T	HE INHIBITION	CONSTANT	$(K_i)$
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Potencies of these drugs for blocking the uptake of <sup>3</sup>H-NE or <sup>3</sup>H-5-HT into rat brain synaptosomes were taken primarily from Bolden-Watson and Richelson, 1993 (167). These values tend to be in good agreement with those reported by others. Potencies for drugs to inhibit the binding of radioligands to the NET or SERT in rat brain synaptosomes were taken from Owens et al., 1997 (175) for the same reason. Potencies of drugs to inhibit the binding of selective radioligands to the *h*NET and *h*SERT were averaged from results in Owens et al., 1997 (175) and Tatsumi et al., 1997 (176). In general, the results obtained in these two studies are in remarkably close agreement. Finally, potencies of drugs to inhibit uptake of <sup>3</sup>H-NE and <sup>3</sup>H-5-HT by the *h*NET and *h*SERT, respectively, were taken from Owens et al., 1997 (175). Such values tend to be in good agreement with those obtained by others using transfected cell systems, such as Eshleman et al., 1999 (177).

<sup>a</sup>From Hyttel and Larsen, 1985 (174).

Drug	<sup>3</sup> H-5-HT Uptake (Rat)	<i>r</i> SERT Binding	<sup>3</sup> H-5-HT Uptake (Human)	hSERT Binding
	,			
Amitriptyline	84	16	36	4
Citalopram	1.4 <sup>a</sup>	0.8	9	1
Desipramine	180	129	163	20
Fluoxetine	14	2	20	0.9
Imipramine	41	9	20	1
Nefazodone	137	220	549	330
Nortriptyline	154	60	279	16
Paroxetine	0.7	0.05	0.8	0.1
Sertraline	3	0.3	3	0.2
Venlafaxine	39	19	102	8

#### TABLE 79.3. VALUES (nM) OF THE INHIBITION CONSTANT (K<sub>i</sub>)

Potencies of these drugs for blocking the uptake of <sup>3</sup>H-NE or <sup>3</sup>H-5-HT into rat brain synaptosomes were taken primarily from Bolden-Watson and Richelson, 1993 (167). These values tend to be in good agreement with those reported by others. Potencies for drugs to inhibit the binding of radioligands to the NET or SERT in rat brain synaptosomes were taken from Owens et al., 1997 (175) for the same reason. Potencies of drugs to inhibit the binding of selective radioligands to the *h*NET and *h*SERT were averaged from results in Owens et al., 1997 (175) and Tatsumi et al., 1997 (176). In general, the results obtained in these two studies are in remarkably close agreement. Finally, potencies of drugs to inhibit the *h*NET and *h*SERT, respectively were taken from Owens et al., 1997 (175). Such values tend to be in good agreement with those obtained by others using transfected cell systems, such as Eshleman et al., 1999 (177).

5-HT, 5-hydroxytryptamine (serotonin); NET, norepinephrine transporter; SERT, serotonin transporter. <sup>a</sup>Calculated from Hyttel, 1978 (178). specific effect occurs clinically depends on how much drug reaches its presumed site(s) of action (i.e., a function of pharmacokinetics). Because these drugs must act on brain to exert their beneficial effects, a factor that substantially influences how much reaches the brain is the extent to which they are protein-bound. Because of the blood-brain barrier, the amount of drug in the extracellular fluid of brain (i.e., CSF) tends to be equivalent at steady state to the concentration of non-protein-bound drug in plasma (i.e., "free" drug). Normal CSF contains so little protein that it may be regarded as an ultrafiltrate of serum. Because most, but not all, antidepressants are extensively bound to plasma proteins (180,181), their concentration in CSF is only a small fraction of the total concentration in serum.

Table 79.4 shows the percentages of protein binding of certain antidepressants. Also shown are steady-state total plasma concentrations of drug and concentrations in CSF. It is apparent that drug actually measured in CSF approximates what would be calculated to be the non-protein-bound concentration in plasma. For this reason, also shown in Table 79.4 are some antidepressants with concentrations

in CSF that have not been reported. It is possible, then, to compare these CSF concentrations of drugs with their Ki values for the inhibition of uptake or ligand binding, shown in Tables 79.2 and 79.3. For a drug such as citalopram, it is obvious that its concentration in CSF is much greater than that required to inhibit serotoninergic uptake or binding to the SERT, irrespective of whether one is obtaining measurements with rat synaptosomes or *b*SERT. It is also obvious that citalopram does not reach sufficient concentration in CSF to block NE reuptake, again irrespective of the noradrenergic parameter or type of tissue. Considerable data indicate that citalopram maintains selectivity as a 5-HT uptake inhibitor in vivo (162). It is also apparent that desipramine reaches concentrations in CSF sufficient to block NE uptake, irrespective of the parameter or tissue used for measurement. However, the potency of desipramine to block ligand binding to the hSERT (20 nM) is sufficient to indicate that it may have a substantial effect on 5-HT uptake in the brain of patients. Although treatment with desipramine does lower concentrations of the serotonin metabolite 5-hydroxyindolacetic acid (5-H1AA) in the CSF of patients

	Concentration (nM) in				
Drug	Protein Binding (%) <sup>a</sup>	Plasma	CSF (measured)	CSF (estimated)	Reference
Amitriptyline	95	512	33		Hanin et al., 1985 (182)
(Nortriptyline) <sup>b</sup>	92	524	48		
Citalopram	50	40–750 <sup>c</sup>	_	20-375	
Fluoxetine	95	854	26		Martensson et al., 1989 (183)
(Norfluoxetine) <sup>b</sup>	_	1,006	17		
Imipramine	90	433	40		Muscettola et al., 1978 (184)
(Desipramine) <sup>b</sup>	82–92	431	56		
Imipramine	90	475	36		Hanin et al., 1985 (182)
(Desipramine) <sup>b</sup>	82–92	642	79		
Nortriptyline	92	443	39		Nordin et al., 1985 (185)
Paroxetine	95	275	7		Lundmark et al., 1994 (186)
Venlafaxine <sup>d</sup>	27–30	370–3,000	_	100-850	

TABLE 79.4. TOTAL PLASMA AND CEREBROSPINAL FLUID CONCENTRATIONS OF SOME ANTIDEPRESSANTS

These numbers do not take into account concentrations of hydroxylated metabolites in CSF, which can have pharmacologic activity [Nordin and Bertilsson, 1995 (190); Nordin et al., 1987 (191); Potter et al., 1979 (192)]. Even though such hydrophylic metabolites may have diminished lipid solubility, the penetration of some hydroxylated metabolites into CSF may be somewhat greater than that of the parent compounds, presumably because of decreased protein binding [Nordin et al., 1985 (185); Sallee and Pollock, 1990 (181)]. Nevertheless, such metabolites more often than not are more weakly potent than their parent compounds, so it is not likely as a rule that such metabolites contribute substantially to pharmacologic activity in brain.

<sup>a</sup>Values taken from van Harten, 1993 (180); Sallee and Pollock, 1990 (181); and Benet et al., 1996 (187). <sup>b</sup>Parentheses indicate measurements were taken of the drug as a metabolite of the parent antidepressant.

<sup>c</sup>Values taken from Bjerkenstedt et al., 1985 (188) and Fredricson-Overo, 1982 (189).

<sup>d</sup>Range of values for venlafaxine refers to venlafaxine plus O-desmethylvenlafaxine.

(192), it is unlikely that this observation directly reflects the ability of the drug to block 5-HT uptake. Rather, it is more likely to be some indirect effect. The clinical efficacy of desipramine does not appear to depend on the availability of 5-HT (194). Furthermore, considerable preclinical data demonstrate little to no effect of desipramine on serotoninergic function (195-197). Given this, any of the other three serotoninergic values for desipramine, which are quite similar, would seem to be a better indicator of what happens clinically. The situation with nortriptyline appears to be similar to that with desipramine. Even at low concentrations, nortriptyline is likely to block NE uptake. Nortriptyline maintains reasonable selectivity in vivo as an inhibitor of NE reuptake (198-201). Because its concentration in CSF exceeds its potency to block ligand binding to the *b*SERT (and approaches its potency to inhibit binding at the rSERT), it seems doubtful that these potencies have relevance for functional inhibition of 5-HT uptake by nortriptyline clinically. Irrespective of the parameters used to assess the effects of fluoxetine, the interpretation would be the same—namely, levels of fluoxetine in CSF are likely sufficient to block 5-HT uptake, but not NE uptake. Again, considerable clinical and preclinical data indicate that fluoxetine maintains selectivity in vivo as a 5-HT reuptake inhibitor (202–204).

Given the great potency of paroxetine on any of the serotoninergic parameters in Table 79.3, its concentration in CSF is sufficient to cause functional blockade of 5-HT uptake. Also, concentrations of paroxetine in CSF appear insufficient to cause much, if any, functional blockade of NE reuptake. Its greatest potency on a noradrenergic parameter (33 nM for inhibition of <sup>3</sup>H-NE uptake by rat brain synaptosomes) is considerably higher than its highest concentration in CSF (Table 79.2). Considerable data in vivo indicate that paroxetine maintains selectivity as an inhibitor of 5-HT reuptake (205-207). Other than its ability to decrease concentrations of methoxyhydroxyphenylglycol (MHPG) in the CSF of depressed patients (186), an effect produced by all SSRIs, including citalopram (186,188,208), no other clinical data are available from which it may be inferred that paroxetine blocks NE reuptake.

Venlafaxine (and its metabolite *O*-desmethylvenlafaxine) appears to be likely to inhibit 5-HT uptake, even at the low end of its concentration in CSF. This conclusion is reached by comparing its concentration in CSF with any of its "serotoninergic" potencies, with the exception of its potency in inhibiting <sup>3</sup>H-5-HT uptake by *h*SERT. Its low concentration in CSF is only equivalent to this potency. Interestingly, one might conclude that venlafaxine blocks NE uptake at higher concentrations only if one considers its potency in blocking <sup>3</sup>H-NE uptake by rat brain synaptosomes (210 n*M*). Even its highest concentration in CSF (850 n*M*) is only about half its potency on either of the *h*NET parameters and comparable with its potency to inhibit ligand binding to the *r*NET. Data show that venlafaxine is likely to block NE uptake *in vivo*, especially at higher doses (206, 209). Thus, it seems reasonable to speculate that the most clinically relevant potency for venlafaxine at a noradrenergic parameter is its potency to block <sup>3</sup>H-NE uptake by rat brain synaptosomes.

For many of the drugs, then, the same conclusion is reached about selectivity (or nonselectivity) in vivo based on concentrations achieved in CSF and any of the noradrenergic or serotoninergic parameters. For some of the drugs, though, the parameter chosen influences the prediction of what will happen clinically. If one examines potencies to inhibit ligand binding to the hSERT, one might predict that both desipramine and nortriptyline are nonselective inhibitors of both NE and 5-HT reuptake in vivo. This does not seem to be so (194). For these drugs, then, the values for the hSERT should be viewed cautiously. As discussed, the clinical situation with venlafaxine causes some concern about its potency to inhibit <sup>3</sup>H-5-HT uptake by *b*SERT or to inhibit ligand binding to either *r*NET or *b*NET. This analysis demonstrates that  $K_i$  values measured in vitro allow only a prediction of what will occur in vivo-they offer no proof. Experiments must be carried out in vivo to prove (or disprove) the predictions.

### **Regulatory Effects**

The pharmacologic effects of uptake inhibitors, just described in detail, are acute and direct effects of the drugs. As mentioned previously, the optimal behavioral effects of antidepressants on mood may not be evident immediately after initiation of treatment; rather, they are delayed from 2 to 3 weeks (210,211), although some symptoms may show early improvement (212–214). Furthermore, until this past decade, even though UPD was recognized as a recurrent illness in some patients, antidepressants were used primarily on a short-term basis (e.g., 2 to 4 months). However, evidence accumulated during the past several years has caused a fundamental shift in the treatment of UPD, so that prophylaxis is emphasized in addition to acute treatment. Such evidence includes the following: (a) UPD is widely recurrent, with more than 50% of patients having a recurrence sometime during their lifetime (215); (b) long-term (years) treatment of patients with recurrent UPD with different classes of antidepressants is effective in preventing depressive recurrences (215,216); (c) antidepressants (SSRIs, venlafaxine, mirtazapine) have been developed that have a much better side effect (and toxicity) profile than the TCAs, so that they are much better tolerated by patients (217). Such realizations have led to an extensive study of the longerterm and more slowly developing effects of antidepressants, particularly on central monoamine systems. It is beyond the scope of this chapter to review this area in detail. The interested reader can find more exhaustive reviews elsewhere (218-222). Rather, we emphasize the long-term effects of antidepressants that would be expected to continue or markedly enhance the increase in serotoninergic and noradrenergic transmission initiated by the inhibition of uptake. Further, we emphasize some issues we believe to be important in long-term studies of antidepressant effects in laboratory animals such as rats.

#### **Receptors/Transporters**

Clearly, a key assumption is that an understanding of delayed pharmacologic effects and the mechanisms that produce them can lead to the development of drugs (or drug combinations) that produce such effects earlier, with consequent earlier clinical improvement. For example, early research showed that although uptake inhibitors do acutely block uptake, they also rapidly decrease the firing rate of serotoninergic or noradrenergic soma (200,223). For this reason, it was speculated that appreciable enhancement of neurotransmission does not occur with these drugs early in treatment. With serotonin, this was thought to be a consequence of a rise in 5-HT in the raphe nucleus during 5-HT uptake inhibition, which activates inhibitory somatodendritic autoreceptors so as to restrain the rise in serotonin in terminal fields (224-226). A similar mechanism was thought to underlie the decrease in firing in the locus ceruleus (227–229).

With time, though, regulatory changes occur that can enhance transmission, especially in the presence of continued inhibition of uptake. Perhaps chief among these is a time-dependent desensitization of inhibitory serotoninergic autoreceptors. In general, the consensus is that long-term administration of inhibitors of 5-HT uptake cause a desensitization of somatodendritic 5-HT<sub>1A</sub> receptors (230-232), although whether terminal autoreceptors become desensitized is more controversial (233,234; see references in 235). The time-dependent desensitization of inhibitory somatodendritic autoreceptors enhances serotoninergic neurotransmission in terminal fields during the long-term administration of 5-HT uptake inhibitors (231,236,237). Such observations led to the idea that concomitant administration of pindolol, a 5-HT<sub>1A</sub>-receptor antagonist, with an SSRI would enhance the rate of response. Unfortunately, data about whether this happens are controversial (232,238, 239). The data are somewhat more contradictory regarding whether desensitization of inhibitory somatodendritic noradrenergic  $\alpha_2$  autoreceptors occurs after long-term administration of NE reuptake inhibitors (227,229,240,241). Less attention has been focused on whether concomitant administration of an  $\alpha_2$ -adrenoceptor antagonist would improve the speed of response of a noradrenergic reuptake inhibitor.

Many of the studies of long-term effects of antidepressants have focused on presynaptic or postsynaptic changes in 5-HT and NE receptors (such as those just described) and their physiologic or behavioral consequences. Even though the SERT and NET are the initial cellular targets for reuptake inhibitors, few early studies examined whether such treatment has regulatory effects on these proteins. However, the cloning of the SERT and NET in the early 1990s (172) made it possible to determine whether these integral plasma membrane proteins exhibit plasticity. This work culminated in studies of mice with knockouts of these transporters. In heterozygotes, in which transporter density is reduced by 50%, the impact on transporter capacity is marginal, which suggests that powerful post-transcriptional events regulate transporter function (242). Studies of the mechanisms of such events have in general been carried out in vitro, either with cells that naturally express these transporters or with cells into which the transporters have been stably transfected. The realization that transporters can be regulated stimulated considerable research during the last decade to determine whether long-term treatment of rats with reuptake inhibitors produces regulatory effects on the SERT or NET. Unfortunately, no consistent picture has emerged (243). We think some of the inconsistency may be a consequence of such factors as route of drug administration and tissue preparation. Because this area has not been reviewed in detail previously, we do so here.

In 1990, Marcusson and Ross (244) reviewed the literature about the effect of antidepressants on the SERT. At that time, the approach to measuring SERT function was to measure <sup>3</sup>H-5-HT uptake in vitro. Factors that may contribute to regulatory effects can be lost during tissue preparation (slices, synaptosomes) and the use of artificial incubation media. Other techniques, such as binding a radioligand to the SERT or quantifying mRNA for the SERT, do not measure SERT function. Another important factor that can affect results is how the drugs are administered. Assessment of the literature and the experience of one of the authors (A. F.) with sertraline caused us to believe that sustained antagonism of the SERT throughout the day is needed to demonstrate down-regulation of the SERT. When sertraline was administered intraperitoneally to rats at a dose of 5 mg/ kg twice daily for 21 days (245), quantitative autoradiographic analysis of <sup>3</sup>H-cyanoimipramine (<sup>3</sup>H-CN-IMI) binding to the SERT in 23 areas of brain revealed small (15% to 21%) decreases in binding in only four areas. By contrast, when the drug was administered subcutaneously by minipump for 21 days (at a daily dose of 7.5 mg/d, which is even less than that used in the previous study), a large (70% to 75%) decrease in the binding of <sup>3</sup>H-CN-IMI was seen throughout brain (246). Given that the analytic methodology was essentially identical in the two studies, as was the time of drug administration, the factor that most likely accounts for the difference in results is the route of drug administration.

In general, the metabolism of drugs is faster in rats than in humans. Most uptake inhibitors (or their active metabolites) have half-lives in humans that average about 1 day or even longer (247). Given this, even the administration of antidepressants once a day with doses producing recommended "therapeutic" plasma concentrations (usually measured at the trough of the daily variation in concentration) is sufficient to maintain consistent blockade of the transporter. In the rat, though, sertraline has a half-life of about 4.5 hours (248). Thus, twice-daily and especially once-daily injections of this drug may not be sufficient to maintain adequate occupancy of the SERT in brain throughout the day, and continuous adequate occupancy may be necessary to obtain regulatory effects. Such considerations are even more important with a drug such as citalopram, which has an elimination half-life in the rat of 3 to 5 hours (189,249), or venlafaxine (or its metabolite *O*-desmethylvenlafaxine), which has a half-life of about 1 hour (250). All these considerations are relevant in a review of effects of long-term administration of uptake inhibitors on the SERT (251).

In general, studies of long-term citalopram given intraperitoneally either once or twice daily have found little to no regulatory effects on the SERT (234,245,252-254). In addition to the lack of effect of long-term intraperitoneal administration of citalopram on SERT parameters, comparable administration of this drug has produced inconsistent effects on somatodendritic 5-HT<sub>1A</sub>-receptor sensitivity (233,254–256). A recent report is illustrative (235). The investigators speculated that positive results with long-term citalopram administration might be obtained if the administration and dosage were adequate to maintain plasma levels in a "therapeutic" range. This was achieved by giving the drug subcutaneously by minipump at a dose of 20 mg/kg per day for 15 days. This regimen produced stable plasma citalopram levels of about 300 nM. After a 48-hour washout, when analytic experiments were carried out, plasma levels of citalopram had dropped to levels that were not pharmacologically active. At this time, evidence for marked subsensitivity of somatodendritic 5-HT<sub>1A</sub> receptors was obtained. Their conclusion was that if a proper, pharmacokinetically validated, long-term regimen with citalopram is used, 5-HT<sub>1A</sub>-autoreceptor desensitization can be observed. We think that these observations account for why those who gave long-term paroxetine by minipump consistently obtained evidence of decreases in SERT function (246, 257-259).

Fluoxetine has a half-life in the rat of just 5 to 8 hours, but that of its metabolite, norfluoxetine, is about 15 hours (260). Inconsistent results have been obtained in studies of the effects of long-term administration of this drug on SERT parameters. Gobbi et al. (234) reported no effect in rats of 3 weeks of treatment with fluoxetine (15 mg/kg orally twice daily) on either <sup>3</sup>H-5-HT uptake into synaptosomes or <sup>3</sup>H-citalopram binding. In this study, measurements were made after 7 days of drug washout. Similar results were obtained by Dean et al. (261), who used homogenates; they gave the drug at a dose of 10 mg/kg per day intraperitoneally for either 10 or 28 days. By contrast, in the study of Durand et al. (262), the same dose of fluoxetine, when given for 21 days, markedly lowered <sup>3</sup>H-citalopram binding in brain homogenates. Berton et al. (263) also reported a significant but more modest (25%) reduction in <sup>3</sup>H-citalopram binding in the midbrain of rats treated once daily with 7.5 mg of fluoxetine per kilogram for 21 days. It is interesting that such dose schedules for fluoxetine produce inconsistent results on SERT measures because comparable schedules cause consistent effects on other measures of serotoninergic function, such as 5-HT<sub>1A</sub>-receptor sensitivity (231,264, 265). It does appear, then, that stable, nonfluctuating plasma levels of 5-HT uptake inhibitors over time are needed to show regulatory effects on the SERT, whereas this may not be so for other serotoninergic parameters.

Several investigators have examined mRNA for the SERT at different times after long-term administration of 5-HT uptake inhibitors. Here, also, the results have been inconsistent (246,266–271). This may be a consequence not only of the route of drug administration but also of the duration. Although no change in mRNA for the SERT after 21 days of treatment with paroxetine or sertraline by minipump has been reported (246,272), changes in mRNA are found earlier in treatment, with peak effects after about 10 days (272). It seems that only if the drugs are given in a regimen that causes decreases in SERT binding can changes in its mRNA be observed, and even then only if one looks at the proper times (i.e., early in treatment).

As with the SERT, a number of investigators have studied the effect of long-term treatment of rats with NE uptake inhibitors on NET binding sites. In more recent work, <sup>3</sup>Hnisoxetine, a radioligand that binds specifically to the NET, has been used (273,274). In the study of Bauer and Tejani-Butt (275), 21 days of treatment with desipramine (10 mg/ kg intraperitoneally once daily) caused modest (20% to 40%) but significant decreases in <sup>3</sup>H-nisoxetine binding in some areas of brain, but not in others. More robust and widespread changes were obtained when desipramine was given by osmotic minipump for 21 days (272). We think it likely that the greater effect seen is a result of giving the drug by minipump to obtain consistent daily plasma levels in the "therapeutic" range. It does seem likely that longterm desipramine treatment can down-regulate the NET; its addition in vitro to PC12 cells in culture at concentrations above 100 nM caused a decrease in the  $B_{\text{max}}$  of (i.e., the maximum density of binding sites) <sup>3</sup>H-nisoxetine binding, with a maximum effect occurring after 3 days of exposure (276). The uptake of  ${}^{3}$ H-NE was also decreased. These effects occurred after exposure of the cells to desipramine for as little as 4 hours, but always after desipramine had been removed from the incubation medium. In a followup study in which cells transfected with hNET were used, similar results were obtained with desipramine; in addition, less NET protein was measured in the desipramine-treated cells. Interestingly, desipramine did not cause any change in mRNA for the NET in these cells. In contrast, in the study of Zavosh et al. (277), addition of desipramine to the human neuroblastoma cell line SK-N-SHSY 5Y not only decreased the  $B_{\text{max}}$  of <sup>3</sup>H-nisoxetine binding by 24 hours

but also decreased message, although only after 72 hours of treatment, not after 24 hours. For this reason, Zavosh et al. (277) concluded that the decreases in ligand binding were not a consequence of changes in message. Interestingly, Szot et al. (278) found that both 2-day and 4-week treatment of rats with desipramine (10 mg/kg intraperitoneally once daily) increased mRNA for the NET in the locus ceruleus.

The antidepressant-induced loss of SERT binding sites (and presumably also NET binding sites) may have important functional consequences relevant to the behavioral improvement produced by reuptake inhibitors. The changes that acute local administration of an SSRI has on the clearance of 5-HT in vivo, measured by chronoamperometry, are significant but modest. Variable and quite small effects are produced on the peak amplitude of the electrochemical signal caused by 5-HT, and clearance of the indolalkylamine is inhibited by 30% to 40% (246). However, when antidepressant treatment causes a marked reduction in SERT binding sites, then the peak amplitude of the 5-HT signal is substantially increased and the clearance time is more than doubled (246). Similar effects on the 5-HT chronoamperometric signal were also observed in rats treated with a serotoninergic neurotoxin to cause more than a 70% loss of SERT binding sites (279). Thus, acute blockade of the SERT by SSRIs may not produce the same enhancement of serotoninergic transmission as that caused by the loss of SERT after longer-term administration of drug. Because sertraline treatment has been found to cause a marked loss of SERT binding sites only after 10 to 15 days of treatment (272), which corresponds to the time when drug-induced behavioral improvement becomes obvious, it may be that such loss of SERT binding sites is among the effects necessary to obtain marked enhancement of serotoninergic transmission and consequent behavioral improvement.

# **Signal Transduction**

In recent years, there has been considerable speculation that the beneficial behavioral effects of antidepressants are a consequence of changes in the intracellular signaling pathways linked to noradrenergic or serotoninergic receptors. In other words, behavioral improvement is not a direct consequence of antidepressant-induced receptor activation (which may occur quickly); rather, it results when such receptor activation alters signaling pathways to cause more slowly developing changes in gene expression. Two major areas have been studied. One deals with effects of antidepressants on second messenger-regulated protein kinases in brain. The other deals with changes in activities of protein kinases that result in changes in gene expression and perhaps even neurogenesis. Such effects are reviewed in this section.

# Protein Kinases

Phosphorylation of proteins may well be the primary regulatory mechanism for intracellular events. Such phosphorylation is controlled by protein kinases, which catalyze the binding of phosphate groups to substrate proteins, or by protein phosphatases, which catalyze the removal or release of such groups. Most often, these enzymes are the primary sites of action of the intracellular second messengers in many signaling cascades. Importantly, many kinases can regulate different and independent functions within a cell, presumably by selective co-localization with necessary substrates (280). This implies that drug effects on translocation of kinases, in addition to direct effects on their activity, may have important functional consequences.

The long-term administration of either fluoxetine or desipramine decreases the basal activity of both soluble and particulate PKC in cerebral cortex and hippocampus (281). Because PKC may be involved in the desensitization of 5-HT<sub>2A</sub> receptors (282) and cell surface expression of the SERT (283), antidepressant-induced effects on PKC activity may cause changes in 5-HT<sub>2A</sub>-receptor sensitivity or SERT expression (246,257).

Long-term, but not acute, treatment of rats with various antidepressants activates two other protein kinases in brain, namely PKA in the microtubule fraction and calcium/calmodulin-dependent protein kinase II (CaMKII) in the synaptic vesicle fraction. Such activation results in phosphate incorporation into selected substrates (284,285). With respect to PKA, Nestler et al. (286) had earlier reported a result consistent with the idea that antidepressants cause a translocation of PKA. They found that long-term antidepressant administration decreases the activity of PKA in the cytosol but increases enzyme activity in the nuclear fraction. Other data have also been reported suggestive of an antidepressant-induced translocation of PKA within intracellular compartments (287). Interestingly, long-term desipramine treatment increases the phosphorylation of MAP-2, a substrate for PKA (288); the increased phosphorylation is coupled to inhibition of the microtubule assembly. The effects on PKA may be caused when long-term treatment with antidepressants increases the binding of cAMP to the regulatory II subunit of PKA in brain homogenates (287,289).

Thus, one site affected by long-term antidepressant treatment may be cAMP-dependent phosphorylation, mediated by PKA, in microtubules. It may be speculated that such phosphorylation causes cytoskeletal changes that result in a modification of neurotransmission and antidepressantinduced changes in gene expression (see below), as PKA translocation to the nucleus is microtubule-dependent (290). Antidepressant-induced activation of PKA is interesting in light of findings of decreased PKA activity in cultured fibroblasts of melancholic patients with major depression (291), perhaps a consequence of reduced binding of cAMP to the regulatory subunit of PKA (292). Given the established facilitative function of CaMKII on neurotransmitter release (293), the effect of long-term antidepressant treatment on CMKII, with increased phosphorylation of substrates such as synapsin 1 and synaptotagmin, may underlie the facilitation of monoamine transmitter release produced by these drugs.

#### Gene Expression/Neuroplasticity

Although the precise mechanism is not understood, longterm but not acute treatment with antidepressants has effects on the expression of specific genes that may be a consequence of the activation of protein kinases, particularly PKA. It is known, for example, that PKA can phosphorylate the transcription factor CREB. CREB binds to specific promoter sites (cAMP response elements) to produce changes in the expression of specific genes, such as those for brainderived neurotrophic factor (BDNF) and its receptor, trkB. Relevant, then, to this signaling cascade is the observation that long-term but not acute administration of various types of antidepressants increases the mRNA for CREB in addition to CREB protein in brain (294; see 221,295). More recently, it was shown that such treatments increase CREB expression and CREB phosphorylation, indicative of functional activation of CREB (296). Furthermore, long-term antidepressant treatment increases BDNF and trkB expression in hippocampus (294,297). The increase in BDNF expression is likely to be a consequence of the increase in CREB expression (298,299). Finally, exogenous BDNF has been shown to have antidepressant-like activity in behavioral tests sensitive to antidepressant treatment (301).

Such results are viewed as evidence that long-term antidepressant treatment causes sustained activation of the cAMP system and the intracellular events described. The noradrenergic and serotoninergic receptors producing increases in cAMP are  $\beta$ -adrenoceptors and 5-HT<sub>4</sub>, 5-HT<sub>6</sub>, and 5-HT<sub>7</sub> receptors. If such intracellular effects are responsible for clinical improvement, then these receptors may be the important ones triggering such improvement.

Finally, these slowly developing intracellular effects of antidepressants have been put into an interesting hypothesis to explain antidepressant actions (221,295). The hypothesis is fundamentally different from earlier views of antidepressant action, in which depression was a problem of synaptic transmission and drugs acted within the synapse to improve behavior directly (301,302). The new view is more morphologic in nature. It posits that depression may be caused by chronic, stress-induced atrophy of neurons in certain areas of brain, particularly the hippocampus. It is well established that such atrophy occurs (303,304). Further, more recent data indicate a decrease in hippocampal volume in some depressive patients (305-307), although this need not indicate a loss of neurons. However, postmortem studies have revealed a loss of glia and neurons in the cortex of depressed patients (137,308). The theory then proceeds to make use of the fact that BDNF is known to be involved not only in the differentiation and growth of neurons in the developing brain but also in neuron maintenance and survival in the adult brain (309-311). Thus, the antidepressant-induced

increase in BDNF can oppose and perhaps overcome the stress-induced cell death pathway. Indeed, long-term antidepressant treatment has been shown recently to increase neurogenesis of dentate gyrus granule cells (312).

## CONCLUSION

Our understanding of the mechanism of action of drugs that treat mood disorders such as depression and manicdepressive illness derives for the most part from their interaction with known signaling systems within the brain. It is evident that intracellular effects initiated by antidepressant or mood stabilizers in synaptic physiology may trigger subsequent neuroplastic changes that result in the long-term regulation of signaling in critical regions of the brain. Although much more research is needed to test this hypothesis and establish whether and how such long-term changes are of physiologic significance, current evidence suggests that such changes in brain may be quite important for the now well-established prophylactic effects of mood stabilizers and antidepressants in the treatment of recurrent mood disorders.

With the advent of new molecular biological strategies that use gene expression arrays, we have the opportunity to examine multiple targets in the brain, both known and unknown, for the action of these drugs. Within this chapter, we have tried to identify the most promising of the candidate targets of mood stabilizers and antidepressants. However, research to determine which current and future targets constitute a profile that is most relevant to the therapeutic action of these agents will continue to be hampered by a lack of animal models for these complex behavioral disorders that have strong construct and predictive validity. Although the field of antidepressant research has used animal models with some of these properties for the development of "like" agents, the development of animal models with which new mood stabilizers can be discovered has proved more challenging. We suggest that the creation of models with both construct and predictive validity to permit the discovery of novel targets directly related to therapeutic efficacy will be significantly enhanced by the identification of susceptibility and protective genes for these illnesses.

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Neuropsychopharmacology: The Fifth Generation of Progress. Edited by Kenneth L. Davis, Dennis Charney, Joseph T. Coyle, and Charles Nemeroff. American College of Neuropsychopharmacology © 2002.