

CHAPTER

60

Neurobiology of Severe Mood and Anxiety Disorders

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MOOD DISORDERS

The most common mood disorder diagnoses are major depressive disorder (MDD); dysthymia, a less severe but more chronic form of depression akin to a mood trait; and bipolar disorder (BD), characterized by both depressive episodes and episodes of mania and hypomania. Lifetime mood disorder prevalence rates in the United States are 20.8% of

the population, comprising 16.6% for MDD and 3.9% for BD (Kessler et al., 2005). Women have higher lifetime rates of mood disorders than men (Kessler et al., 2005). Mood disorders are episodic and are recurrent in about 80% of cases, with the likelihood of recurrence increasing with each successive episode (Mueller et al., 1999). Moreover, the clinical picture of major depression varies greatly from episode to episode within the same patient (Oquendo et al., 2004). The recurrent episodes or

chronicity of the disorders suggests long-term alterations in neurobiological function, while the variability of symptomatic expression between episodes implies that the specific manifestation of an episode of a mood disorder is likely sensitive to state-dependent factors. Thus, the pathophysiology of mood disorders should reflect both a common trait-dependent component and a more variable state-dependent component.

NEUROTRANSMITTER AND NEUROPEPTIDE SYSTEMS AND THE PATHOPHYSIOLOGY OF MOOD DISORDERS

Multiple neurotransmitters have been implicated in the pathophysiology of mood disorders, and more recent research using brain imaging has examined the structure and functioning of neural networks associated with mood regulation and mood disorders.

Serotonergic system

In 1969, the indoleamine hypothesis of depression was proposed wherein the vulnerability to either depression or mania was related to low serotonergic activity, attributable either to less serotonin release, fewer serotonin receptors, or impaired serotonin receptor-mediated signal transduction (Ch. 15). Studies of the serotonergic system over the last 30 years have reinforced its role in mood disorders and identified additional associations with suicidal behavior, impulsivity, aggression, eating disorders, obsessive-compulsive disorder, anxiety disorders, personality disorders, seasonal changes in mood and behavior and alcohol use disorders. Studies of cerebrospinal fluid (CSF) 5-HIAA report lower levels in mood disorders that are not mood-state dependent with respect to severity of depression, and that do not differ between manic and depressed patients, both of which suggest that altered serotonergic function is a stable trait in mood disorders.

Central serotonin system function has been examined in challenge paradigms using hormonal response to the serotonin releasing agent/uptake inhibitor fenfluramine and other related direct and indirect serotonin agonists. Release of serotonin from raphe nuclei projections to the hypothalamus causes in turn the release of prolactin and adrenocorticotrophic hormone (ACTH), and the latter causes the release of cortisol from the adrenal cortex. Thus, prolactin response is an index of central serotonin responsiveness and can be used to measure net serotonin transmission, including elements of presynaptic and postsynaptic serotonergic functioning. Blunted prolactin response to fenfluramine in depressed patients, indicating less serotonin release and/or serotonin 5-HT_{1A} or 5-HT_{2A} receptor signal transduction, has been reported in some but not all studies (Coccaro et al., 1989; Malone et al., 1996). Depletion of serotonin by either inhibition of tryptophan hydroxylase (TPH), the rate-limiting biosynthetic enzyme for serotonin, with parachlorophenylalanine (PCPA), or acute tryptophan depletion, provides another index of serotonergic system function. These studies have demonstrated that in long-term remitted medication-free depressed patients depression recurs after serotonin or tryptophan

depletion. An elevated rate of induction of depression by acute tryptophan depletion has also been observed in the relatives of mood disorder patients, suggesting that serotonin hypofunction may be one pathway for the familial transmission of mood disorders. Similarly, a transient return of depressed symptoms following acute serotonin or tryptophan depletion occurs in patients who have responded to serotonin reuptake inhibitors (SSRIs), indicating that the antidepressant effect of this class of medications is dependent on a continuous enhancement of serotonergic function.

Medications, such as SSRIs, that target the serotonin transporter site and selectively inhibit reuptake of serotonin have proven to be effective antidepressants. Some antidepressant drugs act specifically at one or another of the serotonin receptor subtypes; for example, buspirone and gepirone are 5-HT_{1A} receptor agonists. Less 5-HT_{1A}-receptor signal transduction has been implicated in the pathophysiological mechanism of depression and anxiety. In positron emission tomography (PET) studies of major depression, results of brainstem 5-HT_{1A} autoreceptor binding potential are conflicting, with both higher and lower binding reported, but some of these differences may reflect the method of calculating the binding (Drevets et al., 1999; Parsey et al., 2006).

Imaging studies of 5-HT_{2A} receptor binding in depressed subjects have also reported conflicting results, with some observing increased 5-HT_{2A} receptor binding in the prefrontal cortex; others reporting lower binding potential in the cingulate, insular, and inferior frontal cortex; and others observing no alterations (see Stockmeier, 2003 for a review). The variation in results is possibly explained by downregulation of binding by antidepressant medications (Yatham et al., 1999) or clinical population differences. There is disagreement about 5-HT_{2A} receptor binding in medication-free depressed subjects in imaging (Mintun et al., 2004) and postmortem studies (Rosel et al., 2000). The cause of higher 5-HT_{2A} receptor binding in depression is unclear, but animal studies show that stress can upregulate 5-HT_{2A} receptor binding.

Given the heritability of MDD, a genetic contribution to serotonergic dysfunction seems likely. Animal studies have demonstrated that CSF 5-HIAA levels are partly under genetic control (Higley et al., 1993). As noted above, linkage and association studies of candidate genes related to the serotonin system are numerous. In one such study, Mann et al., 2000 assayed postmortem prefrontal cortical (PFC) serotonin transporter binding in major depression and suicide and examined the relationship to the functional 5-HTTLPR allele in the promoter region. Lower binding to the serotonin transporter (5-HTT) throughout the PFC of subjects with a history of a major depressive episode was observed, and the 5-HTTLPR genotype was associated with major depression, but not with suicide or 5-HTT binding. The authors consider that a diffuse reduction of transporter binding in the PFC of individuals with major depression may be indicative of a widespread impairment of serotonergic function. It was unrelated to genotype at the level of the cortex, although Heinz et al., (1998) reported a relationship in the brainstem using single photon emission computed tomography (SPECT).

Studies of serotonin function in major depression suggest both hypofunction and accompanying compensatory alterations to increase serotonergic activity. A role for serotonergic

system hypoactivity in the pathogenesis of depression is suggested by findings such as lower serotonin and 5-HIAA levels in postmortem brainstem of suicides, relapse of depression with acute depletion of tryptophan, fewer serotonin transporter sites in prefrontal cortex and other brain regions on PET scanning, a blunted prolactin response to fenfluramine during and between episodes of major depression, and the antidepressant properties of medications that enhance serotonergic transmission selectively. Note that more 5-HT_{2A} receptor binding in the frontal cortex of depressed individuals who committed suicide, fewer brainstem 5-HT_{1A} auto-receptors, and fewer serotonin transporters in the cortex (described below) would be consistent with homeostatic changes designed to increase deficient serotonergic transmission in major depression. Future investigations of serotonergic activity in mood disorders will need to further differentiate primary pathogenesis from such compensatory changes.

Noradrenergic system

Early evidence that the noradrenergic system is crucial in the pathophysiology of depression appeared in the mid-1950s, when a number of patients became profoundly depressed when treated for hypertension with tetraabenazine and reserpine, which deplete catecholamines in the central and peripheral nervous system (Muller et al., 1955). More recently, evidence of the role of the noradrenergic system in depressive disorders was seen in the effect of α-methyl-p-tyrosine (AMPT), which induces a more biochemically specific catecholamine depletion by inhibiting tyrosine hydroxylase, the rate-limiting step in catecholamine synthesis. AMPT has negligible effects on mood in healthy subjects, but produces a return of depressive symptoms in recovered depression patients treated with noradrenaline reuptake inhibitors.

Multiple lines of investigation provide evidence of a role for the noradrenergic system in depressive disorder, although considerable variability in findings indicates that much remains to be learned regarding specific mechanisms. The norepinephrine (NE) metabolite 3-methoxy-4-hydroxyphenyl-glycol (MHPG) has been investigated in plasma, urine, and CSF studies. Early studies of urinary MHPG reported lower levels in depressed patients compared with controls and higher MHPG levels in manic states in BD patients. However, subsequent studies have shown considerable variability in MHPG levels in patients with depression (Potter et al., 1993). These inconsistencies may in part be due to the low contribution of CNS catecholamines to urinary metabolite levels.

Investigators have sought more valid and direct measures of brain noradrenergic activity by measuring CSF metabolite levels. Again, significant heterogeneity of results appears in studies of major depression. For example, of six studies of CSF noradrenergic metabolites, three found higher levels in depression, one found lower levels, and two reported no difference (Potter et al., 1993). Studies of BD have yielded somewhat more consistency, with bipolar I patients generally showing lower levels of noradrenergic metabolites in the depressed state; however, results also vary (Maas et al., 1982). While there is some contribution to CSF from the spinal cord and possibly even from blood, most MHPG comes from the brain. The observation in rodents that lower CNS noradren-

ergic activity is associated with greater sympathetic nervous system (SNS) activity suggests that mood disorders may be characterized by lower CNS noradrenergic activity and greater SNS activity. Thus, CSF MHPG levels in mood disorders may reflect the balance of changes in brain and SNS noradrenergic activity (Bunney, 1975).

NE turnover decreases with antidepressant treatment, indicating a compensatory effect of elevated intrasynaptic noradrenaline causing feedback inhibition of tyrosine hydroxylase. This effect has been consistently observed with noradrenergic-specific antidepressants, as expected, but has also been reported with serotonergic-specific agents and with ECT (Owens et al., 1997). The latter effect may be due to the action of the raphe serotonergic neurons on activity of locus ceruleus noradrenergic neurons.

In receptor studies no consistent changes in α₁-adrenergic receptor numbers have been observed in unmedicated depressed patients; however, downregulation and hyposensitivity of β-adrenergic, and possibly α₂-adrenergic, receptors have been reported (Dubovsky & Ruzan, 1999). Antidepressant treatment decreased the number of α₂ and β₁-adrenergic receptors and increased the density of α₁-adrenergic receptors in animal studies (Dubovsky & Ruzan, 1999). In challenge studies, following administration of the α₂-receptor agonist clonidine, which induces growth hormone secretion primarily through an action on postsynaptic receptors, attenuated growth hormone secretion, indicating decreased responsiveness of postsynaptic α₂-adrenergic receptors, was observed in depressed patients (Siever et al., 1992). Likewise indicative of subsensitivity of postsynaptic α₂-adrenergic receptors, elevated plasma cortisol levels have been observed in depressed patients following administration of yohimbine, an α₂-adrenergic receptor agonist (Price et al., 1986).

Postmortem brain studies report greater cortical noradrenaline, and less high affinity beta₁-adrenergic receptor binding. More tyrosine hydroxylase in the locus ceruleus (LC) may result from depletion of stores of NE, and that may thereby trigger a compensatory increase in NE synthesis. There are fewer noradrenergic neurons reported in depressed suicides (Underwood et al., 2004), which may indicate a lower functional reserve of the noradrenergic system in suicide and/or major depression. Thus, there is greater likelihood of noradrenergic depletion in the face of severe or prolonged stress, such as the stress of a depressive illness. Noradrenergic response to stress in adulthood appears to be greater in those reporting an adverse experience in childhood, potentially putting them at greater risk in adulthood for noradrenaline depletion in the context of current life stresses because of fewer noradrenergic neurons, and therefore triggering major depression analogous to AMPT depletion studies (Heim & Nemeroff, 2001).

There is empirical evidence supporting the hypothesis of noradrenergic system dysfunction in major depression; however, the inconsistencies in findings rule out any simple model of increased or decreased noradrenergic activity. It is important to determine which noradrenergic system abnormalities relate specifically to the pathogenesis of mood disorders, and which are related to nonspecific effects of stress, homeostatic mechanisms or comorbid psychopathology. More work is needed on the mood state dependence of noradrenergic function.

Dopaminergic system

Patients with major depression have been reported to have lower CSF levels of the dopamine metabolite homovanillic acid (HVA) compared to nondepressed controls, with more severely depressed patients or the subgroup with psychomotor retardation having still lower CSF HVA levels (Kapur & Mann, 1992; Goodwin et al., 1973). However, not all studies have observed lower CSF HVA in patients with depression. In other measures of dopaminergic activity, lower serum dopamine β -hydroxylase activity and higher plasma dopamine and HVA concentrations have been observed in psychotically depressed patients compared with nonpsychotic depressed patients (Devanand et al., 1985). Adding support to the hypothesis that the dopaminergic system is implicated in depressive disorders are the mood-elevating properties of the dopamine-releasing stimulants methylphenidate and dextroamphetamine, which are sometimes used in the treatment of depression. More recent PET and SPECT imaging studies disagree as to whether D2 receptor binding and dopamine release after amphetamine are altered in major depression.

Cholinergic system

Cholinergic neurons project diffusely throughout the cortex, and it has been proposed that cholinergic hyperactivity could contribute to depression (see Ch. 13). Support for overactivity of the cholinergic system in the pathogenesis of depression is based on findings such as these: (1) cholinergic input reduces REM latency (decreased REM latency is seen in major depression) and the anticholinergic properties of some antidepressants; (2) in some cases, mania is reduced and depression induced by lecithin, an acetylcholine precursor; and (3) following abrupt withdrawal of anticholinergic medications, cholinergic rebound can cause a relapse of depression (Dubovsky & Ruzan, 1999).

Glutamatergic system

Glutamate is the major excitatory neurotransmitter in the CNS, and binds to the N-methyl-D-aspartate (NMDA) receptor (see Ch. 17). The antidepressant effects documented in emerging data on drugs that antagonize NMDA receptors, such as ketamine, suggest a role for the glutamatergic system in depressive disorders. Lower glucose uptake in dorsolateral prefrontal cortex in depression, as indicated by PET imaging studies of the brain using the glucose analogue [^{18}F]-FDG, could reflect less glutamate–glutamine turnover as a result of less glutaminergic activity in the cortex (Kegeles et al., 2003). Morphometric studies of pyramidal cells in neocortex and magnetic resonance spectroscopic analysis of glutamate will help clarify the state of the glutaminergic system in mood disorders (See also Box).

GABAergic system

γ -Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in almost all areas of the CNS, and regulates many CNS functions (see Ch. 18). Lower GABAergic activity may play a role in major depression. In depressed patients,

magnetic resonance spectroscopy studies have observed lower GABA levels in occipital cortex, and lower CSF GABA levels and plasma concentrations of GABA have also been reported (Petty & Schlessser, 1981). We have found that low CSF GABA may be inversely proportional to severity of anxiety in major depression. Lamotrigine enhances GABAergic activity, and has demonstrated antidepressant effects and prevention of recurrent depressions in BD patients (Bowden, 2001). That anticonvulsant mood stabilizers act by enhancing GABAergic function and the anticonvulsant effects of ECT suggests GABA plays a role in mood stability, and a GABA deficiency may favor mood swings. These pathophysiological contributions of GABA and therapeutic effects of GABAergic medications in mood disorders may also be mediated via effects on other neurotransmitter systems (Dubovsky & Ruzan, 1999).

Cortical-hypothalamic-pituitary-adrenal axis

Multiple methods of measuring cortical-hypothalamic-pituitary-adrenal axis activity have provided evidence of altered function in patients with major depression, including elevated corticotropin-releasing factor (CRF) concentrations in CSF; blunted adrenocorticotropic hormone (ACTH) and β -endorphin responses after intravenous CRF administration; lower CRF binding in prefrontal cortex in depressed suicides; pituitary gland enlargement; adrenal gland enlargement; hypercortisolism and elevated CSF cortisol concentrations; blunted plasma glucocorticoid, ACTH, and β -endorphin nonsuppression after dexamethasone administration; higher urinary free cortisol concentrations; elevated 5-hydroxytryptophan-induced cortisol secretion; higher ACTH-induced cortisol secretion; and higher ACTH and cortisol responses to CRF after dexamethasone pretreatment (Amsterdam et al., 1988).

In depressed patients, cortical-hypothalamic-pituitary-adrenal axis hyperactivity can be explained by the hypersecretion of CRF, as well as secondary pituitary and adrenal gland hypertrophy leading to autonomous heightened end-organ responses. Impaired negative feedback at various CNS sites including the hippocampus and pituitary are also likely to contribute. Downregulation of hippocampal mineralocorticoid receptors and expression is reported in depressed suicides (Lopez et al., 1998). Glucocorticoid receptor gene methylation and expression changes contribute to the dysregulation of the HPA axis resulting from childhood adversity. In BD, hyperactivity of the cortical-hypothalamic-pituitary-adrenal axis has been observed (Kirilke et al., 1988). This increase in cortical-hypothalamic-pituitary-adrenal axis activity has also been observed in mixed mood states, in mania, and in depression in rapid-cycling patients. Partial reversal of HPA overactivity is associated with treatment and recovery from depression (see also in Ch. 55).

Thyroid axis

Approximately 5 to 10% of individuals evaluated for depression have previously undetected or subclinical thyroid dysfunction. Jackson (Jackson, 1998) suggests that most patients with depression, although generally viewed

as chemically euthyroid, have altered thyroid function, including slight elevation of the serum thyroxine (T4), loss of the nocturnal TSH rise, and blunted thyrotropin (TSH) response to thyrotropin-releasing hormone (TRH) stimulation. A downregulation of TRH receptors in the pituitary in response to the increased levels of TRH secreted into the hypophyseal portal circulation might explain the blunting of the TSH response to TRH challenge. CSF transthyretin levels are low in major depression (Sullivan et al.) and, as a transporter of T4 into the brain, a deficiency of transthyretin may contribute to intracerebral hypothyroidism.

BD patients have shown abnormalities of the hypothalamic-pituitary-thyroid axis, demonstrated by an exaggerated TSH response to TRH and elevated basal plasma concentrations of TSH (Haggerty et al., 1987). In contrast, a blunted TSH response to TRH, the presence of antithyroid microsomal or antithyroglobulin antibodies (Lazarus et al., 1986), and a blunted or absent nocturnal surge in levels of plasma TSH (Souetre et al., 1988) have also been observed in patients with BD.

Other neuropeptides

Mood disorders are associated with alterations in the activity of the growth hormone axis (see Ch. 20). A blunted growth hormone response to clonidine, an α_2 -receptor agonist, has been consistently found in depression. Increased growth hormone secretion during the day and decreased nocturnal growth hormone secretion have also been observed in depressed patients. Depressed patients have lower CSF concentrations of somatostatin, compared to those with schizophrenia and normal controls. While lower CSF somatostatin is a state-dependent marker of depression, it occurs in a number of unrelated nonpsychiatric conditions, and thus appears to be relatively nonspecific.

Brain growth factors

Brain growth and development are affected by multiple neurochemical factors, including thyroid hormones, somatostatin, growth hormone and brain-derived neurotrophic factor (BDNF) (see Ch. 29). BDNF, a major neurotrophic factor in the brain, is involved in survival and guidance of neurons during development, and is also required for the survival and function of neurons in the adult brain (Duman et al., 2000). For example, it has been shown to play a role in long-term potentiation, a cellular model of learning and memory, thus influencing plasticity (Figurov et al., 1996).

Antidepressant treatment has, in recent studies, been shown to upregulate the cyclic adenosine monophosphate (cAMP)-response element-binding protein (CREB) cascade and expression of BDNF (Duman et al., 1999). This upregulation of CREB and BDNF raises the possibility that antidepressant treatment could oppose the cell death pathway, possibly via increased expression of the onco-gene Bcl-2. Increased expression of Bcl-2 in brain and cultured cells and inhibition of apoptosis of cultured cerebellar granule neurons have been reported with lithium treatment (Duman et al., 2000). Mice lacking the BDNF TrkB receptor fail to show behavioral and neurogenic responses to antidepressants.

Substance P

Substance P, an undecapeptide, is abundant both in the periphery and in the central nervous system. It is usually colocalized with some of the classical neurotransmitters, most commonly serotonin. Substance P is thought to have a role in the regulation of pain, asthma, psoriasis and inflammatory bowel disease and, in the CNS, emesis, migraine, schizophrenia, depression and anxiety. The substance P-prefering receptor neurokinin-1 has been focused on most intensively in drug development, and existing preclinical and clinical literature is suggestive, but not conclusive, concerning a role of substance P and neurokinin-1 receptors in the pathophysiology of depression and/or anxiety disorders. Originally studied as potential analgesic compounds, recent evidence suggested that neurokinin-1 receptor antagonists might possess antidepressant and anxiolytic properties. Thus far, this has not been confirmed by controlled clinical studies. Evidence of impaired substance P-mediated neurotransmission in depression is lacking and CSF studies of substance P in depressed subjects are not in agreement.

NEUROANATOMICAL AND NEUROPATHOLOGICAL CORRELATES OF MOOD DISORDERS

Neuroimaging studies of major depression have identified structural and functional abnormalities in multiple areas of the orbital and dorsal lateral prefrontal cortex, hippocampus and amygdala and in related parts of the striatum and thalamus. Neuromorphology and neuromorphometry in primary mood disorders, and localization of pathology in major depressive episodes arising secondary to cerebral lesions, have been assessed with structural magnetic resonance imaging (MRI) (see Ch. 42 for a review). Briefly, although the specific structure affected may vary among studies and among patients, there is a convergence of findings from structural imaging studies implicating a circuit involving the medial and dorsolateral prefrontal cortex, anterior cingulate, ventral striatum, pallidum, thalamus and hippocampus. There has been no clear evidence of global atrophy in mood disorders, although there are reports that patients with major depression have smaller basal ganglia, cerebellum, and possibly frontal lobe, perhaps indicating local atrophy. BD is associated with smaller cerebellum, possibly smaller temporal lobe, and changes in the hippocampus. BD and elderly major depression patients both show increased rates of subcortical white matter and periventricular hyperintensities. Lesions in these selected brain regions incurred as a result of injuries, such as anterior tumors or stroke, can be involved in the pathogenesis of mood disorders. The aging process may also contribute to the emergence of depression by causing lesions in these anatomic areas.

Functional neuroimaging methods

The emergence of functional neuroimaging methods permits the study of patients across the course of their illness.

Functional imaging tools such as PET, functional magnetic resonance imaging (fMRI), and SPECT allow *in vivo* characterization of neurophysiologic and/or neurotransmitter correlates of normal and pathologic emotional states, chronic or recurrent illness, and treatment response and resistance.

Functional neuroimaging findings have consistently observed prefrontal lobe dysfunction, indicated by lower blood flow and glucose metabolism in dorsal and lateral prefrontal cortex, and increased activity in some ventral structures in mood disorders. There is evidence of abnormalities in basal ganglia, temporal lobe and related limbic structures, that accompany or are independent of structural changes. MDD is primarily associated with dysfunction in the prefrontal cortex, amygdala, anterior cingulate and basal ganglia, while BD depression appears to be also associated with dysfunction in the temporal lobe, in addition to these other areas (Drevets et al., 2008). Some of these functional abnormalities appear mood state-dependent and are located in brain regions involved in normal mood regulation and some pathologic emotional states. Such state-dependent neurophysiologic differences between depressives and control subjects may thus indicate areas where physiologic activity changes to mediate, or respond to, the emotional, behavioral and cognitive manifestations of major depressive episodes.

Other abnormalities are more trait-like, and persist following symptom remission. They are found in orbital and medial prefrontal cortex areas where postmortem studies have also documented reductions in cortex volume and histopathologic changes in primary mood disorders (Mann & Arango, 1999). Receptor binding studies with PET scanning identify 5-HT_{1A} receptor binding changes in brainstem, prefrontal cortex, amygdala and temporal cortex in major depression that are also apparent during remission between episodes, indicating a biologic trait. Evidence from brain mapping, lesion analysis and electrophysiologic studies of humans and experimental animals suggests that these areas appear to modulate emotional behavior and stress responses. Thus, it is hypothesized that dysfunction involving these regions plays a role in the pathogenesis of depressive symptoms. Taken together, these findings implicate interconnected neural circuits in which pathologic patterns of neurotransmission may result in the emotional, cognitive, motivational and behavioral manifestations of primary and secondary mood disorders.

Brain banks with well-characterized postmortem human tissue permit study of the brain changes in mood disorders, and such findings can then be correlated with those of functional neuroimaging studies (Mann & Arango, 1999). *In vivo* neuroimaging data, delimiting areas where gray matter volume is abnormal and characterizing the clinical conditions under which such abnormalities are evident, are beginning to also guide postmortem studies of mood disorders.

The development of selective ligands for neuropeptidergic imaging provides expanding capabilities for noninvasive quantitation of *in vivo* receptor binding and neurotransmitter function. For example, PET and SPECT studies have yielded important information regarding the role of serotonergic receptors in the pathogenesis of mood disorders (see "Serotonergic System" above). Such studies facilitate a more complete characterization of the neurotransmitter abnormalities suggested by

studies of postmortem tissue, body fluids and neuroendocrine function. Combining PET and SPECT technology with genotyping of functional polymorphisms in serotonin receptor genes allows for more precise characterization of potentially important biological intermediate endophenotypes that are more sensitive indices of the pathology of mood disorders. Repeated studies in different mood states and examination of familial and developmental effects using such methods will help understand the pathophysiology of mood disorders and perhaps provide useful biomarkers for measuring and monitoring treatment effects.

Stress, glucocorticoids and neuroplasticity

The potential hyperactivation of the HPA axis in mood disorders has been revisited in recent years, in large part due to the growing recognition of the specific brain areas in which atrophy (loss), or a neuroplastic event, may be present in many patients (see Chapter 55). It remains to be fully elucidated to what extent these findings of atrophy represent the sequelae of biochemical changes (for example, in glucocorticoid levels) accompanying repeated affective episodes *per se*.

The latter suggestion receives support from the observation that chronic stress or glucocorticoid administration has been demonstrated to produce atrophy and death of vulnerable hippocampal neurons in rodents and primates. Furthermore, MRI studies have revealed reduced hippocampal volumes in patients with Cushing's disease and post-traumatic stress disorder (PTSD), conditions associated with hypercortisolism. One of the most consistent effects of stress on cellular morphology is atrophy of hippocampal neurons. This atrophy is observed in the CA3 pyramidal neurons, but not in other hippocampal cell groups (Sapolsky, 2000) (i.e., CA1 pyramidal and dentate gyrus granule neurons). Atrophy of CA3 pyramidal neurons also occurs upon exposure to high levels of glucocorticoids, suggesting that activation of the HPA axis likely plays a major role in mediating stress-induced atrophy. In addition, long-term exposure to stress (i.e., for several months) has also been associated with loss of hippocampal neurons in the CA3 pyramidal cell layer (Sapolsky, 2000) (and see Chapter 55).

Thus, it is possible that recurrent mood disorders, such as bipolar disorder, may lower the threshold for cell death and/or atrophy in response to a variety of other physiological (e.g., normal aging) and pathological (e.g., ischemic) events, and thereby contribute to a variety of deleterious health-related effects.

INTRACELLULAR SIGNALING PATHWAYS

Multi-component cellular signaling pathways interact at various levels forming complex signaling networks that allow the cell to receive internal and external cues, process these cues, and respond to information. They also play a crucial role in the integration and fine-tuning of physiologic processes, and thus it is not surprising that abnormalities in signaling pathways have now been identified in a variety of human

diseases. Furthermore, signaling pathways represent major targets for a number of hormones, including glucocorticoids, thyroid hormones, and gonadal steroids. The biochemical effects of these signaling pathways may play a role in mediating certain clinical manifestations of altered hormonal levels in mood disorder subjects, e.g., the frequent onset of bipolar disorder in puberty, triggering of depressive episodes in the postpartum period, and triggering of mood episodes in response to exogenous glucocorticoids.

Complex signaling networks may be especially important in the CNS, where they “weigh” and integrate diverse neuronal signals and then transmit them to effectors, thereby forming the basis for a complex information-processing network (Figure 60-1). The high degree of complexity generated by these signaling networks may be one mechanism by which neurons acquire the flexibility to generate the wide range of responses observed in the nervous system. 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 mood disorder.

The G-protein–subunit/cyclic adenosine monophosphate (cAMP)–generating signaling pathway

Postmortem brain studies in bipolar patients show increased levels of the stimulatory G protein ($G_{\alpha s}$) accompanied by increases in post-receptor-stimulated adenylyl cyclase (AC) activity in bipolar disorder (see Chs. 21 and 22). These observations are further supported by a study showing increased agonist-activated [^{35}S]GTP γ S binding to G protein α subunits in the frontal cortical membranes of bipolar disorder patients (Wang & Friedman, 1996). Several studies have also found elevated $G_{\alpha s}$ protein levels and mRNA levels in peripheral circulating cells in bipolar disorder, although the dependency of this finding on clinical state remains unclear. However, there is at present no evidence to suggest that the alterations in the levels of $G_{\alpha s}$ are due to a mutation in the $G_{\alpha s}$ gene itself. There are numerous transcriptional and post-transcriptional mechanisms that regulate the levels of G-protein subunits, and the elevated levels of $G_{\alpha s}$ could potentially represent the indirect sequelae of alterations in any one of these other biochemical pathways.

There is growing consensus that the ability of the “simple” monovalent cation lithium to treat multiple aspects of an illness as complex as bipolar disorder arises from its major effects on intracellular signaling pathways, rather than on any single neurotransmitter system *per se*. Although it appears that the lithium ion (at therapeutic concentrations) does not directly affect G-protein function, there is considerable evidence that chronic lithium administration affects G-protein function (Jope, 1999). It might be postulated that these G-protein effects of lithium, which would theoretically attenuate excessive signaling through multiple pathways, likely contribute to lithium’s long-term prophylactic efficacy in protecting susceptible individuals from spontaneous, stress-induced, and drug- (e.g., antidepressant and stimulant) induced cyclical mood episodes.

The protein kinase C signaling pathway

Protein kinase C (PKC) exists as a family of closely related kinase subspecies, has a heterogeneous distribution in the brain (with particularly high levels in presynaptic nerve terminals), and, together with other kinases, appears to play a crucial role in the regulation of synaptic plasticity and various forms of learning and memory (see Ch. 25). PKC is one of the major intracellular mediators of signals generated upon external stimulation of cells via a variety of neurotransmitter receptors that induce the hydrolysis of various membrane phospholipids; these neurotransmitter receptors include muscarinic M1, M3, and M5 receptors; noradrenergic $\alpha 1$ receptors; metabotropic glutamatergic receptors; and the serotonergic 5HT_{2A} receptor.

To date, there have only been a limited number of studies directly examining PKC in bipolar disorders. Although this view is undoubtedly an oversimplification, particulate (membrane) PKC is sometimes viewed as the more active form of PKC, and thus an examination of the subcellular partitioning of this enzyme can be used as an index of the degree of activation. Friedman et al., (1993) investigated PKC activity and PKC translocation in response to serotonin in platelets obtained from bipolar disorder patients before and during lithium treatment. They reported that the ratios of platelet membrane-bound to cytosolic PKC activities were elevated in the manic patients. In addition, serotonin-elicited platelet PKC translocation was enhanced in those patients. In postmortem brain tissue from bipolar patients, (Wang & Friedman, 1996) found increased PKC activity and translocation compared with controls, effects which were accompanied by elevated levels of selected PKC isozymes.

Lithium, at therapeutically relevant concentrations, attenuates PKC activity and downregulates the expression of PKC isozymes α and ϵ in the frontal cortex and hippocampus (Manji & Lenox, 2000). Chronic lithium administration has also been demonstrated to dramatically reduce the hippocampal levels of a major PKC substrate, myristoylated alanine-rich C kinase substrate (MARCKS), which has been implicated in regulating long-term neuroplastic events. Although these effects of lithium on PKC isozymes and MARCKS are striking, a major problem inherent in neuropharmacologic research is the difficulty in attributing therapeutic relevance to any observed biochemical finding.

Glycogen synthase kinase

A crucial kinase that functions as an intermediary in numerous intracellular signaling pathways is the enzyme glycogen synthase kinase-3 (GSK-3). GSK-3, a highly conserved enzyme in evolution, is found in two nearly identical isoforms (variations) in mammals, α and β (see Ch. 25). This enzyme was first discovered (and named) based on its ability to phosphorylate, and thereby inactivate, the enzyme glycogen synthase, an action that leads to a decrease in the synthesis of glycogen. GSK-3 is unique among kinases in that most intracellular signals to GSK-3 inactivate this enzyme. Signals deactivating GSK-3 arise from insulin stimulation, numerous growth factors (e.g., phosphoinositide [PI] 3-kinase), and

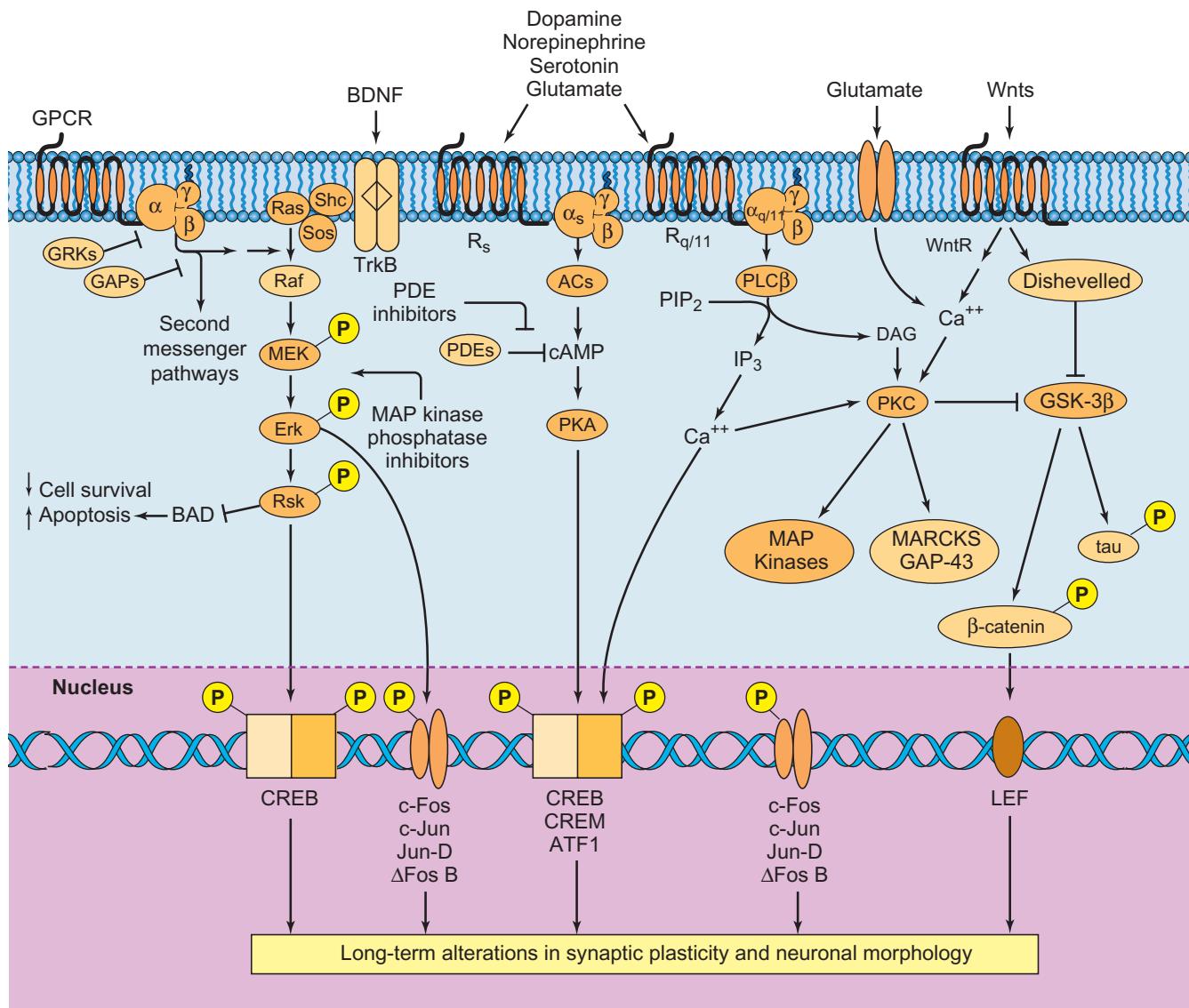


FIGURE 60-1 Major intracellular signaling pathways involved in neural and behavioral plasticity. The figure depicts some of the major intracellular signaling pathways involved in neural and behavioral plasticity. Cell surface receptors transduce extracellular signals such as neurotransmitters and neuropeptides into the interior of the cell. Most neurotransmitters and neuropeptides communicate with other cells by activating seven transmembrane spanning G-protein-coupled receptors (GPCRs). As their name implies, GPCRs activate selected G proteins, which are composed of α and $\beta\gamma$ subunits. Two families of proteins turn off the GPCR signal, and may therefore represent attractive targets for new medication development. G-protein-coupled receptor kinases (GRKs) phosphorylate GPCRs and thereby uncouple them from their respective G proteins. GTPase activating proteins (GAPs, also called RGS or regulators of G-protein-signaling proteins) accelerate the G-protein turn-off reaction (an intrinsic GTPase activity). Two major signaling cascades activated by GPCRs are the cAMP generating second messenger system and the phosphoinositide (PI) system. cAMP activates protein kinase A (PKA), a pathway that has been implicated in the therapeutic effects of antidepressants. Among the potential targets for the development of new antidepressants are certain phosphodiesterases (PDEs). PDEs catalyze the breakdown of cAMP; thus PDE inhibitors would be expected to sustain the cAMP signal, and might represent an antidepressant augmenting strategy. Activation of receptors coupled to PI hydrolysis results in the breakdown of phosphoinositide 4,5-biphosphate (PIP_2) into two second messengers—inositol 4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 mobilizes Ca⁺⁺ from intracellular stores, whereas DAG is an endogenous activator of protein kinase C (PKC), which is also directly activated by Ca⁺⁺. PKC, PKA, and other Ca⁺⁺-dependent kinases directly or indirectly activate several important transcription factors, including CREB, CREM, ATF-1, c-Fos, c-Jun, Jun-D and Δ Fos B. Endogenous growth factors such as brain-derived neurotrophic factor (BDNF) utilize different types of signaling pathways. BDNF binds to and activates its tyrosine kinase receptor (TrkB); this facilitates the recruitment of other proteins (SHC, SOS), which results in the activation of the ERK-MAP kinase cascade (via sequential activation of Ras, Raf, MEK, Erk and Rsk). In addition to regulating several transcription factors, the ERK-MAP kinase cascade, via Rsk, downregulates BAD, a pro-apoptotic protein. Enhancement of the ERK-MAP kinase cascade may have effects similar to those of endogenous neurotrophic factors; one potential strategy is to utilize inhibitors of MAP kinase phosphatases (which would inhibit the turn-off reaction) as potential drugs with neurotrophic properties. In addition to utilizing GPCRs, many neurotransmitters (e.g., glutamate and GABA) produce their responses via ligand-gated ion channels. Although these responses are very rapid, they also bring about more stable

developmental signals. A number of endogenous growth factors (e.g., nerve growth factor and brain-derived neurotrophic factor [BDNF]) use the PI 3-kinase signaling cascade as a major effector system. Thus, growth factors may bring about many of their neurotrophic and/or neuroprotective effects, at least in part, by GSK-3 inhibition. GSK-3 phosphorylates—and thereby inactivates—many transcription factors and modulates the function of cytoskeletal proteins. Inhibition of GSK-3 thus results in the release of this inhibition and activation of multiple cellular targets.

Rapidly increasing evidence suggests that GSK-3 plays an important role in regulating neuroplasticity and cellular resilience. Studies have suggested that changes in GSK-3-mediated MAP-1B (a cytoskeletal protein) phosphorylation are associated with the loss and/or unbundling of stable axonal microtubules. Furthermore, GSK-3 inhibition results in the accumulation of synapsin I, a protein involved in synaptic vesicle docking and release of growth cone-like areas.

In addition to its putative role in regulating synapse formation and axonal growth, there is the role of GSK-3 in regulating cell death (apoptosis) in mature neuronal tissue and the development of GSK-3 inhibitors as novel therapeutic agents for bipolar disorder and classical neurodegenerative diseases.

BDNF and Bcl-2

Activation of the HPA axis appears to play a critical role in mediating hippocampal atrophy. In addition to directly causing neuronal atrophy, stress and glucocorticoids also appear to reduce cellular resilience, thereby making certain neurons more vulnerable to other insults such as ischemia, hypoglycemia and excitatory amino acid toxicity (see Chs. 29 and 37). The reduction in the resilience of hippocampal neurons may also reflect the propensity for various stressors to decrease the expression of BDNF in this region (Duman, 2002). BDNF and other neurotrophic factors are necessary for the survival and function of neurons, implying that a sustained reduction of these factors could affect neuronal viability. Increasing evidence suggests that neurotrophic factors inhibit cell death cascades by (in large part) activating the mitogen-activated protein (MAP) kinase signaling cascade, and upregulating major cell survival proteins such as bcl-2 (Manji & Chen, 2002).

Bcl-2 is a major neuroprotective protein, and bcl-2 overexpression protects neurons against diverse insults, including ischemia, the neurotoxic agent methyl-phenyl-tetrahydropyridine (MPTP), β -amyloid, free radicals, excessive glutamate and growth factor deprivation. Accumulating data suggest that bcl-2 is not only neuroprotective, but also exerts neurotrophic

effects and promotes neurite sprouting, neurite outgrowth and axonal regeneration. If enhanced bcl-2 expression appears to be capable of offsetting the potentially deleterious consequences of stress-induced neuronal endangerment, then pharmacologically induced upregulation of bcl-2 may have considerable utility. Overall, it is clear that the neurotrophic factor/MAP kinase/bcl-2 signaling cascade plays a critical role in cell survival in the CNS, and that there is a fine balance maintained between the levels and activities of cell survival and cell death factors. Modest changes in this signaling cascade or in the levels of the bcl-2 family of proteins (potentially due to genetic, illness, or insult-related factors) may therefore profoundly affect cellular viability.

Intracellular calcium signaling

Impaired regulation of Ca^{2+} cascades has been found to be the most reproducible biological measure abnormality described in bipolar disorder research (see Ch. 24). Calcium ions play a critical role in regulating the synthesis and release of neurotransmitters, in neuronal excitability, and in long-term neuroplastic events, and it is thus not surprising that a number of studies have investigated intracellular Ca^{2+} in peripheral cells, particularly in bipolar disorder.

To date, numerous studies (approximately 15) have consistently revealed elevations in basal intracellular Ca^{2+} levels in platelets, lymphocytes, or neutrophils of patients with bipolar disorder, with only few studies reporting negative results. This elevation in basal Ca^{2+} represents one of the most replicated findings in bipolar disorder research. Higher platelet intracellular Ca^{2+} elevations have also been found in response to stimulation with thrombin, platelet activator factor (PAF), serotonin, dopamine and thapsigargin. In lymphocytes, the same higher elevations were observed when the cells were stimulated with phytohemagglutinin, concavalin A, thrombin and (as in platelets) with thapsigargin and serotonin. However, a few caveats need to be applied to these positive studies. There is considerable evidence that a variety of circulatory factors may influence the activity of blood cells and elements, and bipolar disorder patients are known to have numerous neurohormonal abnormalities (such as catecholamine and cortisol level abnormalities). Furthermore, many studies did not employ an extensive medication washout period, raising the possibility that the elevations in Ca^{2+} in circulating cells are simply secondary manifestations. The regulation of free intracellular Ca^{2+} is a complex, multifaceted process, and the abnormalities observed in bipolar disorder could arise from abnormalities at a variety of levels. Ongoing

changes via regulation of gene transcription. One pathway gaining increasing recent attention in adult mammalian neurobiology is the Wnt signaling pathway. **Wnts** are a group of glycoproteins active in development, but now known to play important roles in the mature brain. Binding of Wnts to the Wnt receptor (**WntR**) activates an intermediary protein, **Disheveled**, which regulates a glycogen synthase kinase (**GSK-3 β**). GSK-3 β exerts many cellular effects; it regulates cytoskeletal proteins, including **tau**, and also plays an important role in determining cell survival/cell death decisions. GSK-3 β has recently been identified as a target for Li $^{+}$'s actions. GSK-3 β also regulates phosphorylation of **β -catenin**, a protein which, when dephosphorylated, acts as a transcription factor at LEF (lymphoid enhancer factor) sites. **CREB**, cAMP response element binding protein; **R_q** and **R_s**, extracellular GPCRs coupled to stimulation or inhibition of adenylyl cyclases (**ACs**), respectively. **R_{q/11}**, GPCR coupled to activation of phospholipase C (**PLC**), **MARCKS**, myristoylated alanine rich C kinase substrate, a protein associated with several neuroplastic events. Modified and reproduced with permission from Payne, J.L. et al. (2004) Cellular biology of bipolar disorder. In *Neurobiology of Mental Illness*. Charney, D.S. and Nestler, E.J. (eds) Oxford University Press, Oxford, UK.

studies should serve to delineate the specific regulatory sites at which the impairment occurs in bipolar disorder.

ANXIETY DISORDERS

The development of mild forms of anxiety and neurovegetative and/or cognitive responses to stress may represent an adaptive evolutionary step against environmentally (external) or self-triggered (internal) threats, but maladaptive reactions have also emerged in human evolution. Thus, anxiety disorders are maladaptive conditions in which disproportionate responses to stress, or even self-evoked responses, are displayed. Anxiety disorders are one of the most frequent psychiatric illnesses, and have a lifetime prevalence of almost 29% (Kessler et al., 2005). The most common presentations are generalized anxiety disorder, with a lifetime prevalence rate of close to 6%; social phobia, 12%; panic disorder, almost 5% (236, 237); and PTSD, 6.8% (Kessler et al., 2005). Specific phobias, acute stress and obsessive-compulsive behavior are other clinical presentations of anxiety disorders.

THE NEUROCHEMISTRY OF FEAR AND ANXIETY

Much of our knowledge of the human neural substrates of fear and anxiety is derived from pioneering work using cat and rodent models. As techniques have advanced, our understanding of the anatomy, neurochemistry and physiology of these responses has progressed. Particularly, the development of functional imaging techniques has allowed us to confirm that observations made in a number of animal species may also apply to humans.

The fear response necessarily begins with perception. Most sensory information is relayed via the thalamus to the sensory cortices, which are responsible for recognition and cognitive appraisal of a threat. Two exceptions to this pathway are olfactory input, which may reach the amygdala and entorhinal cortex directly, and visceral organ input, which proceeds from nuclei in the brainstem to the LC. The LC appears to have several roles that are important in the regulation of anxiety. On one level, noradrenergic efferents appear to have a key role in regulating the peripheral sympathetic response; LC firing appears necessary for the generation of a physiological response to anxiogenic stimuli. Additionally, the LC, perhaps as a mediator of sensory information in the cortex-to-thalamus pathway, also plays a role in directing attention towards salient, threatening stimuli. Notably, there is evidence that the release of CRH in the LC is necessary for the enhanced firing seen in high-anxiety conditions.

The amygdala is perhaps the best-studied, and most strongly implicated, brain structure in anxiety and fear. Electrical stimulation of the amygdala produces fear-like behavioral and physiological responses in animals, and increases the experience of fear in human subjects. Additionally, amygdala stimulation leads to cortisol secretion and HPA axis activation in animals, probably via

outputs to the hypothalamus and the bed nucleus of the stria terminalis. It has been suggested that the amygdala may represent a sort of ‘master switch’ of fear, which projects to a variety of areas. This unitary-mediator hypothesis explains the constellation of behavioral and physiological responses that co-occur so consistently.

Multiple studies suggest that particular pharmacological agents can provoke symptoms of anxiety in susceptible individuals. These agents can be generally classified into two categories: those that reduce available oxygen, and appear to act on peripheral areas; and those that directly manipulate neurotransmitter systems. A number of pharmacological agents have been noted to increase anxiety (generally measured as panic symptoms in patients with panic disorder) in susceptible individuals.

These findings provide important clues in understanding the underlying neurobiology of anxiety disorders. However, the broad spectrum of agents that can produce anxiety symptoms makes it difficult to define one system, or pathway, in the brain most responsible for anxiety. It is possible, however, to broadly define the targets of compounds that may have general mechanisms of action. In this regard, carbon dioxide, sodium lactate and bicarbonate fall into the first category—all act peripherally, resulting in increased respiration, heart rate and other signs of sympathetic nervous system activity. These mechanisms of action helped form a hypothesis in which patients with anxiety disorders may interpret physiological changes (such as increased heart rate, increased respiration, et cetera) as more serious than they really are, and thus the patients respond with increasing anxiety.

It may be that any peripherally aversive stimulus—especially one that stimulates sympathetic activity—thus has the potential to activate brain areas of prime importance in the formation of anxiety symptoms. As a result of pharmacological challenge studies, biochemical assays, neuroimaging and studies of animal models, a number of centrally acting neurotransmitters and their relevant neural circuits have been implicated in anxiety. These neurotransmitters include NE, serotonin, GABA, neuropeptide Y, cholecystokin and substance P.

Noradrenergic systems

Preclinical studies using pharmacological manipulation and electrical stimulation have suggested the involvement of noradrenaline and the LC in anxiety- and fear-like behaviors. The strongest human evidence comes from studies of the anxiogenic and anxiolytic properties of centrally acting selective noradrenergic drugs, some of which exert these actions by increasing LC activity. The selective β -agonist isoproterenol induces anxiety and panic attacks in some patients. However, directly attributing the effects of isoproterenol to enhanced central β -adrenergic receptor throughput is problematic, because this drug does not appear to cross the blood-brain barrier (see adrenergic receptors in Ch. 14).

More direct evidence of noradrenergic effects comes from studies using the α_2 adrenergic receptor antagonist yohimbine. α_2 adrenergic receptors are known to be present as auto-receptors on the cell bodies and terminals of noradrenaline

neurons, where they regulate the firing rate and noradrenaline released per nerve impulse, respectively. Thus, blockade of $\alpha 2$ receptors increases LC noradrenergic neuron firing, as well as NE release; this may explain how in patients with panic disorder, yohimbine increases anxiety and the frequency of panic attacks (Charney et al., 1984). Consistent with the behavioral data are neurobiological data showing that yohimbine increases cardiovascular responses and plasma MHPG and cortisol in panic patients relative to control subjects. Yohimbine has also been reported to produce a decrease in frontal blood flow in this patient population, effects which were not seen in control subjects.

Additional pharmacologic support for the role of the LC noradrenergic system in mediating anxiety symptoms comes from studies using clonidine, a centrally active $\alpha 2$ adrenoreceptor agonist, which decreases LC firing and NE release. Thus, clonidine has been shown to have some efficacy in the treatment of anxiety and in both spontaneous and induced panic disorder. Interestingly, the beneficial effects of clonidine subside over time, perhaps due to desensitization of the $\alpha 2$ receptor with continuous stimulation by a direct agonist. Furthermore, clonidine administration results in a greater degree of hypotension and larger reductions in plasma MHPG in patients with panic disorder relative to control subjects, raising the possibility of altered $\alpha 2$ adrenoreceptor sensitivity in panic disorder. Consistent with such a contention, NE and its metabolites measured in urine, blood, and CSF are elevated in panic disorder, PTSD, specific phobias, social anxiety and GAD, suggesting a dysregulation of the central and peripheral noradrenaline systems.

It has been postulated that the inhibitory inputs from the frontal cortex to limbic regions may be disrupted in anxiety disorders, resulting in unrestrained amygdala activity and thereby an increase in anxiety. In this context, yohimbine decreases metabolism in the cortical brain areas in PTSD patients compared with control subjects. Additionally, as mentioned, functional studies in panic disorder suggest an abnormal blood flow following administration of agents that result in panic symptoms. In addition to yohimbine (Woods et al., 1988), these studies have also been performed with lactate infusion. These findings in the cortex are consistent with the model by which cortical input to the amygdala provides a ‘top-down’ inhibition that is lacking in patients with anxiety disorders—and functions as a contributor to anxiety in patients. Also consistent with these data is the general finding that while low levels of anxiety increase cortical metabolism in humans, high levels of anxiety have been shown to decrease it (Gur et al., 1987). Thus, abnormal cerebral metabolism in anxiety may allow for abnormal amygdala activity. In this regard, recent preclinical work in rats has shown that stimulating specific areas of the medial PFC can increase the extinction of fear responses (Milad & Quirk, 2002).

Serotonergic system

Evidence on several levels implicates the serotonergic system in the treatment of anxiety, although not conclusively. The raphe nuclei presumably play an important role

in the serotonergic aspects of fear and anxiety. An excitatory projection from the LC to the dorsal raphe may be important in the serotonin release observed in the PFC, amygdala and hypothalamus in response to anxiogenic stimuli. Additionally, projections from the dorsal raphe also extend to and inhibit the LC, suggesting a possible negative feedback mechanism. Chronic, but not acute, SSRI administration suppresses LC firing in rats. The median raphe may also have some opposing effects; there is some evidence that output from this nucleus to the dorsal hippocampus acts to increase stress resistance, whereas LC projections to the median raphe suppress its firing.

A number of medications used in the treatment of anxiety have effects on serotonin neurotransmission. These medications include tricyclic antidepressant medications, SSRIs, and monoamine oxidase inhibitors (MAOIs). However, because these medications take weeks to exert their full anxiolytic effects, it is unlikely that blocking the reuptake (and thus increasing synaptic levels) of either serotonin or noradrenaline selectively is responsible for their anxiolytic properties—rather, it is suspected that the therapeutic effects are due to changes in gene expression, protein levels, and eventually changes in synaptic connections between neurons (see also in Ch. 15).

In spite of the large number of medications that target serotonin neurotransmission, consistent evidence implicating serotonin neurotransmission in the pathophysiology of anxiety is lacking. As reviewed by Charney and Drevets (2002), markers of the density and/or activity of serotonin reuptake transporters has been found to be increased, unchanged, or decreased in panic disorders depending on the study and experimental conditions. Furthermore, the results of pharmacological challenges to patients with anxiety disorders have been ambiguous. Thus, responses to serotonin precursors (such as L-tryptophan and 5-hydroxytryptophan) do not appear to be different between control subjects and those with panic disorder, or to be anxiogenic. Challenges with other more direct serotonin agonists are also inconclusive. In this regard, the 5-HT releasing agent fenfluramine has been reported to be anxiogenic and produce greater increases in plasma prolactin and cortisol in patients with panic disorder compared with control subjects. The 5-HT agonist m-chlorophenyl-piperazine (mCPP) has been shown to increase anxiety and plasma cortisol in some studies of panic disorder patients compared with control subjects. Tryptophan depletion in anxiety disorders has also not been generally informative. Indirect evidence also implicates serotonergic dysfunction in PTSD, but more work needs to be done (Charney & Drevets, 2002).

GABAergic system

The GABA system is the primary target for the acute treatment of anxiety. The benzodiazepines (BDZs) function by binding to a potentiator site on the GABA-A receptor, increasing the amplitude and duration of inhibitory postsynaptic currents in response to GABA binding. Unfortunately, the BDZs have problematic side effects, including sedation, cognitive impairment and addictive liability. Our growing

understanding of the GABA system may enable us to design more tolerable medications.

The GABA-A receptor displays enormous heterogeneity; it is composed of a combination of five subunits, of which there are at least 18 subtypes. The various receptors display variation in functional pharmacology, hinting at the multiple finely tuned roles that inhibitory neurotransmission plays in brain function. The majority of GABA-A receptors in the brain are targets of diazepam and other BDZs. For this reason, there has been considerable interest in determining if the desirable and undesirable effects of BDZs might be based on the differing receptor subtypes.

Much work has been focused in this direction, particularly using gene knockout technology. For instance, mutation of the BDZ-binding site of the GABA-A α -1 subunit in mice blocks the sedative, anticonvulsive, and amnesic, but not the anxiolytic, effects of diazepam. In contrast, the α -2 subunit (expressed highly in the cortex and hippocampus) is necessary for diazepam anxiolysis and myorelaxation. Thus, there is now optimism that an α -2 selective ligand will soon provide effective, acute treatment of anxiety disorders without the unfavorable side-effect profile of current BDZs. A compound with this preferential affinity has already been demonstrated to exert fewer sedative and depressant effects than diazepam in rat behavioral studies.

Current GABAergic drugs exert their actions almost immediately upon initiation of treatment, and there is optimism that novel medications of this class will also have immediate benefit, thereby circumventing one of the primary problems of many current non-BZD anxiolytic drugs, namely the delay in onset of action.

CRH and stress axes

The HPA axis is a major pathway by which stress exerts its effects on the brain and the rest of the body. The HPA axis is also believed to be relevant to development of anxiety and anxiety disorders (Bremner & Charney, 2002). In the HPA axis pathway, the lateral paraventricular nucleus of the hypothalamus releases CRH, which in turn stimulates the production of ACTH by the pituitary gland. This hormone (ACTH) stimulates the production of cortisol by the adrenal gland. Cortisol is considered a primary stress hormone of the body, having varied effects on metabolism, neurovegetative behaviors of organisms, and functions of neurons and neuronal systems. A primary receptor for cortisol—the glucocorticoid receptor—is localized to many brain regions important in the stress response, including the hippocampus (Ch. 55).

CRH and its receptors have generated increasing interest as a target for medications. In addition to the pituitary, CRH receptors are in the cortex, amygdala, LC, and regions of the hypothalamus. It has also been reported that CRH increases LC activity, and that local injection of CRH into the LC increases behavioral responses consistent with increased anxiety (Bremner & Charney, 2002). Furthermore, CRH antagonists have been repeatedly shown to have anxiolytic effects in animal models.

Consistent with overactivation of the HPA axis in PTSD are reports of a smaller hippocampal volume, a finding observed in animal models of stress. To date, a single study does

suggest the presence of abnormalities in the temporal lobe in panic disorder (Ontiveros et al., 1989). Studies also suggest an increase in cortisol release in response to stress in PTSD and panic disorder. Furthermore, a generally consistent finding is a chronic increase of CRH in patients with anxiety. Additional studies suggest changes in other mediators of the HPA axis, but these are generally less consistent.

A great deal of effort has gone into the development of antagonists to the types 1 and 2 CRH receptors, because CRH agonism has proven anxiogenic in a variety of animal models, and CRH antagonists have demonstrated anxiolytic properties in rodent and primate studies. Drugs targeting the CRH receptors are perhaps the most promising new class of anxiolytics and antidepressants. The orally active CRH antagonist antalarmin significantly reduces fear and anxiety responses in nonhuman primates and may be a candidate for further development. Several pharmaceutical companies have developed substituted-pyrimidine small-molecule CRH antagonists. Clinical trials are underway to examine the efficacy of these treatments in depression, and it is likely that complementary trials for the treatment of anxiety will also be undertaken. Targeting glucocorticoid receptors may also represent a possible mechanism to prevent stress-mediated neuronal plasticity changes.

Thus, the evidence that stress, and the stress hormone cortisol, may cause changes in hippocampal structure, hippocampal connections, and gene and protein expression suggests that viable targets of anxiolytic agents are cellular signaling pathways involved in neuroplasticity and the maintenance of cellular resilience.

Other neuropeptides

Neuropeptide Y

Neuropeptide Y (NPY) and its receptors may also be important in the regulation of anxiety and stress. NPY is synthesized in the arcuate nucleus, which receives LC input. In a number of rodent models, NPY administration has anxiolytic and, at somewhat higher doses, sedative effects. Likewise, NPY antagonizes CRH-induced stress responses, and suppresses LC firing when injected into the brainstem. There is some evidence to suggest that NPY is low under unstressed conditions, but is stimulated as a counteradaptation to stress. Additionally, NPY projections to the central amygdala, the nucleus accumbens, septum, periaqueductal gray (PAG), hippocampus, and other regions may be involved in NPY anxiolysis. As our understanding of the differing roles played by the various NPY receptors improves, NPY receptor agonists are likely to become a goal of anxiolytic development.

Cholecystokinin

Cholecystokinin (CCK) is another neuropeptide with a role in anxiety disorders. CCK-B receptor agonists reportedly have an anxiogenic effect in animals, and are anxi- and panicogenic in normal subjects and panic disorder patients (albeit patients are more sensitive). Likewise, suppression of CCK-B receptor expression or pharmacological antagonism blocks the acquisition of conditioned-fear response. CCK antagonists have yet to succeed in clinical trials (Pande et al., 1999).

Nevertheless, the CCK system remains an attractive target for drug development, particularly for panic disorders.

Substance P

The neuropeptide substance P (Sub P) binds to the neurokinin-1 receptor (NK-1; also known as tachykinin-1 receptor). Although originally targeted as a nociceptive pathway, antagonists of this receptor may exert anxiolytic and antidepressant effects. There is also evidence for an anxiolytic effect of Sub P when it is injected into the cholinergic nucleus basalis magnocellularis, suggesting region-specific effects. Furthermore, NK-1 activation in the hypothalamus inhibits CRH secretion. Several NK-1 antagonists appear anxiolytic in animal studies, and in a preliminary clinical trial the antagonist MK-869 was found to be as effective as paroxetine in treating anxiety and depression. Subsequent studies in depression have been negative.

INTRACELLULAR TARGETS FOR ANXIETY DISORDERS

Whereas genetic and pharmacological studies have focused predominantly on the receptors, transporters, and metabolic enzymes of neurotransmitters already implicated in anxiety, there also exists a more diverse group of gene knockouts with anxiety-related phenotypes. A number of animal studies have investigated anxiety-like behavior in knockouts of key intracellular signaling enzymes, including calcium/calmodulin-dependent protein kinase-II (CaMK-II) α , adenylylate cyclase type VIII, and PKC ϵ and γ . These studies raise the possibility that pharmacological manipulation of signaling cascades within cells might be exploited in the development of new anxiolytics.

Studies of PKC and anxiety provide a particularly good example. The knockout of either the ϵ or γ isoform confers resistance to anxiety and potentiates the action of benzodiazepines. Furthermore, PKC inhibition may have anxiolytic effects in humans; in a study of psychosocial functioning in women on long-term tamoxifen, which possesses both PKC- and estrogen-inhibiting properties, there was a statistical trend towards reduced anxiety in the treatment group. Likewise, valproic acid, an anticonvulsant and mood stabilizer that reduces expression of several PKC isoforms, may have efficacy in the treatment of panic disorder (Baetz & Bowen, 1998) and PTSD (Clark et al., 1999). The specific roles of PKC isozymes in anxiety behavior require study, but specific inhibitors may someday serve as anxiolytics or anxiolytic potentiators.

Inositol, the building block of the phosphoinositide intracellular signaling pathway, has been examined as a potential anxiolytic. In clinical trials, inositol has reported to be effective in both panic disorder (Benjamin et al., 1995) and depression (Levine et al., 1995), and animal data are also favorable. Because of inositol's status as a dietary supplement, there is little financial backing for studies of its efficacy and safety. The mechanism of action of inositol is not entirely clear, but it is believed to facilitate phosphoinositide signaling-coupled neurotransmission.

FUTURE DIRECTIONS AND THE DEVELOPMENT OF NOVEL THERAPEUTICS

This chapter describes neurobiological findings that are generating considerable scientific excitement about the development of novel agents for the treatment of mood and anxiety disorders. Most notably, there is a considerable body of evidence—both conceptual and experimental—suggesting that impairments in neuroplasticity and cellular resilience are demonstrable in certain mood and anxiety disorders. Thus, in addition to a variety of neurochemical changes, many patients also have pronounced structural alterations (e.g., reduced spine density, neurite retraction, overall neuropil reductions and/or volumetric changes on neuroimaging measures) in critical neuronal circuits. Therefore, for these disorders, optimal treatment may only be attained by providing both trophic and neurochemical support. The trophic support would enhance and maintain normal synaptic connectivity, thereby allowing the chemical signal to reinstate the optimal functioning of critical circuits necessary for normal affective functioning. It is thus noteworthy that many novel strategies currently being investigated for these disorders—including CRH antagonists, GR antagonists, glutamatergic agents and phosphodiesterase inhibitors—can be conceptualized as ‘plasticity enhancers’ that would be expected to exert trophic effects in addition to their effects on specific neurochemical systems.

It is hoped that our ever-expanding knowledge of the neurobiology of anxiety and anxiety disorders will yield entirely new pharmacological approaches to the treatment of these disorders. Despite some concerns with their validity, animal models continue to provide a useful screening mechanism for the development of novel anxiolytics. There are presently a number of promising new targets for anxiety disorders, and a new generation of drugs directed at some of these may come to market in the coming years. Traditionally, the neurochemical systems targeted in the treatment of anxiety disorders have been GABA, serotonin, and noradrenaline. Continuing the development of drugs modulating these transmitters will probably yield modestly more effective and/or better-tolerated medications. However, the results achieved with more innovative targets mentioned above promise both markedly better drugs and a more sophisticated understanding of pathological anxiety. Nongenetic mechanisms of gene regulation, termed epigenetic mechanisms, probably play a role in the formation of cellular memory and the modulation of neural circuitry. The interplay of transcription factors interacting with covalent DNA modifications is probably involved in modulating how previous experience may regulate subsequent behavioral responses.

In conclusion, emerging results from a variety of clinical and preclinical experimental and naturalistic paradigms suggest that a reconceptualization of the pathophysiology, course and optimal long-term treatment of severe mood and anxiety disorders may be warranted. An increasing number of strategies are being investigated to develop small-molecule agents to enhance cellular plasticity; this progress holds promise for the development of novel therapeutics for the long-term treatment of these devastating disorders.

INFLAMMATION, CYTOKINES AND GLUTAMATE: A NEW PATHWAY TO DEPRESSION

Joseph T. Coyle

Recent years have witnessed the evolution of the concept of inflammation from a vague descriptor of ill health to a fundamental disease process that links major depressive disorder to such diverse conditions as diabetes, cardiovascular disease and dementia. Meta-analyses of studies on community and clinical samples have revealed that the plasma levels of inflammatory markers including IL-6, IL-1, TNF- α and C-reactive protein (CRP) are significantly elevated in major depressive disorder (MDD) (Maes et al., 2010). Elevated levels of these cytokines are particularly prevalent in depression with a late life onset (Alexopoulos & Morimoto, 2011).

The occurrence of depression in the elderly is often the harbinger for Alzheimer's dementia, a condition associated with marked increase in brain inflammation. Obesity is also associated with elevated inflammatory markers and type II diabetes. The prevalence of major depressive disorder is significantly increased in type II diabetes and is associated with increased risk for diabetic complications including vascular disease and blindness. The risk for depression is significantly increased with myocardial infarction, a disorder also associated with elevated plasma cytokines and CRP. The co-occurrence of depression with myocardial infarction is a robust predictor of subsequent death. MDD with elevated plasma cytokines that occurs in these various medical illnesses is often poorly responsive to treatment with serotonin specific reuptake inhibitors (SSRIs). An important question concerns the direction of causality between inflammation and depression. Recent clinical studies strongly suggest that inflammation is the causal factor (Capuron et al., 2009). Chronic viral hepatitis C is now treated with a combination of interferon- α (IFN- α) and riboflavin. IFN- α is a cytokine of the early immune system, which induces the cellular release of other proinflammatory mediators such as IL-6. A significant proportion of patients treated with IFN- α develop major depressive disorder, and their cerebral spinal fluid levels of IL-6 correlate with depression symptoms.

What are the neurochemical processes that bridge the gap between elevated inflammatory markers in plasma and depression? Studies by Maes et al., (2010) indicate that proinflammatory cytokines induce indole amine 2,3-dioxygenase (IDO), which catabolizes tryptophan into the kynurenine pathway. Elevated IDO reduces plasma tryptophan levels, which ultimately attenuates the synthesis of serotonin in brain because tryptophan hydroxylase is not saturated by tryptophan. Of perhaps greater significance are the downstream metabolites of tryptophan, kynurenine and quinolinic acid. Kynurenine has anxiogenic effects, whereas quinolinic acid, an NMDA receptor

agonist, has prodepressive effects and at high concentrations has excitotoxic effects.

The potential role of excessive NMDA receptor agonism by quinolinic acid as an alternative pathway to depression has received clinical support from recent studies with the NMDA receptor antagonist ketamine (Diazgranados et al., 2010). In placebo-controlled trials a single dose of ketamine produced a rapid and persistent antidepressant effect lasting up to 10 days in patients selected for being poorly responsive to SSRI treatment. Li et al., (2010) have developed the evidence that the acute NMDA receptor blockade activates the mammalian target of the rapamycin (mTOR) pathway via rebound AMPA receptors activation, thereby promoting synaptogenesis in the prefrontal cortex (Li et al., 2010). Regardless of the precise mechanisms, this robust therapeutic effect of ketamine in cases of major depression unresponsive to traditional antidepressant treatment (approximately 50% of MDD patients are unresponsive) suggests the existence of a distinct pathophysiology for depression involving glutamatergic mechanisms and unrelated to the classical biogenic amine hypothesis.

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