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MOLECULAR AND CELLULAR MECHANISMS IN DEPRESSION

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Over the past three decades, considerable progress has been made in our understanding of the biology of depressive disorders. Still, there are a great number of unanswered questions regarding the relative roles specific biological systems may play in pathogenesis. This debate in part reflects a number of methodologic factors: a possibly over broad definition of the clinical syndrome of major depression; limitations inherent in studies using indirect measurement of brain neuronal activity; problems inherent in postmortem studies; and an overemphasis on cross-sectional rather than longitudinal studies. In this chapter, we review the current status of the neurochemical and cellular features of depressive disorders.

BACKGROUND

Although Freud put forth a hypothesis for understanding the psychological causes of depression in his classic paper, ''Mourning and Melancholia,'' he noted that some depressions were clearly biological in etiology. Research over the past 40 years has done much to point to likely ''culprits'' that are involved in the etiology of the disorder as well as in the mediation of treatment response; these have been reviewed several times recently (1,2).

Early research revolved around monoaminergic theories with particular emphasis first on norepinephrine and later serotonin. The basis for invoking these systems rested largely on a number of pharmacologic observations that have been termed ''the psychopharmacologic bridge.'' These observations included: reserpine, an early antihypertensive, caused depression in some patients and depleted monoamine stores in rat brain; iproniazid, a drug studied as an antitubercular agent, elevated depressed mood and inhibited monoamine degradation by the enzyme, monoamine oxidase; imipramine, a tricyclic compound originally studied as an antipsychotic, had potent antidepressant effects and blocked the reuptake of norepinephrine (and to some extent serotonin) into presynaptic neurons.

These observations led two groups of investigators (3,4) to argue that norepinephrine (NE) activity was decreased in depressive disorders and elevated in manic or excited states. Although a low norepinephrine state was the cornerstone of Schildkraut's catecholamine hypothesis (30), he also argued for other types of dysregulation, including altered receptor functioning. Indeed, more recent data have pointed to biological heterogeneity of norepinephrine activity in depression with some patients demonstrating low and others apparently elevated activity (5). Serotonin (5-HT) theories, in contrast, have emphasized decreased production or reuptake in depression.

As research has continued, investigators have noted a number of other alterations in depressed patients, including among others: elevated corticotropin-releasing hormone (CRH); elevated acetylcholine activity; increased γaminobutyric acid (GABA) levels; excessive glucocorticoid activity in psychotic major depression; hippocampal volume loss, perhaps reflecting the effects of excessive glucocorticoids on neurogenesis, and so on. These have in turn led to or been associated with a number of new biological hypotheses regarding why some individuals become depressed or develop specific symptoms. In the following sections we review the current status of these approaches.

NOREPINEPHRINE

Norepinephrine is a catecholamine that is found in various tissues, including brain, plasma, sympathetic nervous system, heart, and so on. It is synthesized from the amino acid tyrosine, which forms L-Dopa via the enzyme tyrosine

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hydroxylase. L-Dopa is converted to dopamine via dopa decarboxylase and then in turn is converted to norepinephrine via dopamine β-hydroxylase. In the adrenal and other tissues, norepinephrine is converted to epinephrine via phenyl-*N*-methyltransferase (PNMT). NE is degraded by the enzymes catechol-o-methyltransferase and monoamine oxidase.

Norepinephrine measured in urine or plasma is largely derived from non-central nervous system (CNS) sources. In contrast, much early work in this area emphasized 3-methoxy-4-hydroxyphenylglycol (MHPG), 20% to 30% of which is derived from brain. The earliest studies on urinary MHPG reported significantly lower levels in depressed patients than healthy controls (6). Further research revealed low urinary MHPG levels were seen, particularly in bipolar depressives and a subgroup of unipolar patients. As diagnostic nomenclature differentiated bipolar I from II patients, investigators reported low MHPG levels were characteristic of bipolar I and not II patients (5,7,8).

Unipolar depressed patients are heterogeneous in their MHPG levels. As indicated previously, a subgroup of unipolar patients demonstrate low MHPG levels, similar to those seen in bipolar I patients. In contrast, some unipolar patients demonstrate elevated MHPG levels (9). In these patients, urinary free cortisol is similarly elevated (10).

Catecholamine levels have been reported to parallel the state of the disorder in bipolar patients. Bipolar patients demonstrate significantly lower plasma NE and E levels when depressed than when euthymic or manic. Manic bipolar patients demonstrate elevated CSF, urine, or plasma MHPG levels than depressives or healthy controls (11–13). These data provide a rationale for measuring catecholamine output in mood disorder and invoking NE as playing an etiologic role; however, critics argue that some of the changes in levels may be secondary to such features as activity or agitation.

Urinary MHPG levels have been explored as possible tests for predicting antidepressant response. The earliest studies (14,15) pointed to low MHPG levels predicting positive responses to imipramine but not amitriptyline. High-MHPG patients responded to amitriptyline (14,15). These findings led Maas (14) to hypothesize that there were two forms of depression—one a low MHPG state reflected a norepinephrine depression; another characterized by high MHPG levels signified a serotonin depression. This hypothesis, although heuristic, has not been borne out. Subsequent studies failed: (a) to demonstrate that high MHPG levels predicted amitriptyline response (16); and (b) in the light of the development of selective serotonin reuptake inhibitors (SSRIs) the serotonergic potency of amitriptyline was also thrown into question. In contrast, several studies have reported that low urinary MHPG levels do indeed predict response to noradrenergic agents—nortriptyline, desipramine, and maprotiline (17,18). Application of urinary MHPG levels has been limited in part because of: difficulty collecting 24-hr urine samples; the need for patients being drug free when studied; and the lack of surety of the optimal treatment for high-MHPG patients.

Tyrosine Hydroxylase/Locus Ceruleus

The locus ceruleus (LC) is the nucleus of the NE system in brain. Neurons project from the LC to various parts of the brain, particularly the frontal cortex. The LC has been the focus of several postmortem studies of depressed patients or suicide victims. Tyrosine hydroxylase activity has been reported to be up-regulated in brains of suicide victims, perhaps reflecting the effect of chronic stress (19). In another study, NE neurons were reported to be modestly decreased in suicide victims relative to controls (20). A third study reported that NE transporter sites were decreased in depressed subjects who committed suicide but NE neurons were not (21). These studies are somewhat contradictory in direction of NE changes in suicide but suggest the system is altered in suicide. A possible unifying hypothesis revolves around up-regulation of TH in some neurons in an attempt to compensate for loss of neurons or transporter sites.

Receptors

Receptors for NE are grouped into α_1, α_2, β_1, and β_2 subtypes. α_2 Receptor numbers and activity can be studied using platelets; β receptors, using leukocytes. Both have also been explored in postmortem brain. α_2 Receptors are found both presynaptically and postsynaptically. Presynaptic α_2 receptors act as thermostats to regulate NE production and release. α_2 Receptors are universally connected to adenylate cyclase second messenger systems such that agonists inhibit cAMP formation. In contrast, β receptors, which are entirely postsynaptic, stimulate adenylate cyclase and cAMP formation.

α_2 Receptor numbers and activity have been reported in multiple studies to be increased in the platelets of depressed patients (22,23), although there is also at least one negative study (24). α_2 Receptor activity can be explored by measuring cAMP responses to challenges with agonists. Mooney and associates (25) reported that epinephrine suppression of prostaglandin-E induced cAMP formation is decreased in the platelets of depressed patients. Siever and colleagues (26) reported norepinephrine stimulation results in blunted adenylate cyclase responses in an E_1–α_2 prostaglandin coupled model. Platelet aggregation that results from α_2 stimulation has also been reported to be altered in depressed patients (27). Mooney and colleagues (25) using stimulation of α_2 receptors with a variety of agents, including NaF, which directly affects G_1 coupled proteins have hypothesized that this down-regulation is not agonist specific and have argued that a fundamental uncoupling of the receptor–G-protein–AC complex occurs in some depressed patients.

Growth hormone (GH) responses to challenge with clonidine, an α_2 agonist, has also been employed as a functional test of α_1 activity. Consistent blunting of GH responses in depressed patients has been reported (28,29). Blunted GH response appears to be a trait marker; it is found in remitted patients (30). The significance of blunted GH responses to clonidine is not entirely clear, however. Clonidine could be affecting presynaptic or postsynaptic receptors (31). Also, somatostatin, an inhibitor of GH release, may play a role in the GH response to clonidine challenge.

α_2 Receptors have also been explored in postmortem brain of suicide victims. Increased binding sites have been reported in several studies (32–34), although findings regarding the specific isoform and location of the increased binding sites have not been consistent.

β Receptors have been studied in both leukocytes and postmortem brain. Results have been less consistent than with the α_2 receptor. Decreased binding in leukocytes of depressives has been reported inconsistently (35,36). Similarly, in postmortem brain tissue, increased β-adrenergic receptor density has been reported by Mann and colleagues (37); however, decreased Bmax was reported by Crow and co-workers (38) in the hippocampus of depressives. Effects of previous medication may enter into these discrepant findings, as could biological heterogeneity of catecholamine secretion in depressed patients.

Depletion Strategies

α-Methylparatyrosine (AMPT) inhibits TH and ultimately synthesis of norepinephrine. When given to healthy controls or depressives it does little to lower mood (39,40); however, remitted depressed patients on noradrenergic antidepressants show a worsening of symptoms when challenged with AMPT, suggesting that norepinephrine availability or tone is needed for maintaining response to NE agents (41). In contrast, patients on SSRIs do not relapse when challenged with AMPT challenge. AMPT in previously depressed patients who are not currently on medication causes a recurrence of depressive symptoms (42). Taken together, these data suggest the test could be a possible trait marker for depressive vulnerability and that maintaining NE tone is important for sustaining responses to noradrenergic drugs.

SEROTONIN

Serotonin (5-HT) is a monoamine neurotransmitter involved in mood and appetite regulation. In brain, it is synthesized within the raphe from l-tryptophan. Serotonin itself does not cross the blood–brain barrier. Synthesis includes an initial conversion to 5-hydroxytryptophan (5 HTP) via the enzyme, tryptophan hydroxylase. 5-HTP is decarboxylated by L-aromatic-amino acid decarboxylase to form 5-HT. The principal metabolite of 5-HT is 5-hydroxyindole acetic acid (5-HIAA), which is easily measurable in cerebrospinal fluid (CSF) and urine. MAO mediates part of the metabolism of serotonin.

Metabolite Studies

Much of the early interest in serotonin was generated by observations that low CSF 5-HIAA levels in hospitalized depressives were associated with an increased risk for suicide (43). Further studies revealed a relationship, particularly with violent methods of suicide (e.g., hanging) (44) and subsequently with difficulties with impulse control in subjects with antisocial personality (45). Current theories emphasize a more general relationship between low serotonin metabolite concentrations and impulse control problems; the latter may predispose to suicide in subjects who become depressed (46).

Transporter

The serotonin transporter (SERT), a 12-transmembrane molecule, actively pumps 5HT into the presynaptic neuron. Originally, the transporter was studied in platelets using tritiated (^{3}H) imipramine and more recently with the higher affinity (^{3}H)-paroxetine. Numerous studies have reported decreased binding (Bmax) in the platelets of depressed patients as compared to healthy controls. A metaanalysis by Ellis and Salmond (47) of 70 studies demonstrated an overall significant difference between patients and controls, although not all studies concur. Medication did not appear to account for these differences. Although mean values appear to differ between patients and controls, there is considerable overlap in values among patients and controls such that there are numbers of patients who do not appear to have decreased binding; this overlap limits the use of the test as a diagnostic measure.

Decreased ^{3}H imipramine binding was once thought to be a trait marker, that is, did not normalize with treatment. Further study, however, has revealed that decreased ^{3}H-imipramine binding does normalize with treatment but one must wait for sufficient periods to allow for protein regeneration.

The transporter has also been the subject of examination in postmortem brain tissue. Early studies in this area pointed to decreased binding in suicide brains (48); however, more recent studies have failed to confirm these findings (49). These data raise questions regarding the significance of abnormalities in the activity SERT in the pathophysiology of depression and the relationship of peripheral and central forms of the transporter. There are a number of methodologic problems inherent in postmortem tissue that may account for differences among studies, including accuracy of diagnosis, time from death to collection of brain tissue preparation of tissue, and so on.

One approach to studying the activity of the transporter has been to apply functional imaging (e.g., SPECT to determine relative activity). The development of ligands (e.g., ^{123}I-β-CIT), that bind selectively to the transporter has allowed *in vivo* study in humans. In one study, a significant difference in binding using SPECT was observed between depressed patients and controls (50). In this study, significant differences were not observed in platelet binding to ^{3}H-paroxetine, raising questions regarding whether the transporter is regulated differently in the two tissues.

Receptors

Presynaptic and postsynaptic 5HT receptors have also been studied in depressed patients. Over a dozen serotonin receptors have been identified, although the possible roles for many have not. Two that have attracted most study for the longest periods are the $5HT_{1A}$ and $5HT_{2a}$ types.

$5HT_{2a}$ receptors are located postsynaptically in the CNS and can also be found in platelets as well as in other non-CNS tissue. As with the transporter, multiple studies have investigated $5HT_{2a}$ binding sites in the platelets of depressed patients. An increase in binding sites (B-max) has been reported in depressed and suicidal patients with some suggestion that increased binding in suicidal patients may be independent of a diagnosis of major depression (51–53). Generally, $5HT_{2a}$ binding has been thought to be a state marker, although one recent study has suggested binding may not normalize with SSRI treatment (54).

$5HT_{2a}$ binding has also been studied in postmortem brain tissue. As with the serotonin transporter, results here have been mixed with some studies demonstrating increased prefrontal cortical binding but others not (37,55–57). $5HT_{2a}$ receptors are found in frontal cortex suggesting a role in the cognitive aspects of depression.

PET ligands have been developed to study $5HT_{2a}$ activity in brain. One study employed [^{18}F]- altanserin and reported a reduction in activity in right posterolateral frontal, orbitofrontal, and anterior cingulate regions in depressives (58). In another study, no differences were found between nonsuicidal depressives and controls using [^{18}F-] setoperone (59). The exclusion of patients with recent suicidal ideation may have played a role in not finding differences between patients and controls. Studies on effects of antidepressants on $5HT_{2a}$ binding using PET have also yielded mix results. One group reported that SSRIs appear to increase ^{18}F-setoperone binding (60), whereas another recently reported that 3 to 4 weeks of desipramine treatment resulted in a significant decrease in $5HT_2$ activity in multiple areas, particularly in frontal cortex (61). This group was unable to conclude whether the ligand was binding to $5HT_{2a}$ or $5HT_{2c}$ receptors.

$5HT_{2A}$ receptors are coupled to the phosphoinositide second messenger system. When $5HT_{2a}$ receptors are activated by agonists, phosphatidyl inositol 4,5 bisphosphate is hydrolyzed by phospholipase C to form two second messengers, diacylglycerol and inositol 1,4,5-triphosphate. Protein kinase C is activated by diacylglycerol. This system has been studied in the brains of suicide victims. Pandey and associates reported [^{3}H] phorbol dibutyrate binding to protein kinase C in prefrontal cortex was lower in teenage suicide victims (62). More recently they observed that both phospholipase C activity and the β_1 isozyme protein level were decreased of teenage suicide victims (63). Depression per se did not appear to affect the differences between suicide victims and controls. In contrast Hrdina and associates (64) reported unaltered protein kinase C activity in antidepressant free depressives who suicided, and Coull and colleagues (65) reported that phorbol dibutyrate binding sites were increased in the prefrontal cortex of adults with similar histories. Age, diagnosis of depression, antidepressant use, and time to collection of brain may play a role in these disparate findings.

The $5HT_{1a}$ autoreceptor controls release of serotonin from the presynaptic neuron. Over the past few years, multiple groups have explored the potential use of pindolol, a $5HT_{1a}$ antagonist, to hasten response to antidepressants or bring out responses in refractory patients. These studies have yielded mixed results suggesting that pindolol may hasten response to antidepressants in milder or first-episode patients seen in primary care settings. $5HT_{1a}$ receptor number and activity have been studied in postmortem brain. Increased $5HT_{1a}$ Bmax has been reported in suicide victims using nonviolent means compared to violent completers or controls but others have failed to find alterations in $5HT_{1a}$ activity in suicide victims (66–68).

Genetic Studies

A number of studies have explored the possible role of genetics may play, particularly vis-à-vis transporter activity. Long and short forms of the transporter gene appear to be relatively common in the general population. An early study indicated a relationship of the short form with an increased frequency of a variety of neurotic or behavioral traits (69). Allelic variation has also been applied to predicting drug response. In three studies in Europe and the United States, homozygotes or heterozygotes for the S-form were reported to show sluggish responses to SSRIs (70–72). The opposite was found in a Korean study (73). Clearly, further work is needed to understand the importance of genetic forms of the transporter in major depression. More recently, Mann and colleagues (69) reported that the short form genotype was associated with a diagnosis of major depression but not with suicide or 5HT-transporter binding in postmortem tissue.

Depletion Studies

Brain concentrations of serotonin are highly dependent on circulating levels of tryptophan, which competes with other

amino acids for transport into the brain. Charney and Delgado have pioneered in the use of an amino acid cocktail that is relatively devoid of L-tryptophan to rapidly decrease plasma tryptophan and ultimately brain serotonin. In these studies, the drink was first administered to subjects who had responded to various antidepressants and who were being maintained on medication. Diphenhydramine has been commonly used as the comparison cocktail. Euthymic patients on SSRIs but not TCAs rapidly experienced depressive symptoms when depleted of L-tryptophan, suggesting the need for maintaining adequate serotonin levels to ensure continued drug response (74,75). Parallel decreases in glucose utilization in frontal and thalamic regions using PET have also been reported in depressives who experience a relapse in symptoms (76). In contrast, there are multiple reports of depletion not causing a clear recurrence of symptoms in patients treated with bupropion or electroconvulsive therapy (75,77–79). Studies have used a variety of different methods (e.g., patients' being on or off medication, inclusion or exclusion of suicidal patients, etc.), and these differences may account for the discrepant findings. The degree and duration of response observed before the depletion challenge is administered may be of particular importance (79). Patients who are in remission or have shown a prolonged response are unlikely to demonstrate significant worsening of moods (79). These data suggest recent responders are those who are susceptible to experiencing relapse with depletion strategies. Depletion of unmedicated euthymic depressives does not appear to induce recurrence, indicating maintaining serotonin tone is important primarily for continuance of response in recently remitted patients (79).

Of interest is a recent report that women controls show much lower rates of 5HT synthesis and a greater decrease in response to depletion than men do (80). This gender-based difference is consistent with a recent observation that chronically depressed women are more responsive to an SSRI than men are (81).

Fenfluramine Challenge

Fenfluramine, previously marketed as an appetite suppressant, causes a release of serotonin from presynaptic neurons and results in an elevation of prolactin. Prolactin responses to fenfluramine challenge are blunted in depressed patients (82,83) and there are some data to suggest this may be a trait marker (84). However, bipolar manic and axis II patients may also demonstrate blunted prolactin responses, raising questions regarding the specificity of the test. (See refs. 1 and 2 for further review.)

DOPAMINE

As indicated, dopamine (DA) is a precursor for norepinephrine. Although NE has played a central role in etiologic theories of depression, DA has been emphasized far less in depression in spite of its being widely distributed in brain.

CSF levels of homovanillic acid (HVA), a major metabolite, are decreased in depressives (2,85,86), although some studies have reported elevated CSF DA, but not HVA levels (87). Urinary DOPAC levels are decreased in depressives compared with controls (88); in one study, DOPAC levels appeared associated with suicidal behavior (85). Dopaminergic agents such as psychostimulants, nomifensine, and the dopamine agonist pramipexole all have antidepressant effects in nondelusional patients.

In contrast, elevated mesolimbic DA activity has been hypothesized to play a role in delusional depression (89). Elevated CSF HVA levels have been associated with psychotic symptoms and agitation in major depression (89), and increased plasma DA and HVA levels have also been reported in delusional depression (90,91). Increased mesolimbic DA activity has been postulated to occur secondary to elevated hypothalamic–pituitary–adrenal (HPA) axis activity (89). Recent studies in rats, nonhuman primates, and psychotic depressives suggest elevated glucocorticoid activity could also result in altered or decreased prefrontal cortical dopamine metabolism and to alterations in attention and response inhibition (92,93). These data suggest increased HPA axis activity could affect DA turnover differently in specific brain regions—alterations that have been suggested in schizophrenia. Antipsychotic drugs appear to play a key role in treatment of delusional depression and glucocorticoid receptor antagonists are being actively studied in the disorder.

GABA

GABA has become a focus of greater study over the past several years with the increasing use of anticonvulsants in mood disorders. GABA is a major inhibitory neurotransmitter in brain and regulates seizure threshold as well as norepinephrine and dopamine turnover. There are two types of GABA receptors. $GABA_A$ receptors have been studied in anxiolysis because of their location within a benzodiazepine–GABA receptor complex that is coupled to chloride channels. $GABA_B$ receptors are coupled to Ca^{+2} channels. In rats, antidepressants and mood stabilizers appear to up-regulate frontal-cortical $GABA_B$, but not $GABA_A$, receptors (94,95). $GABA_B$ agonists may enhance cAMP responses to norepinephrine and β-adrenergic down-regulation in response to tricyclic antidepressants, suggesting a facilitative role for $GABA_B$.

GABA is also enhanced by anticonvulsants such as valproic acid, which act as mood stabilizers. GABA levels have been reported to be decreased in the CSF of depressed patients in some but not all studies (96,97). Plasma GABA levels have also been reported to be lower in unipolar depressives (98,99), and this may not normalize with treatment

(100). Alcoholism can also be associated with low plasma GABA levels (101). In refractory depressed patients undergoing cingulotomy, GABA levels are inversely related to degree of depression (102). A number of groups are actively exploring using fMRI to image GABA in the brains of patients with mood disorders, both before and after treatment.

NEUROENDOCRINE SYSTEMS

Neuroendocrine systems were originally studied as gateways to the exploration of neurotransmitter activity, such as norepinephrine and serotonin, in depression. Over time, emphasis has shifted to exploring the roles components of several of these axes may play in the pathogenesis of specific symptoms or disease states. Three axes, hypothalamic–pituitary–adrenal (HPA), hypothalamic–pituitary–thyroid (HPT), human growth hormone (HGH), in particular have been examined in major depression.

HPA Axis

The hypothalamic–pituitary–adrenal (HPA) axis is frequently activated during periods of stress and depression. The axis consists of three major components: (a) corticotropin releasing hormone (CRH), which is located in the paraventricular nucleus of the hypothalamus and stimulates the pituitary to release adrenocorticotropin hormone (ACTH); (b) ACTH which stimulates the adrenal cortex to release cortisol; and (c) cortisol, which feeds back to the pituitary, hypothalamus, and hippocampus to decrease release of CRH and ACTH.

Multiple lines of evidence point to abnormalities of the axis in depression. Initial studies focused on excretion of cortisol and its precursors in patients with depression. Activation of the axis was also associated with suicidal ideation. Sachar in a classic study reported elevated serum cortisol levels over a 24-hour period in severely depressed patients (103). Elevations were particularly seen in the evening and overnight, times when the axis should be quiescent. These data generally were interpreted as indicating that the depressed patient was highly stressed.

One method for challenging the axis is to administer the synthetic steroid dexamethasone (DEX) (104). The expected response is to suppress the axis because the pituitary and perhaps the hypothalamus read the DEX signal as indicating sufficient glucocorticoid activity, and shuts off production or release of cortisol. Depressed patients demonstrate a significantly higher nonsuppression rate than do controls, although the rates of nonsuppression are relatively low in many studies (105). Patients with severe or psychotic depression demonstrate relatively high rates of nonsuppression or high postdexamethasone cortisol levels (106). Indeed, psychosis appears to be the greatest symptom or syndrome contributor to DEX nonsuppression, greater than the effect of severity or melancholia (107). Outpatients with milder and nonpsychotic disorders show much lower rates of nonsuppression. This difference in types of patients studied may help explain the variability in DEX nonsuppression rates across studies. DEX responses have also been used to assess adequacy of treatment with patients who are nonsuppressors after treatment showing a significantly increased risk for relapse (108).

Glucocorticoid overactivity has been hypothesized to play a direct role in the development of cognitive impairment and delusions in patients with psychotic major depression (89). Trials are currently underway exploring the efficacy of glucocorticoid antagonists in psychotic depression (109). Moreover, glucocorticoids have been hypothesized to cause increases in glutamate activity, decrease nerve growth factor activity, and hippocampal volume loss on MRI in patients with a history of severe depression, but there are no studies that have simultaneously explored these various dimensions in depressed patients (110). Recently, Rojkowska and colleagues did report that neuronal size and density and glial densities were reduced in prefrontal cortical regions in postmortem tissue from subjects with major depression as compared to controls (111). Overall, there has been a shift from viewing excessive glucocorticoid activity in major depression as an epiphenomenon to its having specific effects on cognition or symptom formation.

Study of more proximal components of the axis have also pointed to marked abnormalities in major depression. In most of the relevant studies, CRH levels have been reported to be elevated in the CSF or plasma of depressed patients (1,112). Challenge with ovine or human forms of CRH results in blunted ACTH responses in depressives suggesting increased central CRH release (113). Remission of episodes appears to be associated with normalization of CRH studies. Postmortem studies have reported that CRH mRNA expression was increased (114) and CRH Bmax was decreased in the frontal cortex of suicide victims (115). These data suggest CRH release from the hypothalamus may be associated with a down-regulation of CRH in other brain regions (2).

Imaging studies have reported increased pituitary and adrenal size during depression, which appear to normalize with recovery (116,117). Increased pituitary size and elevated CSF CRH levels are associated with DEX nonsuppression (118). Elevated plasma ACTH levels have been reported in psychotic depression (119).

ACTH release is not only stimulated by CRH. For example, arginine vasopressin (AVP) may enhance CRH's stimulation of ACTH. AVP neurons are increased in the PVN of suicide victims (120) and serum AVP has been reported in one study to be elevated in hospitalized depressives (121).

CRH is also found in extrahypothalamic brain regions. In the amygdala, CRH appears to play a key role in fear responses and over-activation of these systems may lead to panic and depression (2). Amygdala CRH has been reported

to be under positive (stimulatory) feedback by cortisol and this observation has spurred on much research to develop specific CRH antagonists to treat anxiety and depressive disorders. A recent report on an open label trial suggested that a CRH antagonist might be effective in hospitalized depressives (122).

Although the literature has emphasized elevated CRH and cortisol activity in major depression (in part because of the emphasis on DST nonsuppression), there is emerging evidence that CRH and cortisol activity may only be elevated in some subtypes of major depression and that some depressed patients may actually have low HPA activity. Recent data suggest that depressed patients with a history of early abuse (as well as those with psychosis) may be most consistently at risk for demonstrating elevated ACTH levels in response to social stress (123). Depressives who were not abused as children did not show similar responses. In a recent study, we reported *decreased* levels of ACTH or cortisol activity over 24 hours in nonpsychotic depressives as compared to controls (119). Similarly, low values have been reported in several other types of patients, including atypical depression, posttraumatic stress disorder, so-called burn out syndromes, and so on. Thus, both decreased and elevated HPA axis activity may be found in specific depressive subtypes. In many ways this parallels the findings in catecholamine activity in depressed patients.

This seeming contradiction in findings or emphasis over time may have several explanations. First, the DST as we use it may not measure cortisol overactivity as much as it does central CRH overdrive in response to suppressing the pituitary because DEX poorly penetrates brain at the doses used in the test. Second, previous studies have often not explored the role of psychosis or early abuse. Third, relatively few studies on the HPA axis in depression have explored cortisol activity over the full 24-hour period.

HPT Axis

The overlap in symptoms between patients with hypothyroidism and those with major depression has led to number of studies on the hypothalamic–pituitary–thyroid (HPT) axis in patients with mood disorders. These studies have yielded intriguing, although at times, conflicting results.

Thyrotropin-releasing hormone (TRH) is released from the hypothalamus and stimulates TRH receptors in the pituitary to release thyroid-stimulating hormone (TSH), which in turn stimulates specific receptors in the pituitary to release triiodothyronine (T_3) and thyroxine (T_4) hormones. Thyroid hormones provide feedback to both the hypothalamus and pituitary to regulate the axis.

Activity of the axis can be measured in several ways: circulating levels of T_3 and T_4—both bound and unbound; TRH levels in the CSF; TSH responses to TRH administration (TRH-stimulation test); and circulating TSH levels. In addition some patients demonstrate antibodies to thyroid tissue suggestive of an autoimmune thyroiditis, often in the face of normal T_4, T_3, or TSH levels.

TRH is found in extra-hypothalamic regions in brain. CSF TRH was increased in two small studies of depressed patients as compared to controls (124,125), although not all studies agree (126). Elevated TRH levels should be accompanied by a blunted TSH response to TRH because TRH levels in the pituitary would be expected to be downregulated in the face of elevated TRH. Indeed, multiple studies have reported such blunting in a relatively high percentage (approximately 25%) of patients with major depression. A recent review concluded that 41 of 45 studies reported blunted TSH responses to TRH in major depression (127). Blunting of TSH responses to TRH in these patients is not owing to clinical or subclinical hypothyroidism because thyroid parameters were generally within normal limits in these patients.

Type I hypothyroidism is characterized by decreased levels of T_3 and/or T_4, increased TSH, and increased TSH responses to TRH (1). Antithyroid antibodies may be present. Type II hypothyroidism is characterized by normal T_3 or T_4 levels but otherwise similar abnormalities as in Type I disease (1). Rates of Type III or IV subclinical hypothyroidism have been reported to be elevated in depressed patients. These syndromes are both characterized by normal circulating levels of T_3, T_4, and TSH but have other abnormalities such as elevated TSH responses to TRH or the presence of antithyroid antibodies. In one study, depressed patients with high normal thyroid levels were also reported to demonstrate exaggerated TSH responses to TRH (128). These data have been interpreted as indicating some patients with major depression may have subclinical hypothyroidism. Indeed, asymptomatic autoimmune thyroiditis with positive antibodies has been reported to be relatively high (9% to 25%) in several surveys of depressed patients (127, 129). Taken together, TSH stimulation test data suggest elevated or decreased TRH activity could be involved in major depression, depending on whether patients met criteria for subclinical hypothyroidism.

T_3 has been reported to be an effective augmentor of responses to antidepressants and appears to exert greater effects than does T_4 (130). Patients with a history of thyroid disease (e.g., adenoma) who were taking suppressing or replacement doses of thyroxine or T_4 demonstrated an improvement in mood and cognitive function when T_3—but not placebo—was added (131). One possible explanation for the differential effects of T_4 and T_3 on mood rests with local tissue conversion of T_4 to T_3 that in brain is mediated by Type II 5′ deiodinase and may be dysregulated in some patients. Depressed patients have been reported to demonstrate increased reversed T_3 levels in CSF (130), which suggests inhibition of the Type II 5′ deiodinase and subsequent increased activity of the Type III of the enzyme. Cortisol can inhibit Type II of the enzyme and may play a role in the increased rT_3 levels. Of interest is a recent report (133)

that in depressed patients low T_3 levels predicted earlier relapse, pointing further to an important role for T_3 in mood relation.

Transthyretin is a protein that transports thyroid hormone from the periphery to the brain via the choroid plexus. Transthyretin levels have been reported to be low in refractory depressed patients (134). This may also help explain possible CNS enhancing effects of T_3 in the face of normal circulating thyroid hormone levels.

Overall, research on the HPT axis has produced some intriguing leads for understanding the pathophysiology of depression and improving its treatment. However, there are still a number of seeming contradictions regarding the direction and specific nature of HPT alterations in depression. Data point to both elevations in central TRH activity and subtle forms of hypothyroidism (suggestive of low T_3 and TRH activity) as playing potential roles in major depression.

Human Growth Hormone

Growth hormone (GH) is synthesized in the anterior pituitary. Two hypothalamic hormones, growth hormone releasing factor (GRF) and somatostatin modulate its release from the pituitary. GRF is stimulating; in contrast, somatostatin inhibits release. Somatostatin is also found in extra-hypothalamic regions, and appears to act as a neurotransmitter. The major neurotransmitters involved in mood regulation (e.g., norepinephrine, serotonin, and dopamine) all affect GH release, and these systems can be challenged by specific compounds (e.g., apomorphine, clonidine, etc.).

Diurnal rhythms of GH, as measured in plasma, are disrupted in depression. Nocturnal GH is elevated in depression (135), but daylight-stimulated GH levels are increased in both unipolar and bipolar depressives (136).

As indicated, GH responses to clonidine are blunted in depression (28). GH responses to GRF have also been explored in patients with major depression with several, but not all, groups reporting blunted GH responses (137–139). CSF levels of somatostatin, which inhibits GH, CRH, and ACTH release, are also reduced in depression (140,141), such that somatostatin does not appear to provide an explanation for the blunted GH responses to GRF in depression. Low somatostatin levels in depression may reflect increased cortisol activity (1,142) and appear to normalize with treatment (2). Low CSF somatostatin has also been observed in various neurological disorders (e.g., Alzheimer's disease).

CONCLUSION

Proliferation of research into the biology of depression has resulted in a number of intriguing leads for understanding the pathophysiology of major depression. Most studies have focused on single biological systems such that there is a dearth of studies that simultaneously explore multiple systems and their complex interactions in depression. Also, research has tended to emphasize cross-sectional rather than longitudinal designs such that we have little understanding of the biological underpinnings of initiation, maintenance, and termination of depressive episodes. Future research that combines genetic risk factors with longitudinal study of multiple systems will likely lead to breakthroughs in our understanding of the biology of the disorder. Also, greater emphasis on the biology of specific depressive subtypes (e.g., delusional depression) or of symptom dimensions may provide greater insights.

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