Biological Basis of Generalized Anxiety Disorder

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Despite the considerable revisions to diagnostic criteria, recent data indicate that generalized anxiety disorder (GAD) is one of the most common anxiety disorders. Growing evidence also indicates that GAD is a serious illness, which frequently causes moderate impairment and often requires prolonged treatment. Thus, investigation of the biological correlates of GAD may be helpful in the development of effective treatments for this disorder. Recent data suggest possible abnormalities in the regulatory mechanisms of several important biological components in GAD patients. Maladaptive responses to stressful stimuli have been observed in the locus-ceruleus-norepinephrine-sympathetic nervous system, the hypothalamic-pituitary-adrenocortical axis, and the cholecystotin system. Abnormalities in other important CNS modulators, such as 5-HT and gamma-aminobutyric acid, may also be involved in the biology of GAD. In the following article, the authors will review the existing information regarding these potential biological abnormalities in GAD.

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Despite the relatively rapid evolution of the human species, it is quite remarkable that the basic mechanisms involved in coping with stress have not changed significantly over the past several thousand years. In fact, our responses to modern-life stressors often resemble those set in motion during situations of physical danger and threat to survival in most vertebrates. These responses include a cascade of adaptive events, such as changes in arousal, alertness, vigilance, and presumably cognition, as well as physiologic changes, such as altered blood flow to important organs, and increased heart rate and blood pressure. Prolonged, maladaptive stress response mechanisms appear to be a part of pathologic anxiety states such as generalized anxiety disorder (GAD). For example, persistently anxious individuals, especially those suffering from GAD, often complain of excessive persistent symptoms characteristic of the acute stress response, such as hypervigilance, arousal, increased muscle tension, tremor, or palpitations. This review will highlight recent data examining neurophysiologic and biochemical aspects of the human stress response with a particular emphasis on the persistent anxiety state of GAD. Additionally, we will suggest possible stress-related neurobiological models of GAD, which may serve as a guide to future research studies.

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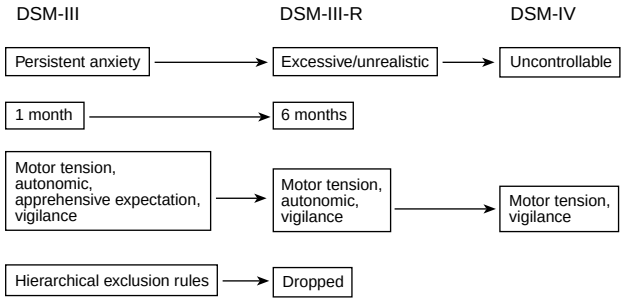
GAD: DEFINITION

GAD was first defined as a separate diagnostic entity in the third edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-III). Diagnostic criteria for GAD in DSM-III required the presence of generalized, persistent anxiety for at least 1 month, as manifested by symptoms from at least three of four categories that included motor tension, autonomic hyperactivity, apprehensive expectation, and vigilance/scanning. However, the diagnosis of GAD could not be assigned if patients met criteria for another mental disorder. Thus, GAD represented a residual diagnosis that was very low in the diagnostic hierarchy of mood and anxiety disorders. DSM-III defined GAD was also associated with low diagnostic reliability, due in part to its residual status.[1] The hierarchical exclusion rules were dropped in DSM-III-R, which further required the presence of psychic anxiety (i.e., excessive and/or unrealistic worry) in two or more areas unrelated to another axis I disorder.[2] Additionally, the DSM-III-R required that the somatic symptom criteria associated with GAD included the presence of at least 6 (from a list of 18) somatic symptoms that formed three clusters: motor tension, autonomic hyperactivity, and vigilance and scanning (Figure 1). Finally, the duration criterion of GAD was extended from 1 to 6 months, which helped differentiate GAD from a transient reaction to negative life events such as adjustment disorder. Using field trial–based data, the DSM-IV Anxiety Task Force recommended that GAD continue to be considered an independent diagnostic cat-

egory, but also proposed considerable revisions to the diagnostic criteria.[3] Some of the more substantial revisions to the earlier DSM-III-R criteria included increased emphasis on uncontrollable worry and less emphasis on autonomic symptoms—requiring only three of six associated physical symptom criteria (restlessness or feeling keyed up or on edge, being easily fatigued, difficulty concentrating or mind going blank, irritability, muscle tension, and sleep disturbance). This recommended revision was accomplished via empirical studies of treatment-seeking GAD patients, which indicated that symptoms constituting the autonomic hyperactivity cluster were relatively infrequently endorsed by patients with GAD.[4,5]

Figure 1. Generalized Anxiety Disorder: Changing Diagnostic Criteria



EPIDEMIOLOGY OF GAD

Despite the considerable revisions to diagnostic criteria, recent data indicate that GAD is one of the most common anxiety disorders (Table 1). For example, the Epidemiologic Catchment Area (ECA) Study examined the prevalence of DSM-III GAD in three of the five ECA sites (Durham, N.C., St. Louis, Mo., and Los Angeles, Calif.).[6] The reported lifetime prevalence of GAD ranged from 4.1% to 6.6% in those three sites. Since the introduction of the DSM-III-R in 1987, several epidemiologic studies reported the prevalence of DSM-III-R GAD. Faravelli et al.[7] evaluated the prevalence of GAD in a population survey (N = 1100 interviews) in Florence, Italy, and found prevalence rates of 2% (2.8% when DSM-III criteria were used) for current GAD and 3.9% (5.4% when DSM-III criteria were used) for lifetime GAD. Wacker and colleagues[8] reported a 1.9% lifetime prevalence of GAD in a population sample in Basel, Switzerland. The most recent epidemiologic survey of DSM-III-R GAD was conducted as a part of the National Comorbidity Survey of psychiatric disorders in the United States by Kessler and colleagues.[9,10] This study evaluated the lifetime and 12-month prevalence of 14 DSM-III-R psychiatric disorders in a representative national sample (N = 8098). The authors found that the prevalence rates in the total sample were 1.6% for current GAD (present for past 6 months), 3.1% for 12-month GAD, and 5.1% for lifetime GAD. These rates were higher than those for panic disorder. The National Comorbidity Survey also found that the majority of individuals with GAD reported substantial interference with their lives (49% of subjects), had a high probability for seeking professional help for GAD symptoms (66% of subjects), and were taking medications for GAD (44% of subjects).

When DSM-III-R GAD criteria were introduced, researchers also specified, for the first time, the importance and the relative frequency of psychiatric comorbidity in GAD. The available epidemiologic data confirm that GAD commonly coexists with other psychiatric disorders—mostly mood and anxiety disorders.[9,10] The most prevalent

Table 1. Lifetime Prevalence of Generalized Anxiety Disorder in the General Population

Study	%
Epidemiologic Catchment Area (ECA)[6]	
Durham, NC	6.6
St. Louis, Mo	6.6
Los Angeles, Calif	4.1
National Comorbidity Survey[9]	5.1

additional current diagnoses were social phobia (16%–59%), simple/specific phobia (21%–55%), panic disorder (3%–27%), and major depression (8%–39%). Despite high rates of concurrent psychiatric disorders, a significant minority of GAD sufferers have no other comorbid disorders. In fact, the National Comorbidity Survey found that one third of the people with current GAD did not have any other recent (within 1 month) diagnoses. Our own findings are in agreement with this perspective.[11]

In summary, current data indicate that GAD is probably one of the most common anxiety disorders. In addition, growing evidence indicates that GAD is a serious illness that frequently causes moderate impairment, and often requires prolonged treatment. Thus, investigation of the biological correlates of GAD may be helpful in the development of effective treatments for this disorder. Despite the attention that has been focused on anxiety disorders by the medical and research communities over the last decade, relatively less attention has been devoted to the investigation of the biology of GAD. Thus, the biological underpinnings of GAD remain largely unexplored. In the following section, the authors will review the existing information regarding the biological abnormalities in GAD, including the few available data from family studies of GAD.

GENETIC FACTORS IN GAD

It has been suggested that the vulnerability to anxiety disorders may be in part determined by genetic factors. Genetic transmission of a disorder suggests that certain gene-encoded changes in proteins or other factors and the

Table 2. Generalized Anxiety Disorder: Biological Studies

Type of Study	Results
Noradrenergic system	
Plasma catecholamine levels	Negative
Platelet α_2-adrenoceptor binding sites	Decreased
Growth hormone response to clonidine stimulation	Blunted
Yohimbine stimulation	Negative
Levels of catecholamine degradation enzymes	Normal
Hypothalamic-pituitary-adrenal (HPA) axis	
Urinary free cortisol	Normal
Dexamethasone suppression test (DST)	Nonsuppression
Thyroid function	Normal
Autonomic function	
Autonomic activity at rest	Normal
Autonomic response to stress (skin conductance)	Lower
Challenge studies	
Lactate infusion, CO_2 inhalation	Increased anxiety symptoms; lower rate of panic attacks
Other neurotransmitter systems	
Benzodiazepine	
Benzodiazepine binding sites	Decreased
Serotonin (5-HT)	
m-Chlorophenylpiperazine (*m*-CPP) administration	Anxiogenic
CSF 5-HT levels	Decreased
Titrated H^3-paroxetine binding	Decreased
Corticotropin-releasing factor (CRF)	
CSF CRF levels	Normal
Cholecystokinin (CCK)	
CCK-B receptor agonist pentagastrin administration	Increased rate of panic attacks

resulting biological consequences of these changes may play a role in the pathophysiology of specific disorders. Although there are numerous family and twin studies of "anxiety neurosis," only few have examined familial patterns of DSM-III and DSM-III-R GAD. For example, Torgersen[12] studied a sample of twins from Norway who were treated for psychiatric illness and found a concordance rate for DSM-III-defined GAD of 0% in monozygotic twins and 5% in dizygotic twins. Similarly, in an Australian twin sample, Andrews et al.[13] found no significant difference in concordance rates for DSM-III-defined GAD in monozygotic and dizygotic twins. In contrast, Noyes et al.[14] studied 20 probands with DSM-III GAD who had no history of panic attacks. Of the 123 first-degree relatives, 19.5% (N = 24) met criteria for GAD compared with 3.5% (4 of 113) of relatives of controls. However, a major limitation of the Noyes et al. study is that the diagnoses were not established blindly. In a more recent study, Skre and collaborators[15] examined 20 monozygotic and 29 dizygotic twins with DSM-III-R-defined GAD. They found that GAD was heritable only in co-twins of probands with both GAD and a lifetime history of mood disorder, suggesting a possible link between the two. Finally, in the largest twin study to date, which included 1033 female twin pairs, Kendler et al.[16] found that genetic factors appear to play a significant, but not overwhelming, role in the etiology of GAD; the heritability of GAD is estimated at around 30% in comparison to 70% heritability in major depression. In addition, the authors found that the vulnerability to GAD and major depression is influenced by the same genetic factors.[17]

In summary, the available data suggest at least a modest genetic contribution to the development of GAD.

NEUROBIOLOGY OF GAD

As mentioned, research of the biology of GAD has generally been based upon paradigms and measures utilized in stress research. These studies have focused on the evaluation of catecholamines, autonomic reactivity, neuroendocrine measures, and other neurotransmitter systems, including, e.g., serotonin, gamma-aminobutyric acid (GABA), and more recently cholecystokinin function (Table 2). Further, advances in computer-assisted imaging technologies, such as single photon emission computed tomography (SPECT) and positron emission tomography (PET), allowed exploration of potential abnormalities in the metabolic/biochemical function of specific brain areas in patients with GAD.

Noradrenergic System

Studies examining stress responses suggested that the locus-ceruleus-norepinephrine-sympathetic nervous system may play an important role in the response to stress.[18] Researchers subsequently hypothesized that abnormalities in catecholamine function may play a role in pathologic anxiety such as GAD. Initially, several investigators reported that GAD patients showed increases in plasma catecholamine concentrations and urinary catecholamine output. Mathew et al.[19] reported that patients with GAD had higher plasma catecholamine levels than normal controls. However, subsequent studies failed to confirm dif-

ferences in baseline (resting) plasma levels of catecholamine in patients with GAD.[20] The authors concluded that the previous findings may have been related to the stress of venipuncture. Munjack et al.[21] controlled for this possibility by using an indwelling catheter instead of venipuncture and found no differences in resting epinephrine and norepinephrine levels between GAD patients and normal controls. Other studies evaluating the levels of enzymes responsible for the degradation of catecholamines (catechol-O-methyl transferase, dopamine-β-hydroxylase, and monoamine oxidase) also failed to detect differences between GAD patients and controls.[22]

In contrast, studies utilizing various challenge paradigms, which examine the functional or dynamic aspects of the adrenergic system, found several differences between GAD patients and controls. Abelson and colleagues[23] found a blunted growth hormone response to clonidine (α_2-partial agonist) stimulation in GAD patients, suggesting a possible decrease in the sensitivity of α_2-adrenergic receptors in these patients. However, the authors found no differences in blood pressure, heart rate, or 3-methoxy-4-hydroxyphenylglycol (MHPG) levels between GAD patients and controls after the clonidine challenge. These results are further supported by studies that found a decrease in platelet α_2-adrenoreceptor binding sites (B_{max}) in GAD patients compared with normal comparison subjects (using the α_2 antagonist yohimbine or clonidine as a ligand), suggesting a possible decrease in the number of catecholamine receptors in GAD patients.[24,25] It was hypothesized that initially higher levels of catecholamines may subsequently lead to a down-regulation of postsynaptic α_2-adrenoreceptors.

In contrast, Charney and colleagues[26] failed to find differences between GAD patients and controls in cardiovascular responses such as blood pressure and heart rate, self-rated anxiety, changes in plasma MHPG and cortisol after yohimbine challenge. However, Charney et al. observed an attenuated increase in MHPG in patients with GAD versus normal controls, which is consistent with the possibility that GAD subjects may have presynaptic α_2-receptor hyposensitivity.

In summary, baseline-resting levels of catecholamine in patients with GAD appear to be normal, but the available data suggest that GAD patients may have subtle abnormalities such as reduced receptor sensitivity in the adrenergic system.

Neuroendocrine Studies

Hypothalamic-pituitary-adrenal (HPA) axis. Activation of the hypothalamic-pituitary-adrenal (HPA) system normally causes increases in plasma cortisol levels and is believed to be a critical component of normal stress responses. The regulation of glucocorticoid secretion is complex. Hypothalamic corticotropin-releasing factor (CRF) regulates the activity of the HPA axis via control of pituitary adrenocorticotropic hormone (ACTH) secretion. Other ACTH secretagogues include epinephrine, vasopressin, and oxytocin. Cortisol exerts negative feedback at the level of the pituitary. The hippocampus also plays an important role in the regulation of HPA activity, predominantly via its inhibitory effects on the basal-circadian and stress-induced secretion of glucocorticoids through type I and II hippocampal corticosteroid receptors.[27] It appears that changes in hippocampal receptor function can amplify or diminish the corticosteroid feedback response. These effects have been elegantly demonstrated in animal models. For example, researchers found that rats exposed to chronic stress or administration of exogenous steroids show decreased hippocampal corticosteroid receptor density.[27] These animals also subsequently exhibit heightened or prolonged adrenocortical responses to stress, implying that a loss of these hippocampal receptors results in reduced sensitivity to corticosteroid feedback as demonstrated by dexamethasone nonsuppression on the dexamethasone nonsuppression test (DST)—the most commonly used probe of HPA function.[27]

Since the activation of the HPA axis may be a critical component in normal stress responses, researchers hypothesized that baseline plasma cortisol levels may be elevated in GAD patients.[28] This hypothesis was not supported by studies measuring 24-hour urinary free cortisol output in GAD patients compared with normal controls.[29] However, challenge paradigms used to examine HPA axis reactivity in GAD subjects (such as the DST) suggested evidence of potential abnormalities. Indeed, some investigators have shown a more abnormal escape from dexamethasone, which was not attributable to the presence of depression in DSM-III-diagnosed GAD patients versus normal controls. Avery et al.[30] found a nonsuppression rate of 38% in GAD patients (by using a cutoff of ≥ 0.4 ng/dL as nonsuppression), which was significantly higher than that for patients with depression (13%). Similarly, Tiller et al.[31] found a nonsuppression rate (by using a cutoff of ≥ 5 ng/dL) of 27% in GAD patients.

In summary, these data suggest possible abnormalities in the regulatory mechanisms of the HPA axis in GAD patients, possibly associated with abnormal stress response in these individuals.

Other. Regulation of growth hormone (GH) secretion may be abnormal in GAD subjects. For example, Abelson and colleagues[23] suggested that the blunted GH response to clonidine in GAD patients may be due to abnormal GH feedback mechanisms instead of or in addition to abnormal adrenoreceptor function.

There is minimal information on thyroid function in GAD. Munjack et al.[32] compared total serum thyroxine, free thyroxine index, triiodothyronine resin uptake, and thyroid-stimulating hormone in 52 patients with GAD, 41 patients with panic disorder, and 14 normal comparison

subjects. The authors found no difference in thyroid function between these groups.

In summary, the available data indicate that there may be abnormalities in HPA function in GAD patients.

Psychophysiology: Autonomic Function

Consistent with the results from studies of HPA function in GAD (i.e., normal baseline measures with abnormal responses to stimulation), studies that assessed peripheral response to stress by measuring autonomic responses such as electrodermal activity (e.g., skin conductance), respiration, and blood pressure found no baseline differences, but GAD patients showed hyporeactivity to stress challenges. Hoehn-Saric et al.[33] did not find differences in electrodermal activity (skin conductance), respiration, blood pressure, and heart rate variability at rest in women diagnosed with DSM-III-R GAD compared with controls. However, the authors observed that women with GAD showed a significantly attenuated skin conductance response and slower habituation (i.e., a more prolonged recovery to baseline) to stress. Cameron et al.[25] also found that patients with DSM-III-diagnosed GAD exhibited lower systolic blood pressure upon standing compared with controls. Although an autonomic hyporesponsivity pattern is not uniformly found, these data may suggest that patients with GAD have less "autonomic flexibility," i.e., weaker autonomic response to stress as well as a more prolonged time to recovery (slower habituation) than normal controls.

Challenge Studies: Lactate Infusion, CO_2 Inhalation

Pharmacologic challenge strategies have become an increasingly important tool for investigating the phenomenology and biology of anxiety disorders. Challenge models involve the assumption that pathophysiologic differences between diagnostic groups can be found more readily during activation of specific neurotransmitter receptor systems than at baseline or resting conditions. The interest in the sodium lactate and CO_2 challenge models evolved from the observation that intravenous administration of sodium lactate or inhalation of CO_2 provokes physiologic and psychological symptoms of panic in patients with panic disorder at a significantly higher rate than in normal comparison subjects.[34] Cowley et al.[34] evaluated the response of patients with GAD to the administration of sodium lactate. They found that patients with GAD (who had never had panic attacks) panicked at a lower rate (11% vs. 41%) after lactate infusion than patients with panic disorder. However, GAD patients were significantly more likely to report increased anxiety symptoms than nonpsychiatric controls.

Unlike panic disorder patients, patients with GAD also have very low rates of panic attacks in response to CO_2 inhalation. Gorman et al.[35] reported that zero of three DSM-III-diagnosed GAD patients had a panic response to inhaled 5% CO_2. Similarly, Holt et al.[36] reported that 5% CO_2 induced panic attacks in none of the 10 DSM-III-diagnosed GAD patients compared with 44% of panic disorder patients (11/25 subjects). The somatic and psychic anxiety responses of GAD patients were significantly less than those seen in panic disorder patients but were consistently, but not significantly, greater than those of normal controls. In contrast, Rapee et al.[37] reported that 5.5% CO_2 inhalation induced panic attacks in 21% of GAD patients compared with 0% of normal controls and 49% of panic disorder patients. Unfortunately, the authors did not exclude GAD patients who had a history of panic attacks or panic disorder. Finally, Verburg et al.[38] compared the effects of 35% CO_2 inhalation in GAD and panic disorder patients. The authors found that GAD patients experienced less anxiety and fewer panic attacks compared with panic disorder patients (0/9 vs. 6/9, respectively) but had a similar increase in somatic symptoms.

In summary, GAD patients appear to resemble normal controls with respect to a panic challenge paradigm (e.g., few or no panic attacks) but appear more sensitive than normal controls in terms of number of symptoms and symptom intensity. This body of evidence further supports GAD as a biologically distinct disorder at least with respect to panic disorder.

Neurotransmitter Abnormalities

Alterations in different neurotransmitter systems have been implicated in the pathophysiology of anxiety disorders. It was previously believed that anxiety disorders may be associated with abnormalities in one neurotransmitter system. However, growing evidence supports the idea that dynamic interactions between various neurotransmitter systems may be involved in the pathophysiology of chronic anxiety, such as GAD. Presently, data suggest that the catecholamine (described earlier), serotonin, cholecystokinin (CCK), and corticotropin-releasing factor (CRF) neurotransmitters and the GABA/benzodiazepine receptor complex may be involved in the pathophysiology of GAD.

Benzodiazepines. Benzodiazepines have traditionally been the treatment of choice for many patients with GAD. They act at specific brain benzodiazepine receptors, which are part of the gamma-aminobutyric acid (GABA) receptor complex. GABA is the major inhibitory neurotransmitter in the brain. Several lines of evidence suggest abnormalities in the GABA-benzodiazepine receptor complex in GAD. The peripheral benzodiazepine receptors on platelets and lymphocytes have been studied most extensively. Recent studies suggest that there is a decrease in the number of benzodiazepine-binding sites on platelets and lymphocytes of patients with GAD. Weizman et al.[39] reported decreased density of platelet benzodiazepine receptor binding sites in patients with GAD and an increase in density of these binding sites after chronic diazepam treatment. Similarly Ferrarese et al.[40] reported decreased

density of lymphocyte benzodiazepine receptors in GAD patients compared with controls. After treatment with diazepam, the lymphocyte benzodiazepine receptor binding increased to normal levels. Similar results were reported by Rocca et al.[41] However, the peripheral benzodiazepine receptors are pharmacologically distinct from central benzodiazepine receptors, and the significance of these findings is unclear. Other researchers examined the function of central benzodiazepine receptors in patients with GAD. Central benzodiazepine sensitivity has been studied by Roy-Byrne and colleagues.[42] In this paradigm, researchers measure the velocity of saccadic eye movements, which is controlled in part by benzodiazepine receptors in the superior colliculus/pons area. These investigators had previously found evidence of reduced sensitivity of saccadic eye movements in panic disorder.[42] When the same paradigm was used, similar results were reported (though less conclusively) in GAD patients by Cowley et al.[43] The authors also examined the effects of intravenous diazepam on the levels of cortisol, ACTH, and GH, but found no differences between GAD patients and controls on these measures.

It appears that GAD patients may have alterations in the sensitivity of the central benzodiazepine receptors. Theoretically, a "shift" in benzodiazepine receptor sensitivity would cause the observed reduction in benzodiazepine receptor agonist effects in these patients, as has been shown in panic disorder. If this finding holds true in GAD, neutral ligands such as the benzodiazepine antagonist flumazenil may act as partial inverse agonists.[44] This hypothesis remains to be tested in GAD.

Serotonin. Serotonin (5-HT) function has been thought to play an important role in anxiety responses in animals and humans.[45] For example, compounds that affect serotonergic activity, such as the 5-HT_{1A} receptor agonists buspirone, ipsapirone, and gepirone, and the 5-HT_2 receptor antagonists ritanserin and serazepine have anxiolytic effects in GAD patients.[45,46]

On the basis of neuroanatomical location of 5-HT neurons, several theoretical models for the involvement of the 5-HT system in the biology of persistent anxiety states have been advanced. The cell bodies of some major 5-HT pathways arise in the raphe nucleus. They innervate the hypothalamus and thalamus (presumably regulating endocrine and other homeostatic functions), the basal ganglia, and the limbic system, particularly the septohippocampal system and the amygdala.[45] Eison[47] hypothesized that pathologic anxiety may represent excessive 5-HT activity in these critical brain areas. Evidence suggests that lesions of the serotonin system or blockade of serotonin synthesis have anxiolytic effects in animal models.[48,49] Agents that selectively affect serotonergic activity, such as the 5-HT_{1A} agonists buspirone and gepirone, decrease the firing rate of serotonergic neurons in the dorsal raphe nucleus in animal models and exert anti-anxiety effects in GAD patients.[50,51] Eison suggests that in patients with pathologic anxiety, buspirone stimulates the presynaptic 5-HT_{1A} autoreceptors and reduces 5-HT release and the activity of serotonergic neurons. In contrast, Graeff[52] postulated that stressful situations activate the 5-HT_{1A} postsynaptic receptors in the hippocampus, initiating adaptive and protective anxiety responses in stressful situations, whereas excessive stimulation of 5-HT_2 receptors in the limbic forebrain gives rise to oversensitivity to aversive stimuli, causing anxiety. In that context, both ritanserin-like drugs, which block postsynaptic 5-HT_2 receptors in the limbic forebrain, and buspirone-like drugs, which stimulate 5-HT_{1A} activity in the hippocampus, decrease anxiety. Finally, in this model, the anxiolytic effects of benzodiazepines may be mediated in part via decreasing the release of 5-HT in forebrain limbic areas by enhancing GABAergic inhibition of 5-HT raphe neuronal firing.[52]

Studies examining 5-HT function in GAD patients have been limited. Germine et al.[53] found that the administration of *m*-chlorophenylpiperazine (*m*-CPP), which activates multiple serotonin receptors, causes greater anxiety and "anger" responses in patients with GAD than in normal controls. da Roza et al.[54] examined the effects of ritanserin treatment on slow-wave sleep in a small number of GAD patients (N = 8) and normal controls and found no differences between the two groups. Because of the small sample size, study results should be viewed with caution. In the only study examining the CSF levels of serotonin in patients with GAD, Brewerton et al.[55] reported decreased levels of 5-HT in GAD patients compared with controls. Finally, in peripheral models, Iny et al.[56] found decreased titrated H^3-paroxetine binding in platelets of GAD patients compared with controls. Thus, the currently available data support the potential abnormality of serotonergic function in GAD, and limited data implicate the involvement of 5-HT_1 and 5-HT_2 receptors.

Corticotropin-releasing factor. Corticotropin-releasing factor (CRF), which modulates ACTH release, is primarily located in cells in the paraventricular nucleus (PVN) of the hypothalamus. However, CRF has also been shown to have extensive extrahypothalamic distribution consistent with its involvement in stress and emotionality. CRF is located in a number of forebrain limbic areas such as the amygdala, the locus ceruleus, and the dorsal vagal complex. CRF-secreting neurons are, in turn, modulated by other neurotransmitters that are implicated in anxiety and stress responses, such as serotonin and norepinephrine (which potentiate release) and GABAergic, opioid, and glucocorticoid neurotransmitters (which inhibit release).[57]

It appears that CRF plays an important role in centrally mediated, anxiety-related behaviors and stress responses.[57] For example, the iontophoretic administration of CRF onto locus ceruleus neurons increases the locus

ceruleus firing rate and the secretion of epinephrine and norepinephrine from the adrenal medulla.[18] In rodents, intracerebral CRF administration is associated with decreased exploration and less contact with novel stimuli in an unfamiliar environment and increased behaviors typical of arousal and stress (e.g., sniffing, locomotion, and grooming).[18] CRF administration also increases stress-induced fighting in rodents and potentiates the acoustic startle response; this effect is reversed by chlordiazepoxide administration.[58] Further, Chappell et al.[57] demonstrated that both chronic and acute stress increase CRF levels in the locus ceruleus and periventricular hypothalamic areas.[57] It is possible that many of the responses observed in animal models, which are affected by CRF, such as increased acoustic startle response, increased locomotor activity (restlessness), and increased autonomic activity, may mimic symptoms observed in patients with GAD. However, Fossey et al.[59] found no difference in CSF CRF levels between GAD patients and patients with obsessive-compulsive disorder, panic disorder, and normal controls. Nevertheless, additional research examining the potential role of CRF in anxiety disorders such as GAD is needed.

Cholecystokinin system. Cholecystokinin (CCK) exists in various forms in mammals. In the CNS, the tetrapeptide (CCK-4) and sulfated octapeptide (CCK-8S) are neurotransmitters, which may also play an important role in the mediation of anxiety responses in animals and humans.[60] CCK is one of the most abundant peptide neurotransmitters in the brain; its receptors are widely distributed through the CNS, with high densities in the hypothalamus, limbic system, basal ganglia, hippocampus, cortex, and the brain stem.[61] To date, two types of CCK receptors have been described and cloned: CCK-A (for alimentary) receptors found in the viscera and in some distinct brain areas, and CCK-B (for brain) receptors widely distributed in the brain of all mammals studied.[61]

Data indicate that the CCK system may be involved in the pathophysiology of anxiety responses in animal models and pathologic anxiety in humans. Peripheral or central administration of CCK receptor agonists, such as the CCK-B receptor agonist CCK-4, induces arousal and fear responses in animal models.[61] Observed responses to CCK-B receptor agonists include decreased exploratory activity in mice and rats (elevated plus-maze test); submissive, restless behavior in monkeys; and defensive attack in cats.[62–64] Pretreatment with CCK-B antagonists such as L-365,260 and CI-988 blocks the anxiogenic-like effects of CCK agonists in these models.[64]

The role of CCK as an endogenous modulator of anxiety/stress responses in humans has been recently explored. Philipp et al.[65] examined plasma levels of CCK neuropeptides in 19 healthy long distance runners 1 hour before a competitive race, immediately after the race, and under control/rest conditions. They found that plasma CCK levels were significantly elevated before the marathon, compared with control conditions, and showed only a small additional increase after the run. The effects of CCK agonist challenge on anxiety responses in humans have been also examined in normal volunteers and patients with panic disorder. Intravenous administration of CCK-4 and pentagastrin to patients who suffer from panic disorder induced panic attacks in a dose-dependent manner in patients with panic disorder and normal controls.[66] Importantly, patients with panic disorder exhibited significantly higher rates of panic attacks than normal controls to the same dose of CCK-4, suggesting increased sensitivity in panic disorder.[66] We[67] have recently demonstrated that an intravenous administration of the CCK-B receptor agonist pentagastrin (a peptide closely resembling CCK-4) to patients suffering from GAD induces panic attacks at higher rates than in normal controls. Panic attacks were reported by 71% (5/7) of GAD patients and 14% (1/7) of age- and sex-matched normal comparison subjects. These studies suggest that CCK may be an important modulator and/or mediator of normal anxiety responses in humans and may be involved in pathologic anxiety such as panic disorder, GAD, and perhaps other pathologic anxiety states.

While the picture is incomplete at this point, there is evidence that CCK-8S and CCK-4 may both modulate anxiety, but also may antagonize each other in critical ways in areas important in anxiety responses.[61]

The potential role of the CCK system in anxiety states such as GAD is particularly intriguing in light of its interactions with other systems that are believed to be important in anxiety disorders. Specifically, CCK increases the activity of catecholaminergic neurons in the locus ceruleus. For example, CCK activates neurons in the locus ceruleus via peripheral CCK receptors in vagal afferent pathways.[68] Additionally, treatment with a neurotoxin (DSP-4), which selectively destroys noradrenergic nerve terminals in projections from the locus ceruleus, results in increased CCK receptor binding density in brain regions such as the frontal cortex and the hippocampus, which receive noradrenergic input from the locus ceruleus.[61] Accumulating evidence also points to intimate interaction of the CCK and GABA neurotransmitter systems. CCK is colocalized in the GABA-synthesizing neurons in the cortex, hippocampus, and basolateral amygdala.[61] Withdrawal from long-term diazepam administration is associated with up-regulation of CCK-8 binding in the frontal cortex of the rats.[61] Bradwejn et al.[66] showed that clinically relevant concentrations of benzodiazepines selectively antagonized CCK-8-induced activation of the rat hippocampal pyramidal cells. The specificity of the response to benzodiazepines was supported by the lack of effect of nonbenzodiazepine agents haloperidol, phenobarbital, and meprobamate.[66] However, in a study to assess whether CCK-4 effects are mediated through benzo-

diazepine receptors, Bradwejn et al.[69] found that flumazenil (a benzodiazepine receptor antagonist) did not antagonize the effects of CCK-4 in healthy volunteers. The authors suggested that both systems may act on GABA receptors via physiologically opposing (i.e., either inducing or blocking anxiety) but separate mechanisms. CCK also interacts with the 5-HT system in animal models of anxiety. For example, the anxiogenic-like antiexploratory effects of CCK agonists in rodents can be blocked by treatment with the anxiolytic 5-HT_3 receptor antagonist ondansetron.[70] Finally, CCK also plays a role in the modulation of the HPA stress axis activity. Administration of a CCK-B receptor agonist in humans increases the secretion of ACTH and cortisol.[71] In animal models, researchers were showed that peripheral CCK-8 administration stimulates ACTH release presumably via vagal afferents.[72] Further, central administration of CCK-8 also induces the release of hypothalamic CRF secretion.[72] In addition, Mannisto et al.[73] found that CCK-A receptor stimulation inhibits the secretion of thyrotropin and probably GH from the anterior pituitary in rats, while stimulation of CCK-B receptors exerts an opposite effect.

In summary, the CCK system appears to exhibit significant "cross talk" with other neurotransmitters. This is one way in which the CCK system may be involved in the pathophysiology of pathologic anxiety states such as GAD.

Cerebral Blood Flow and Metabolism Studies

Important advances have been made in the past several years in the neuroimaging research in anxiety disorders. These techniques allow us to assess the neuroanatomy and more importantly the metabolic activity of distinct brain areas. Neuroimaging techniques use tracer amounts of biochemicals labeled with radiation emitters, which are subsequently localized in different brain areas. They are used to measure cerebral blood flow (CBF), which is highly correlated with cerebral metabolism, as well as more direct measurements of basal cerebral glucose or oxygen metabolic rates.

Several researchers examined changes in CBF in normal volunteers and patients with GAD. It has been found that in normal controls, increased arousal, such as mental tasks or during REM sleep, is associated with increased CBF, whereas decreased arousal during drowsiness or sleep is associated with reduced CBF.[74] During task or stimulus, normal subjects show a diffuse and general increase in CBF, which habituates with repeated exposure, as well as a specific regional increase in activated brain areas.[74] Unfortunately for researchers, CBF varies with different anxiety levels. Gur et al.[75] used xenon-133 inhalation technique and PET and found that normal volunteers with lower baseline anxiety levels had increased CBF with increasing anxiety, and those with higher baseline anxiety showed decreased CBF with increasing anxiety. Gur et al.[75] also found that normal controls with moderate anxiety performed better and had greater increases in CBF than those with low or high anxiety levels.

In a study using xenon inhalation, Mathew et al.[74] examined CBF in nine patients with GAD. They found that under resting conditions, the pattern of global or regional blood flow did not differ significantly between GAD patients and controls. However, they found significant negative correlations between state anxiety and cerebral blood flow in most brain regions. Mathew et al.[77] also assessed changes in CBF in 13 GAD patients and controls after 5% CO_2 inhalation. The authors found that there were no differences in anxiety between the groups; however, those subjects who became anxious (both patients and normal controls) showed significantly less increase in CBF compared with the nonanxious group.

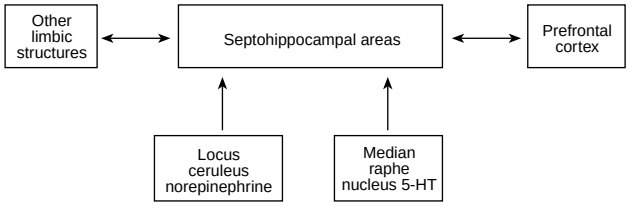
Regional differences in brain activity were also noted in patients with GAD. Wu and colleagues[76] evaluated 18 patients who met DSM-III criteria for GAD by using PET measurements of cerebral glucose metabolism at "baseline" (during a passive viewing task), after a cognitive vigilance task designed to stimulate anxiety, and after 3 weeks of treatment with benzodiazepines or placebo. The authors found higher relative metabolic rates for GAD patients in parts of the occipital, temporal (right posterior temporal lobe), frontal lobes (left inferior frontal gyrus), and the cerebellum relative to normal control subjects during a passive viewing task. They also found a decrease in absolute metabolic activity in the basal ganglia, temporal lobes, and cingulate gyrus in GAD patients. The metabolic rates in the amygdala and hippocampus were also reduced but the difference was not significant. During the vigilance task, GAD patients showed a significant increase in relative basal ganglia and right parietal lobe metabolism whereas right temporal and occipital lobes demonstrated a decrease in metabolic rates and no changes in limbic activity. The authors did not find a global decrease in cortical metabolism, as had been predicted by blood flow studies. Finally, benzodiazepine treatment resulted in a significant decrease in glucose metabolism in the cortical surface (especially the occipital cortex), the limbic system, and basal ganglia, compared with patients receiving placebo, and was not associated with normalization of patterns of regional metabolism. However, in the placebo group, decrease in anxiety scores was associated with relative increase in basal ganglia activity and a decrease in limbic activity.

In summary, although limited in number, the available imaging studies in patients with GAD suggest potential changes in brain activity in this population.

Conclusion: Unifying Neurobiological Model of GAD

The data presented above suggest possible abnormalities in the regulatory mechanisms of several important biological components in GAD patients. Although during

Figure 2. Neuroanatomical Model of Generalized Anxiety Disorder: Behavioral Inhibition System



rest conditions, GAD patients do not appear to differ significantly from normal controls on most measures studied, their response to stressful stimuli appears abnormal. Maladaptive responses to stressful stimuli have been observed in the locus-ceruleus-norepinephrine-sympathetic nervous system, the HPA axis, and the CCK system. Abnormalities in other important CNS modulators, such as 5-HT and GABA, may also be involved in the biology of GAD.

Several unifying neuroanatomic models of persistent anxiety states such as GAD have been proposed. Gray[78] developed an elegant model (the "behavioral inhibition system") for the neuroanatomic circuit that modulates responses to threat in animal models and potentially persistent anxiety states in humans (Figure 2). The behavioral inhibition system includes the septohippocampal areas and its connected structures including the noradrenergic and the 5-HT afferents. Gray hypothesized that in animals the septohippocampal areas are responsible for the processing of threat-relevant stimuli, such as signals of punishment, nonreward, or novel stimuli. The assessment of the presence of danger activates the behavioral inhibition system, which results in increased arousal and the inhibition of all the regular, ongoing behaviors. Noradrenergic and serotonergic stimulation of the septohippocampal area further activates this system. This state of increased vigilance/scanning and hyperarousal mimics persistent anxiety states, such as GAD. Thus, it is possible that certain anxiolytic drugs may exert their effects via reduction of the noradrenergic or the serotonergic inputs into the septohippocampal areas.

In summary, although limited and conflicting, the neurophysiologic and neuroimaging data provide some support for the involvement of the structures postulated by Gray in the mediation of generalized anxiety. However, further research utilizing new emerging research techniques such as measurement of receptors in human brain tissue and state of the art neuroimaging studies is clearly needed to determine the biological correlates of GAD.

Drug names: buspirone (BuSpar), chlordiazepoxide (Librium and others), diazepam (Valium and others), flumazenil (Romazicon), haloperidol (Haldol and others), meprobamate (Equanil and others), ondansetron (Zofran), paroxetine (Paxil), pentagastrin (Peptavlon), phenobarbital (Luminol and others).

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