Neuropeptide Alterations in Depression and Anxiety Disorders

David A. Gutman, Dominique L. Musselman, MD,
and Charles B. Nemeroff, MD, PhD

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Please send all correspondence to:

Charles B. Nemeroff, MD, PhD
Department of Psychiatry and Behavioral Sciences
Emory University School of Medicine, Box AF
1639 Pierce Drive, Suite 4000
Atlanta, GA  30322
Phone: (404) 727-8382
FAX: (404) 727-3233
1. Introduction

In the search for the underlying pathophysiology of the major psychiatric disorders, neuropeptides in general, and hypothalamic releasing factors in particular have been scrutinized closely. Our growing understanding of the brain over the past few decades has suggested complex interactions between classical monoamine neurotransmitters, such as dopamine (DA), norepinephrine (NE) and serotonin (5HT), and the growing numbers of neuropeptides found throughout the brain. Neuropeptides, molecules that contain two or more amino acids linked by peptide bonds, function as critical chemical messengers and are heterogeneously distributed through the peripheral and central nervous system (CNS). Many of these peptides exert diverse actions functioning as hypothalamic hypophysiotropic releasing factors, as neuromodulators, and/or neurotransmitters. As understanding of the behavioral effects of these neuropeptides continues to grow, it has become increasingly apparent that dysregulation of the proper functioning of neuropeptide systems may be relevant to particular psychiatric disorders. In this chapter we briefly review basic neuropeptide neurobiology most relevant to psychiatric disorders, and summarize preclinical and clinical studies implicating neuropeptide alterations in the pathophysiology of mood and anxiety disorders.

2. Rationale for exploring neuropeptide modulation in affective and anxiety disorders

Undoubtedly one early rationale for the intensive study of neuropeptide systems in patients with primary psychiatric disorders was the higher than expected psychiatric morbidity in patients with primary endocrine disorders such as Addison’s disease or Cushing's syndrome. Disturbances in the feedback regulation of the hypothalamic-
pituitary-end organ axes are of considerable interest. Because hypothalamic
hypophysiotropic neuropeptides ultimately control the activity of these neuroendocrine
axes, considerable effort has been expended into the search for evidence of dysfunction in
these systems. However outside of the neuroendocrine axes, the ongoing discovery and
characterization of new neuropeptides and multiple subtypes of neuropeptide receptors
and their heterogeneous distribution has often suggested these peptides may modulate
behaviors directly relevant to psychiatric disease. Because neuropeptides account for
neurotransmission at a large percentage of CNS synapses and indirectly or directly
modulate the activity of a diverse number of hormones and neurotransmitters that have
been implicated in the pathogenesis of affective and anxiety disorders, their scrutiny may
allow for elucidation of the primary pathophysiological deficits in these devastating
illnesses.

One of the primary motivations for the continuing investigation of the major
endocrine axes is the so-called “neuroendocrine window strategy”. This strategy is
based on a large literature which indicates that the secretion of the target endocrine
organs, e.g. the adrenal cortex or thyroid, is largely controlled by their respective
pituitary trophic hormones, which in turn is controlled primarily by the secretion of their
respective hypothalamic release and/or release-inhibiting hormones. There is now
considerable evidence that the secretion of these hypothalamic hypophysiotropic
hormones is controlled, at least in part, by the classical neurotransmitters including 5HT,
aacetylecholine (ACh), and NE, all previously posited to play a preeminent role in the
pathophysiology of affective, anxiety, and/or psychotic disorders.
However, the hypothesis that one can infer information about higher CNS neuronal activity, as for example the activity of serotonergic neurons in a particular disease state solely by measuring the function of a specific endocrine axis, is, however both far from proven and fraught with difficulty. Many other factors then a single neurotransmitter system affect the activity of the various endocrine axes. Nevertheless taken together with other measures of neurotransmitter function, this approach can indeed provide useful information.

The contrasting behavioral and neurobiologic effects of antidepressants (tricyclic antidepressants or monoamine oxidase inhibitors), anxiolytics (SSRIs, SNRIs and benzodiazepines), and antipsychotics as well as those drugs which induce or worsen depression (such as reserpine), anxiety (flumazenil, cholecystokinin), and psychosis (psychostimulants, phencyclidine) have provided yet another impetus for scrutiny of neuroendocrine and neuropeptide pathophysiology in the major psychiatric illnesses because virtually all of these agents alter the activity of one or more endocrine axis. This second core assumption, the “psychopharmacologic bridge technique” posits that if a drug produces therapeutic effects and has specific biochemical actions, an etiologic relationship between the therapeutic effects, biochemical changes, and the primary cause of the syndrome may exist [1]. For example tricyclic antidepressants block reuptake of NE and 5-HT, MAO inhibitors inhibit the metabolism of catecholamines and 5-HT, and moreover, down-regulation (decrease in the number) of β-adrenergic receptors which is associated with most antidepressant treatments occurs in association with the clinically successful treatment of depression. The “pharmacologic bridge technique” and “neuroendocrine window strategy” suggest together that alterations of a variety of
endocrine axes exist within patients with major psychiatric disorders, and that many clinically efficacious agents act upon one or more of the neuropeptide circuits in the CNS. The pharmacologic bridge technique also suggests altered neurotransmitter transporter or receptor-mediated signal transduction in depression and other psychiatric disorders. Whether alterations in peripheral endocrine organ hormone secretion contribute primarily to the pathogenesis of psychiatric disorders, and/or whether altered secretion of pituitary and hypothalamic hormones primarily contribute to the signs and symptoms of a specific mental illness remains a subject of considerable controversy. In this chapter, we briefly outline the major findings concerning the putative roles of neuropeptides and hypothalamic releasing factors in mood and anxiety disorders.

3. Current Strategies for Evaluating a Specific Role for Neuropeptides In Psychiatric Disorders: Preclinical and Clinical Studies

3.1 Preclinical Studies
Preclinical studies have been essential in providing useful and novel peptide circuit targets for drug discovery, and in understanding some of the possible roles of neuropeptides and their interactions with other brain systems. Most of these studies involve directly injecting the peptide of interest into the brains of laboratory animals, and studying its effect on behavior and on other known peptide and hormonal systems. One example of the usefulness of this strategy is in identifying potential behavioral effects of cholecystokinin (CCK), which was originally discovered in the gastrointestinal tract. CCK injections in laboratory animals can induce many behavior responses characteristic of panic attacks, which has led to scrutiny of a potential role for CCK and its receptor systems in panic disorder.

3.2 Clinical Studies

As noted above, in the 1970s and 1980s peripheral neuroendocrine markers were often used to indirectly assess CNS function because the brain was relatively inaccessible for study, with the exception of cerebrospinal fluid (CSF) and postmortem studies. With the emergence of the monoamine theories of mood disorders and schizophrenia, many investigators attempted to draw conclusions about the activity of specific neurotransmitter circuits in patients with various psychiatric disorders by measuring the basal and stimulated secretion of pituitary and end-organ hormones in plasma. Clinical studies are also often confounded by the normal circadian rhythms and the pulsatile release of many of the hypothalamic-pituitary-end-organ axes components which are often not taken into account when these experiments are designed. Further, differences in assay sensitivity, gender, inclusion criteria for patients, and severity of symptoms all may potentially generate confounding or at least quite variable results. Nevertheless
considerable progress about the neurobiology of psychiatric disorders has been discovered through such an approach.

Before proceeding into a review of the relevant clinical literature, a brief overview of the most common methodologies employed merits discussion. Most clinical studies in humans are based on one of three methodologies: (1) postmortem studies in which the concentration of neuropeptides and neuropeptide receptor kinetics are measured, (2) studies measuring the concentrations of peptides in biological fluids such as plasma and CSF, and (3) studies measuring the biological and behavioral changes following administration of the neuropeptides (i.e. challenge tests).

Postmortem studies are advantageous in that they allow peptide levels to be sampled directly in brain tissue and from distinct brain regions. Methodologically there are major obstacles to overcome in obtaining and studying postmortem tissue. Because peptides and their receptors and mRNA are degraded postmortem, differences between the elapsed time between death of the subject and harvesting of the tissue, and hence in postmortem decay, as well as other neurochemical confounds associated with preserving and handling the tissue render conclusions concerning the relation between peptide circuit alterations and psychiatric disorders tentative, and unable to stand alone.

Many investigators have measured peptides in plasma and CSF. The concentration of neuropeptides in CSF is thought by some, but not all, investigators to accurately measure extracellular fluid concentration in brain, and therefore has an advantage over postmortem studies which is thought to measure both the releasable and nonreleasable pools of neuropeptides. Although CSF concentrations may be an index of the mean activity of a neuropeptide system, they may not accurately represent regional
variations in extracellular availability of these peptides that may be of great pathophysiologic importance. Plasma concentrations of neuropeptides are also commonly determined, though they are confounded by the presence of binding proteins as for example with CRF, and the impossibility of differentiating between CNS and peripheral sources of the neuropeptide being measured.

Another important strategy has been the administration of neuropeptides or synthetic neuropeptide agonists or antagonists directly to patients with affective and anxiety disorders, and measuring subsequent neuroendocrine and/or behavioral changes. In these so-called stimulation or provocative tests, hypothalamic and/or pituitary derived factors or their synthetic analogs are exogenously administered, and the hormonal response to this “challenge” is assessed. For example, in the standard corticotropin-releasing-factor (CRF) stimulation test, a 1 μg/kg dose of human or ovine CRF is administered intravenously, and the adrenocorticotropic (ACTH) and cortisol response is measured over a period of 2 or 3 hours. This test is a very sensitive measure of hypothalamic-pituitary-adrenal (HPA) axis activity, and changes in the magnitude and/or duration of the response relative to normal control values are characteristic of one or another type of dysregulation of the HPA axis. One must be cautious, however, about making assumptions about what, if any, CNS deficits are responsible for the pattern of HPA axis activity observed.

4. Corticotropin-Releasing Factor (CRF) in Mood and Anxiety disorders

4.1 Basic Biology

The hypothalamic-pituitary-adrenal (HPA) axis has been extensively scrutinized in patients with psychiatric diseases. An abundance of evidence dating back over 100 years
has demonstrated that dysfunction of the HPA axis may lead to specific psychiatric disturbances. At the pinnacle of this system is the neuropeptide corticotropin-releasing factor (CRF), also known as corticotropin-releasing hormone (CRH), a 41 amino acid peptide which as a hypothalamic releasing factors controls the activity of the HPA axis.

Within the hypothalamus, CRF is primarily synthesized in the parvocellular neurons located in the paraventricular nucleus (PVN) [2]. CRF neurons in the PVN [Figure 1] receive input from a variety of brain nuclei including the amygdala, bed nucleus of the stria terminalis, and other brain stem nuclei [2]. Hypothalamic CRF-containing neurons in turn project to the median eminence [3]. In response to stress, this neural circuit is activated releasing CRF from the median eminence into the hypothalamo-hypophyseal portal system where it binds to CRF receptors on corticotrophs in the anterior pituitary which promotes the synthesis of proopiomelanocortin (POMC) and the release of its post-translation products, adrenocorticotropic hormone (ACTH), β-endorphin, and others. ACTH released from the anterior pituitary into the systemic circulation then stimulates the production and release of cortisol by acting on ACTH receptors in the adrenal cortex. [For a general schematic of HPA axis regulation see Figure 2].

Although Saffron and Schally identified a crude extract that promoted the release of ACTH from the pituitary in 1955 [4], CRF was not isolated and chemically characterized until 1981. Working with extracts derived from 500,000 sheep hypothalami, Vale and colleagues at the Salk institute isolated, synthesized, and elucidated the structure of CRF [5]. This discovery led to the availability of synthetic CRF, which finally permitted a more comprehensive assessment of HPA axis activity to
be developed. It is now clear that CRF coordinates the endocrine, immune, autonomic and behavioral responses of mammals to stress.

Two CRF receptor subtypes, CRF₁ and CRF₂, with distinct anatomical localization and receptor pharmacology have been identified [6-10] in rats and humans. Both receptors are G-protein coupled receptors and are positively coupled to adenylyl cyclase via Gₛ. The CRF₁ receptor is predominant expressed in the pituitary, cerebellum, and neocortex in the rat [11]. A growing body of evidence from animal studies has shown that the CRF₁ receptors may specifically mediate some of the anxiogenic-like behaviors observed after administration of CRF [12, 13]. The CRF₂ receptor family is composed of two primary splice variants, CRF₂A and CRF₂B. The CRF₂A receptor is more prevalent in subcortical regions, such as the ventromedial hypothalamus, lateral septum, and dorsal raphe nucleus, whereas CRF₂B is more abundantly expressed in the periphery. In addition to CRF, several other endogenous peptide ligands for CRF receptors have recently been discovered including urocortin [14], urocortin II, and urocortin III and perhaps others [15, 16]. With the discovery of several new putative endogenous ligands, much of the pharmacology and functional interactions between these ligands and receptors remains to be discovered [17, 18].

4.2 HPA Axis abnormalities in depression

Evidence linking HPA axis abnormalities and psychiatric symptoms, as noted above, dates back more than 100 years. The greater than expected prevalence of depression and other psychiatric symptoms in both Cushing’s and Addison’s disease served as an impetus for researchers to scrutinize HPA axis function in patients with psychiatric disorders. Based on the work of research groups led by Board, Bunney and
Hamburg, as well as by Carroll, Sachar, Stokes and Besser, literally thousands of studies were conducted in this area, most in the 1970’s.

Some of the earliest studies demonstrated abnormalities in glucocorticoid function in depressed patients including elevated plasma and cerebrospinal fluid (CSF) cortisol concentrations in depressed patients [19, 20], increased 24-hour urinary free cortisol concentrations and increased levels of cortisol metabolites in urine [21]. One of the major advances, of course, came with the discovery of the chemical identity of the neuropeptide CRF, the primary physiological secretagogue of ACTH, and ultimately of cortisol.

In order to accurately assess HPA axis function, several functional challenge tests have been developed, both provocative and suppression tests. The most commonly used test to measure HPA axis function is the dexamethasone suppression test (DST). In this standardized test, 1 mg of dexamethasone is administered orally at 11 P.M., and blood samples are obtained at 8 A.M. the following morning for cortisol measurement. Dexamethasone is a synthetic steroid similar to cortisol, and it suppresses ACTH secretion, and subsequently cortisol release, in healthy volunteers. Nonsuppression of plasma glucocorticoid levels following the administration of dexamethasone is common in depressed patients. The rate and magnitude of cortisol nonsuppression after dexamethasone administration generally correlates with the severity of depression [22]; in fact nearly all patients with major depression with psychotic features exhibit DST nonsuppression [23, 24]. Since Carroll’s initial report [25, 26] and subsequent claims for diagnostic utility [27], the dexamethasone suppression test has generated considerable
controversy [28] as to its diagnostic utility. Diagnostic issues notwithstanding, the overwhelming conclusion from the myriad of studies demonstrates that a sizeable percentage of depressed patients exhibit HPA axis hyperactivity.

Shortly after the isolation and characterization of CRF, a standardized CRF stimulation test was developed to further assess HPA axis activity. In this paradigm, CRF (ovine or human) is administered intravenously (usually in a fixed dose of 100 μg or as a 1 μg/kg dose), and the ensuing ACTH (or β-endorphin) and cortisol response is measured at 30-minute intervals over a 2-3 hour period [29]. Numerous studies have now demonstrated a blunted ACTH and β-endorphin response to exogenously administered ovine CRF (oCRF) or human CRF (hCRF) in depressed patients when compared to non-depressed subjects, though the cortisol response in depressed patients and non-depressed control subjects did not consistently differ [30-34]. The blunted ACTH response to CRF occurs in depressed DST non-suppressors, but not in DST suppressors [35]. The attenuated ACTH response to CRF is presumably due to either chronic hypersecretion of CRF from nerve terminals in the median eminence, which results in down-regulation of CRF receptors in the anterior pituitary, and/or to the chronic hypercortisolemia. This receptor down-regulation results in a reduced responsivity of the anterior pituitary to CRF, as has been demonstrated in laboratory animals [36-40]. Following recovery from depression, the documented disturbances in the HPA axis generally remit.

A combined dexamethasone/CRF test has also been developed by Holsboer and his colleagues. In this test, 1.5 mg of dexamethasone is administered orally at night (23:00 h), and subjects receive an iv bolus of 100 μg of human CRF at 15:00 h the
following day. Patients with HPA axis dysfunction, which is frequently encountered in 40-60% of depressed patients, display a paradoxically increased release of ACTH and cortisol following CRF challenge relative to controls. These abnormalities disappear following remission of depression, and normalization of HPA axis function seems to precede full clinical remission [41, 42]. The combined DEX/CRF test appears to have much higher sensitivity for detecting subtle alterations in HPA axis function; approximately 80% of patients with major depression exhibit an abnormal response to the DEX/CRF test. In contrast, only approximately 44% of patients with major depression demonstrate an abnormal response when the dexamethasone suppression test is administered alone [41, 42]. Furthermore, otherwise healthy individuals with first degree relatives with an affective illness, which greatly increases their own risk for psychiatric disorders, demonstrated cortisol and ACTH responses to the DEX/CRF test which were higher than a control group, but less than patients currently suffering from major depression. One interpretation of these findings is that a genetically transmittable defect in corticosteroid receptor function may render these individuals more susceptible to developing affective disorders by increasing baseline and evoked HPA axis activity [43].

4.3 Extrahypothalamic CRF and depression

The studies thus far discussed focused primarily on dysregulation of the HPA axis, but perhaps of even greater relevance to psychiatric disorders is the fact that CRF controls not only the neuroendocrine, but also the autonomic, immune, and behavioral responses to stress in mammals. Moreover, results from both clinical studies, and a rich body of literature conducted primarily in rodents and lower primates have indicated the importance of extrahypothalamic CRF circuits [13, 44]. In rodents, primates, and
humans, CRF and its receptors have been heterogeneously localized in a variety of regions including the amygdala, thalamus, hippocampus, and prefrontal cortex, and others [45-48]. These brain regions are known to be important in regulating many aspects of the mammalian stress response, and in regulating affect. The presence of CRF receptors in both the dorsal raphe (DR) and locus coeruleus (LC), the major serotonergic and noradrenergic cell body-containing regions in the brain, respectively, also deserves comment. Because most available antidepressants including the tricyclics and selective serotonin reuptake inhibitors (SSRI) are believed to primarily act via modulation of noradrenergic and/or serotonergic systems, the neuroanatomical proximity of CRF and monoaminergic systems suggests provides plausibility for interaction between CRF systems and antidepressants.

Involvement of extrahypothalamic CRF systems in the pathophysiology of depression is suggested by numerous studies showing elevated CRF concentrations in the cerebrospinal fluid (CSF) of drug free depressed patients [49-53], though a discrepant report has appeared [54]. Elevated cisternal CSF CRF concentrations have also been detected in depressed suicide victims [49]. A reduction in concentrations of CRF in CSF has been observed in healthy volunteers treated with the tricyclic antidepressant desipramine [55] and in depressed patients following treatment with fluoxetine [56] or amitryptiline [57] providing further evidence of a possible interconnection between antidepressants, noradrenergic neurons and CRF systems. Similar effects have been reported after electroconvulsive therapy (ECT) in depressed patients [58]. Elevated CSF CRF concentrations appear to represent a state, rather than a trait, marker of depression, i.e., a marker of the state of depression rather than a marker of vulnerability to depression
[58]. Furthermore, high and/or increasing CSF CRF concentrations despite symptomatic improvement of major depression during antidepressant treatment may be the harbinger of early relapse [59], as our group previously reported for DST non-suppression [60].

While the exact mechanism contributing to CRF hyperactivity remains obscure, studies from our group and others have documented long-term persistent increases in HPA axis activity and extrahypothalamic CRF neuronal activity after exposure to early untoward life events—for example, neglect and child abuse respectively in both laboratory animals (rat and non-human primates) and patients [43, 61-63]. Early life stress apparently permanently sensitizes the HPA axis and leads to a greater risk of developing depression later in life. To measure HPA axis responsivity to stress in humans, the Trier Social Stress Test (TSST) was developed. This laboratory paradigm involves a simulated 10-minute public speech and a mental arithmetic task. The TSST has been validated as a potent activator of the HPA axis in humans [64]. Recently, our group has reported increased plasma adrenocorticotropic hormone (ACTH) and cortisol concentrations, presumably due to hypersecretion of CRF, after exposure to the TSST in women (both depressed and non-depressed) who were exposed to severe physical and emotional trauma as children [65]. The depressed women both with and without early life stress exhibited a blunted ACTH response to CRF whereas the women with early trauma alone exhibited an exaggerated ACTH response. These data provide further evidence for functional hyperactivity of CRF systems that may be influenced by early adverse life events.

4.4 CRF and anxiety disorders
Involvement of CRF in anxiety disorders has been well documented in both animal and human studies. As reviewed by Arborelius and colleagues [66] patients with posttraumatic stress disorder (PTSD), i.e. Vietnam combat veterans, exhibit significantly elevated CSF CRF concentrations [67, 68], as well as alterations in the ACTH response to CRF challenge [69]. A recent elegant study using an indwelling catheter in the lumbar space which allows repeated sampling of CSF for several hours after the initial, and presumably stressful, lumbar puncture, demonstrated elevated CSF CRF levels in PTSD combat veterans [68]. In contrast, low serum cortisol and urinary free cortisol levels have been repeatedly, yet unexpectedly, been detected in PTSD, especially after dexamethasone administration [70, 71]. One possible mechanism that has been proposed by Yehuda and colleagues to explain these findings is heightened negative glucocorticoid feedback within the HPA axis in chronic PTSD patients [72].

Although CSF CRF concentrations are not increased in panic disorder patients [73, 74], a diminished ACTH response to CRF administration has been observed [75]. Increased [76] or normal concentrations [74, 77] of CSF CRF have been documented in patients with OCD, though significant decreases in CSF CRF concentrations occur with a therapeutic response to clomipramine [78]. Patients with generalized anxiety disorder (GAD), however, exhibit similar CSF CRF concentrations in comparison to normal controls [74, 79]. Not surprisingly increased concentrations of CSF CRF occur in alcohol withdrawal, a condition of sympathetic arousal and increased anxiety [80, 81]. In contrast, CSF CRF concentrations are reduced [82] or are normal [83] in abstinent chronic alcoholics with normal plasma cortisol concentrations. Although HPA axis hyperactivity exists in patients with certain anxiety disorders, such perturbations do not
exist in the patterns suggestive of CRF hypersecretion as documented in patients with major depression [66]. Moreover in the majority of these studies careful assessment of comorbidity of mood and anxiety syndromes and symptoms have not been conducted.

4.5 CRF in Depression and Anxiety: Conclusions

Space constraints do not permit an extensive review of the preclinical literature, however several additional points are worth highlighting. Numerous studies have documented that when CRF is directly injected into the CNS of laboratory animals, it produces effects reminiscent of the cardinal symptoms of depression including decreased libido, reduced appetite and weight loss, sleep disturbances, and neophobia. Based on this premise, newly developed CRF₁ receptor antagonists represent a novel putative class of antidepressants. Such compounds show activity in nearly every preclinical screen for antidepressants and anxiolytics currently employed. Recently, a small open label study examining the effectiveness of R121919, a CRF₁ receptor antagonist, in major depression was completed [84] in which standard severity measures of both anxiety and depression were reduced in the depressed patients after R121919 treatment. Although this drug is no longer in clinical development, it is clear that CRF₁ antagonists represent potentially a new class of psychotherapeutic agents to treat anxiety and affective disorders. Finally the early trauma data raise the question of whether childhood physical and sexual abuse, common among depressed patients, is responsible for some or all of the changes reported in the HPA axis and CRF circuits in patients with major depression.

5. Thyrotropin-Releasing Factor, the Thyroid Axis, and Depression

5.1 Biology
Dysfunction of the hypothalamic-pituitary-thyroid (HPT) axis has been associated with numerous psychiatric disturbances ranging from mild depression to overt psychosis. The HPT axis controls the release of thyroid hormones, $T_3$ and $T_4$, and is regulated by two peptide hormones, thyrotropin releasing hormone (TRH), a tripeptide, and thyroid stimulating hormone (TSH) or thyrotropin, a large protein. TRH was the first of the hypothalamic releasing hormones to be isolated and characterized. TRH is synthesized predominantly in the paraventricular nucleus in the hypothalamus and stored in nerve terminals in the median eminence where it is released into the vessels of the hypothalamic-hypophyseal portal system. TRH is then transported to the sinusoids in the anterior pituitary where it binds to TRH receptors on thyrotropes, and promotes the release of TSH into the systemic circulation.

TSH is a 28 kDA glycoprotein composed of two noncovalently linked protein chains, TSH-$\alpha$, which is identical to the $\alpha$ subunit contained in other pituitary hormones including follicle-stimulating hormone, luteinizing hormone and human chorionic gonadotropin, and TSH-$\beta$. Upon stimulation by TSH, the thyroid gland releases the iodinated amino acids L-triiodothyronine ($T_3$) and thyroxine ($T_4$). Besides their well known metabolic effects, $T_3$ and $T_4$ feedback to the hypothalamus and adenohypophysis to regulate the synthesis and release of TRH and TSH, respectively [85]. Following its initial isolation, the heterogeneous distribution of TRH in extrahypothalamic sites strongly suggested a role for this peptide as a neurotransmitter or neuromodulator, as well as a releasing hormone. It is now established that TRH itself can produce direct effects on the CNS independent of its actions on pituitary thyrotrophs.

5.2 Psychiatric manifestations of thyroid dysfunction
Primary thyroid axis disorders are often associated with numerous psychiatric manifestations ranging from mild depression and anxiety to overt psychosis. Regardless of the etiology, hypothyroidism leads to a number of clinical manifestations including slowed mentation, forgetfulness, decreased hearing, cold intolerance, and ataxia. Decreased energy, weight gain, depression, cognitive impairment or overt psychosis (“myxedema madness”) may also result. Due to the overlapping symptoms with depression, thyroid hormone deficiency must be ruled out when evaluating patients with depression.

Early studies from the late 1960’s and early 1970’s scrutinized psychiatric comorbidity in patients with hypothyroidism, and a substantial number of these patients demonstrated signs of depression, anxiety, and/or cognitive disturbances \[86, 87\]. Furthermore, these symptoms were often improved or resolved following resolution of the thyroid condition alone. Later studies have demonstrated varying degrees of cognitive disturbance in up to 48% of psychiatrically ill hypothyroid cases \[88\], and approximately 50% of unselected hypothyroid patients have symptoms characteristic of depression \[88\]. Anxiety symptoms are also common, occurring in up to 30% of unselected patients. Mania and hypomanic states have been rarely reported in hypothyroid patients. Finally, although psychosis is the most common reported symptom in the case literature on hypothyroidism (52.9%), it only accounts for approximately 5% of the psychiatric morbidity in an unselected sample \[88\], presumably because more severe symptoms are more likely to be documented in the clinical literature.
5.3 TRH and TSH in depression

Considerable evidence has revealed an increased incidence of HPT axis dysfunction in patients with major depression [Table 2]. More than 25 years ago research groups led by Prange [89] and Kastin demonstrated that approximately 25% of patients with major depression exhibit a blunted TSH response to TRH [89, 90]. This is clearly not secondary to hyperthyroidism but presumably due to hypersecretion of TRH from the median eminence, which leads to TRH receptor downregulation in the anterior pituitary and reduced sensitivity of the pituitary to exogenously administered TRH. This hypothesis seems plausible in light of evidence showing elevated TRH concentrations in the cerebrospinal fluid (CSF) of drug-free depressed patients [91] and an inverse relationship between the blunted TSH response to TRH and CSF TRH concentrations [92]. Depressed patients have also been shown to have an increased prevalence rate of symptomless autoimmune thyroiditis (SAT), defined by the abnormal presence of antithyroglobulin and/or antimicrosomal thyroid antibodies consistent with Grade 4 hypothyroidism [93].

Although there is a preponderance of evidence suggesting elevated TRH release in some depressed patients, as of yet it is unclear what precise role this factor plays in depression. Interestingly, a lumbar intrathecal infusion of 500 μg of TRH into medication-free inpatients with depression produced a clinically robust, but short-lived, improvement in mood and suicidality, which led the authors to propose that elevated TRH levels might be a compensatory response in depression [94]. Although this work is
preliminary, it raises the interesting possibility that a systemically administered TRH receptor agonist may represent a novel class of antidepressant agents.

5.4 Bipolar disorder and HPT abnormalities

HPT axis abnormalities have also been reported in bipolar disorders. Both elevated basal plasma concentrations of TSH and an exaggerated TSH response to TRH have been demonstrated [95, 96]. There is also evidence that bipolar patients with the rapid cycling subtype have a higher prevalence rate of hypothyroidism (Grades I, II and III) than bipolar patients who do not [97, 98]. A blunted or absent evening surge of plasma TSH, a blunted TSH response to TRH, [99, 100], and the presence of antithyroid microsomal and/or antithyroglobulin antibodies [101, 102] have also been demonstrated in bipolar patients.

5.5 Thyrotropin-Releasing Hormone Alterations in Anxiety disorders

Evidence demonstrating alterations of TRH circuits in anxiety disorders is relatively limited. A blunted TSH response to TRH has been observed in panic disorder patients [103, 104]. However in one of these studies, this effect was only demonstrated in patients with concurrent major depression [104]. Another study indicated that the TSH response to TRH is normal in panic disorder patients [105]. CSF TRH concentrations in patients with panic disorder, generalized anxiety disorder, or obsessive-compulsive disorder are unchanged compared to control subjects [106].

6. Arginine-vasopressin (AVP)

6.1 Biology

Arginine-vasopressin (AVP), also known as anti-diuretic hormone (ADH), is a nonapeptide synthesized in the lateral magnocellular neurons of the paraventricular and
supraoptic nuclei of the hypothalamus, and is released directly into the bloodstream from axon terminals in the posterior pituitary [107]. These AVP-containing neurons terminate in the neurohypophysis and secrete AVP into the systemic circulation, though they send collaterals to the hypothalamo-hypophyseal portal system as well. Another group of AVP-containing neurons project from the medial parvocellular subdivision of the paraventricular nucleus (PVN) to the median eminence. Within the median eminence, the parvocellular-derived AVP is released from axon terminals, secreted into the hypothalamo-hypophyseal portal circulation, and carried to the anterior lobe of the pituitary gland [3]. Moreover extrahypothalamic AVP-containing neurons lie within limbic structures such as the septum and amygdala, as well as in the brainstem and spinal cord [108]. AVP-containing neurons also receive afferent innervations from many different neuronal cell groups and send axonal projections from the cerebral cortex throughout the CNS. It is thought that AVP and the other well-known posterior pituitary hormone, the nonapeptide oxytocin (OT), play a role in modulating neural activity in hypothalamic, limbic, and autonomic circuits.

AVP has prominent roles in controlling fluid balance via its effects on the kidney, in regulating blood pressure by its vasoconstrictive effects on blood vessels, and can directly promote the sensation of thirst. AVP also can act synergistically with CRF to promote the release of pituitary POMC-derived peptides, i.e. ACTH and β-endorphin in humans [109] and animals [110] following stressful stimuli [111]. Chronic stress or adrenalectomy increases the activity of the parvocellular AVP system [112, 113]. CRF and AVP are colocalized in the parvocellular cells of the human hypothalamus and may be secreted together into the human hypothalamic-hypophyseal portal circulation [114].
The ratio of AVP and CRF released into the hypothalamic-hypophyseal portal circulation varies in different species [110], and according to the nature of the stress [115, 116].

6.2 AVP in anxiety and depression

Similar to the clinical investigations regarding CRF, a variety of patient groups have been studied. Alterations of CSF AVP concentrations have been reported in patients with major depression, bipolar disorder, schizophrenia, anorexia, obesity, alcoholism, Alzheimer’s disease, and Parkinson’s disease [117, 118]. CSF AVP concentrations in patients with major depression are reportedly reduced in comparison to control subjects, though the source of CSF AVP is likely extrahypothalamic and not an index of PVN AVP secretion [119-121]. Basal plasma concentrations of AVP in depressed patients are also reportedly decreased in comparison to age-matched controls [122], though others have found no difference [123]. Interestingly AVP secretion in response to an infusion of hypertonic saline is diminished in depressed patients as compared to controls [123].

A mildly blunted ACTH response to exogenous AVP administration has been reported in depressed patients [33], but the finding was not replicated in two other studies [124, 125]. Remarkably an increase in the number of PVN AVP neurons colocalized with CRF cells has been reported in depressed patients compared to controls in a postmortem tissue study [126, 127]. This is of interest in view of the ability of AVP to potentiate the actions of CRF at the corticotrop. In contrast to the findings suggestive of diminished hypothalamic-vasopressinergic activity in depressed patients are the findings suggestive of hypersecretion of AVP in bipolar patients during their manic phase. Elevations in CSF AVP concentrations have been documented in manic patients [118], as
well as significant increases of plasma AVP in comparison to patients with unipolar depression and controls [128]. Clearly hypothalamic and extrahypothalamic AVP circuits are regulated independently. Whether the perturbations of AVP secretion in patients with neuropsychiatric disorders are state or trait dependent requires further elucidation.

7. Endogenous Opioid Peptides

7.1 Biology

The rationale for studying opioids in depression and anxiety disorders is based on the observation that opiates possess antidepressant and anxiolytic effects. Opium has been known to have mood-elevating properties, and Krapelin proposed opium as a treatment for depressed patients as early as 1905. It is now known that the endogenous opioid peptide system is comprised of three groups of peptides including methionine-enkephalin and leucine-enkephalin, [129], β-endorphin [130], and the dynorphins [131]. These peptides have been shown to play a variety of physiological roles, including regulation of pain, mood, respiration, cardiovascular function, gastrointestinal activity, satiety, and sexual behavior (see review by Smith[132]).

At the genomic level there are three genes responsible for the precursors of opioid peptides: pro-opiomelanocortin (POMC), proenkephalin and prodynorphin. Consequently there are at least three classes of opioid peptides with different biosynthetic and neuronal pathways: the endorphins, enkephalins and dynorphins. In the adenohypophysis [133], POMC is processed to yield only ACTH and β-lipotropin. β-lipotropin is then processed to yield at least 3 compounds, including β-, γ-, and α-endorphin. The second endogenous opioid system is comprised of met- and leu-
enkephalin whose precursor is proenkephalin. Derivatives of prodynorphin are the third group of endogenous opioid peptides including dynorphin A, dynorphin B, and neoendorphin which are located almost exclusively in the posterior pituitary.

7.2 Opioids in depression

Early open trials with β-endorphin indicated an improvement in depressed patients following intravenous β-endorphin administration [134], which were later substantiated by controlled clinical trials [135, 136]. However these results were not confirmed, and there is conflicting evidence to whether intravenously administered β-endorphin permeates the blood-brain barrier (see [137] for a comprehensive review of these studies). Also, the highly addictive nature of opioids limits their usefulness as a potential treatment for depression. Several studies have also shown that the potent mu opiate receptor antagonist naloxone does not directly affect mood [138-140], further weakening a strong role of the endogenous opioid system in affective disorders. Two later studies, using very high doses, showed an increase in anxiety in normal controls [141] or an augmentation of symptom severity in depressed patients. Nevertheless these studies did not provide evidence for a role for opioid peptide systems in depression.

The studies investigating CSF β-endorphin immunoreactivity in depression and anxiety disorders have by and large also not substantiated any major role for endogenous opioid peptides in depression or anxiety disorders. Most investigators [142-146], but not all [147], have reported normal concentrations of CSF β-endorphin in patients with major depression. One study also showed elevated CSF β-endorphin levels in patients diagnosed with panic disorder [148], though this effect was not demonstrated in a later study [149]. Because of these negative findings and a lack of corroborating evidence,
extensive scrutiny of CSF enkephalin and dynorphin concentrations in patients with affective disorder has not been conducted.

Numerous studies have also measured plasma concentrations of β-endorphin in patients with affective disorders. Because both ACTH and β-endorphin are coreleased from the pituitary following stress and share a common precursor, POMC, these studies are particularly interesting in light of the consistent alterations in HPA axis function seen in depression [150]. Studies investing plasma levels of β-endorphin have yielded somewhat discrepant results. Some investigators have shown increased concentrations [147, 151, 152] whereas others have found normal concentrations [34, 153-156].

Not surprisingly, similar to the ACTH response to i.v. CRF challenge (vida supra), the β-endorphin response to exogenously administered oCRF is blunted in depressed patients compared to normal subjects [34]. Moreover nonsuppression of plasma β-endorphin occurs in depressed patients in a manner similar to cortisol non-suppression after dexamethasone administration. β-endorphin nonsuppression to dexamethasone has been observed even in those patients whose baseline β-endorphin levels were similar to those of normal controls [156-158]. In these patients, postdexamethasone levels of cortisol and β-endorphin were strongly correlated [156, 158]. In contrast, depressed patients have been reported to exhibit increased secretion of β-endorphin in response to cholinergic stimulation [147], thyrotropin-releasing hormone (TRH), and luteinizing-hormone-releasing-hormone (LHRH) in comparison to controls [159].

8. Neuropeptide Y
Originally cloned from a pheochromocytoma by Minth and colleagues in 1984, neuropeptide Y (NPY) is a 36 amino acid-containing peptide whose gene is expressed in cells derived from neural crest [160]. Neurons displaying NPY immunoreactivity are abundant within several of the limbic areas of the CNS [161, 162]. NPY is also present within neurons of the hypothalamus, brainstem, and spinal cord. Present in most sympathetic nerve fibers, NPY can be detected in vascular beds throughout the body and occurs in parasympathetic nerves as well [163]. Receptors for NPY are also widely distributed. Not only do NPY-containing neurons innervate CRF-containing cells of the PVN [164] but NPY administration increases hypothalamic CRF levels [165], as well as promote its release [166]. The relationship of NPY to CRF is further substantiated by the partial blockade of the NPY-stimulated ACTH response by a CRF receptor antagonist. Moreover NPY potentiates the effects of exogenously administered CRF in animals [167]. NPY itself may have anxiolytic properties. Recently developed NPY overexpressing mice have demonstrated an attenuated sensitivity to behavioral consequences of stress [168].

Although an initial investigation [169] did not find significantly diminished CSF NPY concentrations in depressed patients, Widerlov and colleagues [170] subsequently reported that patients with major depression do exhibit decreased CSF NPY concentrations in comparison to sex- and age-matched controls. A recent study has also demonstrated decreased levels of NPY in the plasma of patients who attempted suicide, and patients who had repeatedly attempted suicide had the lowest plasma NPY levels [171]. Negative correlations have been also observed between dimensional anxiety ratings and CSF NPY levels in depressed patients [172]. Marked reductions in brain tissue concentrations of NPY have also been reported in suicide victims, with
the most dramatic decreases in those patients diagnosed with major depression [173]. Efforts toward development of NPY receptor-specific agonists and antagonists continue, but the field has been greatly hampered due to the lack of availability of synthetic nonpeptide NPY receptor agonists [174]. NPY-ergic medications may have significant benefit in the treatment of affective disorders and/or eating disorders.

9. Substance P

Mammalian members of the peptide tachykinin family are known as neurokinins [175] and include neurokinin A, neurokinin B, and Substance P. The most well known and abundant of the neurokinins, the undecapeptide Substance P, was discovered in 1931 by von Euler and Gaddum, but not isolated in pure form until 1970 by Chang and Leeman. Substance P (SP) binds to the neurokinin 1 (NK-1) receptor, neurokinin A (NKA) to the neurokinin 2 (NK-2) receptor, and neurokinin B (NKB), to the neurokinin 3 (NK-3) receptor. Within the CNS, SP is localized within the limbic, hypothalamic and brainstem areas (amygdala, hypothalamus, periaqueductal gray, locus coeruleus and parabrachial nucleus) [176] and is colocalized within norepinephrine- and serotonin-containing cell bodies as well [177-180]. Furthermore substance P and other tachykinins serve as pain neurotransmitters in primary afferent neurons [181], and exert a variety of other peripheral actions, including bronchoconstriction, vasodilatation, salivation, and smooth muscle contraction in the gut [182, 183].

Preclinical studies have provided much of the impetus to continue investigation of the potential efficacy of substance P receptor antagonism in psychiatric disorders, particularly when these agents have not been effective as analgesics [184]. Substance P (or substance P agonist) administration to animals elicits behavioral and cardiovascular
effects resembling the stress response and the so-called “defense reaction” [185]. Moreover preclinical studies documented reduction of behavioral and cardiovascular stress responses by administration of substance-P receptor antagonists [186, 187]. A breakthrough study had indicated that the substance P receptor antagonist MK-869 is more effective than placebo, and is as effective as paroxetine in patients with major depression with moderate to severe symptom severity [187].

Future clinical investigations will determine whether brain and CSF substance P concentrations are altered in patients with major depression [188], one study showed elevated levels, [189] and whether there are significant changes in CSF substance P concentrations after treatment [190]. Moreover we await convincing studies revealing if substance P antagonists will play a substantive therapeutic role in patients with anxiety disorders or schizophrenia [191] or asthma, irritable bowel syndrome, and migraine.

10. Growth Hormone and Somatostatin

10.1 Biology

A great deal of evidence has accumulated demonstrating abnormalities in both growth hormone secretion and somatostatin concentrations in CSF in depression, and to a lesser extent, anxiety disorders. Growth hormone (GH) is synthesized and secreted from somatotrophs located in the anterior pituitary. Its release is unique in that it is controlled by two peptidic hypothalamic hypophysiotropic hormones, growth-hormone-releasing factor (GHRF) and somatostatin, and secondarily by classical neurotransmitters such as Ach, DA, NE and 5HT, that innervate the releasing factor-containing neurons.

Somatostatin, also known as growth hormone-release-inhibiting hormone (GHIH) or somatotrophin-release inhibiting factor (SRIF), was first isolated from ovine hypothalamus in 1974 [192]. It is a tetradecapeptide, containing a disulfide bridge
linking the two cysteine residues. Somatostatin is released predominantly from the periventricular and paraventricular nucleus of the hypothalamus and inhibits GH release. Somatostatin also inhibits the release of both CRF and ACTH [193-195]. Somatostatin has a wide extrahypothalamic distribution in brain regions including the median eminence [196, 197], limbic system, cerebral cortex, hippocampus, hypothalamus [198] and amygdala.

GHRF was characterized and sequenced in 1981 after considerable difficulty. The long-postulated GHRF was discovered several years after the elucidation of the structure of somatostatin, from extracts of an ectopic tumor associated with acromegaly. GHRF is a 44 amino acid peptide, and has the most limited CNS distribution of all the hypothalamic-releasing hormones that have been identified. GHRF containing neurons are concentrated in the infundibular and arcuate nuclei of the hypothalamus and stimulate the synthesis and release of GH. Dopamine, NE and 5HT innervate GHRF-containing neurons thereby modulating GH release. Both GHRF and SRIF are released from the median eminence into the hypothalamo-hypophyseal portal system where they act on somatotrophs in the anterior pituitary to regulate GH release. Negative feedback is provided by GH, which stimulates somatostatin release preventing further GH release. The GH axis is unique in that it does not have a single target endocrine gland but instead growth hormone acts directly on multiple tissue targets including bone, muscle and liver.

Growth hormone is released in a pulsatile fashion, with its highest release occurring around the time of sleep onset and extending into the first non-REM period of sleep [199]. A variety of stressors including starvation, exertion, or emotional stress also promote growth hormone release in humans [200]. Growth hormone is necessary for the
longitudinal bone growth which occurs during late childhood; accordingly GH levels are high in children, reach their peak during adolescence and decline throughout adulthood. In additions to its effects on the long bones, growth hormone has predominantly anabolic effects and leads to increased muscle mass and decreased body fat. GH also stimulates the release of somatomedin from the liver and insulin like growth factors as well.

Growth hormone is released after treatment with L-Dopa, a DA precursor [201], apomorphine, a centrally active DA agonist [202, 203], clonidine [204], a central $\alpha_2$ adrenergic receptor agonist, NE [205], and the serotonin precursors L-tryptophan [206] and 5-hydroxytryptophan [207]. Serotonin receptor antagonists, methysergide and cyproheptadine, interfere with the GH response to hypoglycemia [205]. In contrast, phentolamine, a nonspecific $\alpha$-adrenergic receptor antagonist, inhibits GH secretion [208].

10.2 Growth Hormone and Depression

Several findings indicate dysregulation of GH secretion in depression. A blunted nocturnal GH surge in depression has been reported [209], whereas daylight GH secretion seems to be exaggerated in both unipolar and bipolar depressed patients [210]. A number of studies have also demonstrated a blunted GH response to noradrenergic agents (clonidine, desipramine) in depressed patients [211-217] and dopaminergic agonists (apomorphine) [218] in depressed patients. Siever et al. (1982) [217] demonstrated that the blunted GH response to clonidine was not related to age or sex, and this study provided evidence that the diminished GH response to clonidine may be secondary to decreased $\alpha_2$-adrenergic receptor sensitivity in depression [217].
A GHRF stimulation test has also been developed and studied in depressed patients. Two groups observed a blunted GH response to GHRF in depressed patients [219-221]. However Krishnan and colleagues [222, 223] found minimal differences in serum GH response to GHRH between depressed and control patients. A comprehensive review of GHRF stimulation tests in depression, anorexia nervosa, bulimia, panic disorder, schizophrenia, and Alzheimer’s disease suggested that the results of this test are not always consistent and in some cases contradictory [224]. Contributing factors include the variability of GHRF-stimulated GH among controls, lack of standard outcome measures, and age and gender related effects. Further studies using GHRF will help develop a standard stimulation test to further clarify the GH response to GHRF in depression and other psychiatric disorders. With the characterization of the genes encoding GHRF and its receptor, alterations in the CNS of depressed patients that underlie the diminished GH response to NE and DA agonists can now be studied in postmortem tissue.

Using a GHRF stimulation test, our group has demonstrated a slight exaggeration of GH response to GHRF in depressed patients compared to controls, though this group difference was mainly attributable to 3 of the 19 depressed patients who exhibited markedly high GH responses to GHRF [222]. Others, however, have reported a blunted GH response to GHRF in depressed patients. Thus it is unclear whether the blunted GH response to clonidine seen in depression is due to a pituitary defect in GH secretion, further implicating a subsensitivity of \( \alpha \)-adrenergic receptors in depression, or to a primary GHRF deficit which leads to a secondary blunted GH response. Recently a
diminished GH response to clonidine was demonstrated in children and adolescents at high risk for major depressive disorder. When considered with evidence demonstrating GH dysregulation in childhood depression [225], it suggests the blunted GH response seen in high-risk adolescents may represent a trait marker for depression in children and adolescents [226]. Arguably, the blunted GH response to clonidine seen in depression may be the most reproducible and specific finding in the biology of affective disorders.

10.3 Somatostatin and depression

Several studies have demonstrated decreased SRIF concentrations in the CSF of patients suffering with depression [143, 227-231], schizophrenia [227] and dementia including Alzheimer’s disease [232, 233]. Reduced CSF SRIF concentrations have been reported in patients exhibiting dexamethasone nonsuppression (whether schizophrenic or depressed), and are negatively correlated with the maximum postdexamethasone cortisol plasma concentration in patients with major depression [234]. Although some investigators have reported normalization of CSF SRIF concentrations after recovery from depression [228, 235, 236], others have noted no significant changes in CSF SRIF concentrations of depressed patients after clinical improvement with antidepressants [59] or ECT treatment [58]. Interestingly, administration of certain psychotropic medications is known to either decrease CSF SRIF concentrations, e.g. carbamazepine [235], diphenylhydantoin, and fluphenazine [237], increase CSF SRIF concentrations, e.g. haloperidol [238], or have no effect, e.g. desipramine or lithium [239]. Somatostatin also inhibits the release of both CRF and ACTH [193-195] indicating a direct interaction between the growth hormone and HPA axes. No differences in CSF SRIF levels have been observed in patients with generalized anxiety disorder compared to normal controls.
No published studies measuring GHRH concentration and GHRH mRNA expression have been conducted in postmortem tissue of depressed patients and matched controls which, in light of the evidence presented here, is of interest. Similarly, CSF studies of GHRH are also lacking.

Obvious alterations of GH and SRIF concentrations and function exist in major depression, though whether these changes represent fundamental contributors to this syndrome or are merely epiphenomena remains to be determined. Diminished concentrations of the inhibitory neuropeptide SRIF might plausibly allow CRF hypersecretion and increased HPA axis activity. Further elucidation of SRIF receptor function and the effects/utility of SRIF receptor agonists and antagonists will provide important information regarding the pathophysiology of major depression and neurodegenerative disorders such as AD.

11. Cholecystokinin

11.1 Biology

Cholecystokinin (CCK) was first identified in the gastrointestinal tract as a 33 amino acid peptide [240] and was later discovered in the mammalian CNS in 1975. Utilizing gastrin antisera that avidly cross-reacts with CCK, Vanderhaeghen et al. [241] found abundant gastrin-like material in the brain of many vertebrate species, including humans. Amino acid sequence analysis determined this substance to be the carboxyl-terminal amidated peptide CCK 8 [242]. In the gut, CCK exists predominantly in its larger forms of CCK, 22, 33, 39, and 58 with smaller quantities of CCK 8. In the brain its major amidated form is CCK 8. Although first identified in the gastrointestinal track, CCK is found in higher concentrations in the brain than in the gut. In the brain only
neuropeptide Y exists in higher concentrations than CCK. CCK and high densities of its receptors exist in areas of the mammalian brain associated with emotion, motivation and sensory processing, such as the cortex, striatum, hypothalamus, hippocampus, and amygdala [243-248]. CCK is often colocalized with DA in the ventrotegmental neurons that comprise the mesolimbic and mesocortical DA circuits. Of the two major subtypes of CCK receptors that exist, the CCKA subtype is primarily found in the lower gastrointestinal tract, pancreas, and gallbladder, whereas the CCKB receptor predominates in the brain.

CCK has been reported to reduce the release of DA [249-251]; conversely, the release of CCK is modulated by DA [252, 253]. Moreover preclinical studies indicated that DA neuronal activity may be either facilitated or inhibited by CCK [254-257]. Due to the interactions between DA and CCK, initial investigations had focused on a putative role for CCK in the pathophysiology of schizophrenia with little evidence forthcoming to support the hypothesis. Investigation of possible perturbations of CCK function in patients with mood disorders has similarly demonstrated rather disappointing findings. There is a single report of diminished concentrations of CSF CCK in patients with bipolar, [258] but not in unipolar, depression [119, 143, 259, 260]).

11.2 CCK and panic disorders

An impetus for the study of a role of CCK in the pathophysiology of panic disorder (PD) and other anxiety disorders was stimulated by the finding that i.v. injection of cholecystokinin tetrapeptide (CCK-4) induced panic symptoms in healthy individuals [261]. In a subsequent double-blind study, patients with PD experienced panic attacks after i.v. CCK but not following saline challenge [262]. Furthermore in comparison to
normal controls, patients with PD exhibit an increased sensitivity to CCK-4, a preferential CCKB receptor agonist [263-265], though both PD patients and controls experience panic attacks with increasing doses of CCK-4 [263, 264]. These findings have been extended to investigations in which panic attacks were provoked in patients with PD, and to a lesser extent in patients with generalized anxiety disorder [266] and normal controls with pentagastrin, another CCKB receptor agonist [267, 268]. Patients with PD have been reported to exhibit diminished CSF CCK concentrations in comparison to controls [269].

The development of CCKB receptor antagonists may lead to a potentially novel treatment for PD and other anxiety disorders. Certain CCKA or CCKB receptor antagonists have demonstrable anxiolytic [270-273], antidepressant [274], or memory enhancing [275] effects in animals. Moreover in patients with panic disorder, administration of L-365,260, a benzodiazepine-derived CCKB receptor antagonist, blocks CCK-4-induced panic [276]. In normal controls L-365,260 did not exhibit an anxiolytic effect but did not induce adverse changes in mood, appetite, or memory [275]. A larger placebo controlled, double-blind clinical trial with L-365,320 failed to find any clinical improvements in patients with panic disorders [277]. Another compound, CI-988, has been studied in patients with generalized anxiety disorder (GAD) but was no more effective than placebo as an anxiolytic [278]. Nevertheless efforts continue toward the development of an alternative, effective anxiolytic that does not have the adverse sedative, dependence liability and cognitive effects of benzodiazepines.

12. Clinical Implications and Conclusions
The last 3 decades of neuropsychophysiologic exploration has yielded a plethora of new findings regarding alterations of CNS circuits containing neuropeptides and hypothalamic releasing factors in certain psychiatric disorders. These studies have led to major advances in biological psychiatry by helping us further understand the brain circuits involved in the pathophysiology of mood and anxiety disorders. Although the balance of evidence indicates that multiple neuropeptide systems within the CNS are altered in major depression and anxiety disorders, determination of the activity or dysfunction of these systems within the brain remains relatively difficult. Not only may there be differences between hypothalamic and extrahypothalamic circuit involvement in a particular disorder, but it remains unclear to which compartment (or both) does CSF sampling access. Furthermore there is also discordance between CNS and more “peripheral” sources of several neuropeptides, such as CCK. Peripheral plasma concentrations of a neuropeptide or hypothalamic releasing factor are determined by not only the rate of release, but local metabolic degradation, the presence of specific binding proteins (as for example in the case of CRF), and redistribution into other extravascular spaces [279]. For example plasma CRF concentrations can be measured, but may not truly represent CNS secretion, because of the factors noted above and the contribution by the adrenal medulla and spleen which also synthesize the peptide.

The importance of neuropeptides in the pathophysiology of psychiatric illness is most evident in the large literature indicative of CRF hypersecretion in patients with major depression. This theory is supported by evidence from a variety of disciplines and has led to the development of a novel therapeutic approach for the treatment of anxiety and depression, namely CRF receptor antagonists. This is one of the few instances where
preclinical evidence has lead to a rationale target for drug discovery in the hopes of treating psychiatric disease. Further, this work has provided a mechanism to explain the increase in depression seen in patients exposed to trauma early in life, first postulated by Freud in the early part of the twentieth century. If CRF truly is the ‘black bile’ of depression, CRF receptor antagonists may represent a novel class of antidepressants with a unique mechanism of action distinct from other commonly used antidepressants.

---------[INSERT TABLE 5 AROUND HERE]---------

Virtually all of the neuropeptide and neuroendocrine axis alterations in patients with major depression thus far studied are state-dependant. However nearly all the studies noted in this chapter are “cross-sectional” in design, i.e., the psychiatric disorder and alterations of neuropeptide or hypothalamic releasing factor were determined at approximately the same time. Clinical investigators of the 21st century will extend understanding of whether certain neurobiologic alterations provide fundamental pathophysiologic contributions to the behavioral manifestation of a particular psychiatric disorder, or are merely epiphenomena, i.e. diminished CSF concentrations of SRIF in patients with Alzheimer’s disease. Furthermore present efforts guided by the “neuroendocrine window strategy” and the “pharmacologic bridge technique” may provide information as to whether the secretion of neuropeptides and hypothalamic-releasing factors are associated with alterations in the activity of putative neurotransmitters, such as 5HT, DA, and ACh, in a particular disease state. The availability of selective ligands that can be utilized with positron-emission tomography (PET) will mark the next major leap in our understanding of the peptidergic involvement in psychiatric disorders. The ability to determine peptide-receptor alterations in the brain
and pituitary of patients with psychiatric disorders will contribute immensely to our understanding of the neurobiological underpinnings of such disorders.

A clearer understanding of the neuroendocrinology of depression and anxiety may well lead to the development of novel pharmacologic agents for the treatment of these major mental disorders. We await confirmation of the initial report documenting the effectiveness of the substance P (NK-1) receptor antagonist, MK-869, in patients with major depression. Early studies of novel CRF receptor antagonists suggest efficacy in the treatment of depression. A selective CCKB antagonist with anxiolytic activity offers a new psychotropic modality in the treatment of panic disorder. Progress during the last three decades has been nothing short of remarkable and the concatenation of present findings undoubtedly adumbrates further progress in these disorders. In conclusion, the elucidation of biochemical abnormalities in psychiatric disorders has led to an increased comprehension of the neurochemical basis of psychiatric disease, with the ultimate goal being the development of novel pharmacological and behavioral techniques to treat these devastating disorders.

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Bibliography:


106. M.D. Fossey, R.B. Lydiard, J.C. Ballenger, M.T. Laraia, G. Bissette, and C.B. Nemeroff, Cerebrospinal fluid thyrotropin-releasing hormone concentrations in


276. J. Bradwejn, D. Koszycki, A. Couetoux du Tertre, H. van Megen, J. den Boer, and H. Westenberg, The panicogenic effects of cholecystokinin-tetrapeptide are


Figure 2. Overview of the common organizational motif of the neuroendocrine axis. The neurosecretion of hypothalamic factors into hypophyseal portal vessels is regulated by a
set point of activity from higher brain centers. Neurohormones released from the hypothalamus into hypophyseal portal vessels in turn stimulate cells in the pituitary. These adenohypophyseal hormones then regulate the hormone output from the end-organ. The end organ then exerts negative feedback effects at the pituitary and hypothalamus to prevent further neurohormone and pituitary hormone release via “long-loop” negative feedback. Short-loop negative feedback may also occur where pituitary hormones feed back directly on hypothalamic neurons to prevent further neurohormone release.
Figure 1. Diagram of the neurovascular anatomy of the hypothalamic-pituitary axis. PVN, paraventricular nucleus. SON, supraoptic nucleus. POA, preoptic area. ARC, arcuate nucleus. PT, pars tuberalis. PI, pars intermedia. PD, pars distalis. PN, pars nervosa, MB, Mamillary Body, OC, Optic chiasm.
Table 1:
HPA Axis changes in depression

↑ Corticotropin-releasing factor (CRF) concentrations in cerebrospinal fluid\(^a\)

↓ Adrenocorticotropic hormone response (ACTH) to CRF stimulation
↓ Density of CRF receptors in frontal cortex of suicide victims
Enlarged pituitary gland in depressed patients\(^a\)
Adrenal gland enlargement in suicide victims and depressed patients
↑ Plasma cortisol during depression\(^a\)
↑ Urinary free cortisol concentrations\(^a\)
Nonsuppression of plasma cortisol and ACTH after dexamethasone administration\(^a\)

\(^a\) State-dependent

Table 2:
Hypothalamic-pituitary-thyroid (HPT) axis alterations in depression

↑ CSF TRH concentrations in depressed patients
↓ Nocturnal plasma TSH
Blunted or exaggerated TSH in response to TRH stimulation
Presence of antithyroglobulin and/or antimicrosomal thyroid antibodies

Table 3: Releasing and Inhibiting Factors for Growth Hormone

Growth Hormone Releasing Factors

- Growth Hormone-Releasing Hormone (GRF of GHRH)
- Dopamine
- L-DOPA
- Apomorphine (dopamine receptor agonist)
- Norepinephrine
- Clonidine (\(\alpha_2\) adrenergic receptor agonist)
- Serotonin
- L-tryptophan
- 5-hydroxytryptophan (5HTTP)

Factors Which Inhibit Growth Hormone Release
- Somatostatin (SRIF)
- Phentolamine (nonspecific alpha-adrenergic receptor antagonist)
- Methysergide (serotonin 5HT_{1,2} receptor antagonist)
- Cyproheptadine (serotonin 5HT_{1,2a,2c} receptor antagonist)

**Table 4:**

<table>
<thead>
<tr>
<th><strong>Growth hormone (GH) axis changes in depression</strong></th>
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<tr>
<td>↑ Circulating daily GH levels (uni- and bipolar depression)</td>
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<tr>
<td>↓ Nocturnal GH in depression</td>
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<tr>
<td>↓ Response of GH to noradrenergic or dopaminergic agents</td>
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**Table 5:** Alterations of Neuropeptides and Hypothalamic Releasing Factors in Various Psychiatric Disorders

**Major Depression**
- Hyperactivity of the HPA axis
- Dysregulation of GH secretion
- Diminished SRIF activity
- Diminished NPY secretion

**Bipolar Disorder-Manic Phase**
- Hypersecretion of AVP

**Anxiety Disorders**
- Increased sensitivity to CCK-4, a preferential CCK_{B} receptor agonist
Table 1: Hypothalamic Pituitary Adrenal (HPA) Axis Activity in Anxiety and Other Psychiatric Disorders

**Posttraumatic Stress Disorder**
- increased CSF CRF concentrations
- diminished ACTH response to CRF stimulation
- plasma cortisol nonsuppression after low-dose (i.e. 0.5 mg) dexamethasone administration
- increased or decreased 24-hour urinary free cortisol concentrations
- diminished hippocampal volume

**Panic Disorder**
- normal CSF CRF concentrations
- diminished ACTH response to CRF administration

**Obsessive Compulsive Disorder**
- normal or increased CSF CRF concentrations

**Alcohol Dependence**
- increased CSF CRF concentrations in acute alcohol withdrawal

**Increased CSF CRF Concentrations**
- Alzheimer’s disease
- Huntington’s disease
- Parkinson’s disease
- Spinocerebellar Degeneration

**Normal CSF CRF Concentrations**
- Generalized Anxiety Disorder
- Schizophrenia
- Somatization Disorders
- Abstinent patients with Alcohol Dependence
- Alzheimer’s Disease