REVIEW

The role of corticotropin-releasing factor in depression and anxiety disorders

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Abstract

Corticotropin-releasing factor (CRF), a 41 amino acid-containing peptide, appears to mediate not only the endocrine but also the autonomic and behavioral responses to stress. Stress, in particular early-life stress such as childhood abuse and neglect, has been associated with a higher prevalence rate of affective and anxiety disorders in adulthood. In the present review, we describe the evidence suggesting that CRF is hypersecreted from hypothalamic as well as from extrahypothalamic neurons in depression, resulting in hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis and elevations of cerebrospinal fluid (CSF) concentrations of CRF. This increase in CRF neuronal activity is also believed to mediate certain of the behavioral symptoms of depression involving sleep and appetite disturbances, reduced libido, and psychomotor changes. The hyperactivity of CRF neuronal systems appears to be a state marker for depression because HPA axis hyperactivity normalizes following successful antidepressant treatment. Similar biochemical and behavioral findings have been observed in adult rats and monkeys that have been subjected to early-life stress. In contrast, clinical studies have not revealed any consistent changes in CSF CRF concentrations in patients with anxiety disorders; however, preclinical findings strongly implicate a role for CRF in the pathophysiology of certain anxiety disorders, probably through its effects on central noradrenergic systems. The findings reviewed here support the hypothesis that CRF receptor antagonists may represent a novel class of antidepressants and/or anxiolytics.

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Introduction

In recent years a large body of evidence has emerged linking stressful life events with an increased vulnerability for affective and anxiety disorders. Stressful events often precede the onset of depression and stress has also been associated with the severity of the illness (Dunner *et al.* 1979, Brown *et al.* 1987, Hammen *et al.* 1992). Moreover, stressful life events in childhood have been shown to predispose an individual for development of mood and anxiety disorders in adulthood. For example, loss of a parent in childhood was found to increase the risk for major depression and generalized anxiety disorders in a retrospective twin study (Kendler *et al.* 1992). In a recent study including 424 women with a history of childhood abuse, a clear association between early-life stress and adult psychological problems was found. Additionally, women who reported having been physically and/or sexually abused as children had higher scores for both depression and anxiety, lower scores for self-esteem, and were more likely to have attempted suicide than women who had not been abused as children (McCauley *et al.* 1997). Thus, early stressful life events, particularly childhood abuse and neglect, may cause biological 'wounds' that increase an individual's vulnerability to stressors later in life and, thus, predispose an individual to develop mood or anxiety disorders.

Corticotropin-releasing factor and stress

Following a search lasting nearly three decades, corticotropin-releasing factor (CRF), a 41 amino acid peptide, was isolated and structurally characterized by Vale and co-workers in 1981. Subsequently, using immunohistochemical and radioimmunoassay techniques CRF was found to be heterogeneously distributed throughout the central nervous system (CNS; for review see Owens & Nemeroff 1991). The highest density of CRF-containing cell bodies

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is found in the medial parvocellular division of the hypothalamic paraventricular nucleus (PVN) with the majority of cells projecting to the median eminence. This CRF pathway comprises the hypothalamic component of the endocrine stress axis (vide infra). CRF-containing interneurons are widely distributed in the neocortex and are believed to be important in several behavioral actions of the peptide, including effects on cognitive processing. Another brain region with a high density of CRF cell bodies is the bed nucleus of the stria terminalis (BNST) which project to brainstem areas such as the parabrachial nuclei and dorsal vagal complex that are involved in autonomic functioning. CRF perikarya in the central nucleus of the amygdala send terminals to the parabrachial nucleus of the brainstem as well as to the BNST and the medial preoptic area which both, in turn, send terminals to the parvocellular region of the PVN and thus may influence both neuroendocrine and autonomic function (Gray & Bingaman 1996). The presence of CRF immunoreactivity in the raphe nuclei and locus coeruleus (LC), the origin of the major serotonergic and noradrenergic pathways in brain, points to a role for CRF in modulating these monoaminergic systems which have long been implicated in the pathophysiology of depression and anxiety disorders.

Two different CRF receptors have been described, CRF_1 and CRF_2, both of which are positively coupled to adenylate cyclase (De Souza & Grigoriadis 1995, Chalmers *et al.* 1996, Grigoriadis *et al.* 1996). CRF_1 receptors are found in high density in the pituitary, as well as in brain, particularly in the neocortex. CRF_2 receptors are more abundant in the periphery, but are also found in some brain areas such as the septum, ventromedial hypothalamus and dorsal raphe nucleus. The CRF_2 receptor is currently known to exist in two different isoforms in both rat and human; these have been designated $CRF_{2\alpha}$ and $CRF_{2\beta}$ (Chalmers *et al.* 1996). A new CRF-like peptide, urocortin, was recently cloned from rat and human tissue (Vaughan *et al.* 1995, Donaldson *et al.* 1996*a*,*b*). Urocortin is a 40 amino acid peptide with approximately 45% homology in amino acid sequence to CRF. In the rat, urocortin-containing perikarya and urocortin mRNA expression are most prominent in the Edinger-Westphal nucleus and the lateral superior olive, regions that do not contain CRF mRNA (Vaughan *et al.* 1995, Wong *et al.* 1996). Wong *et al.* (1996) reported relatively high expression of urocortin mRNA in several other brain regions including the intermediate lobe of the pituitary, hippocampus, neocortex, hypothalamic PVN, and amygdala. The highest density of urocortin innervation is observed in the lateral septum and the dorsal raphe nucleus. In a recent study, urocortin immunoreactive cells, as well as urocortin mRNA, were found in human anterior pituitary suggesting that urocortin may have a paracrine or autocrine role in the production and/or secretion of adrenohypophysial hormones (Iino *et al.* 1997). Urocortin binds with equal affinity to both CRF receptor subtypes, but possesses much higher affinity for CRF_2 receptors than does CRF and is found in brain regions distinct from CRF. It is of considerable interest to note that the lateral septum and dorsal raphe nucleus almost exclusively express CRF_2 receptor mRNA. Thus, the urocortin–CRF_2 system may comprise a transmitter system separate from, but related to, CRF. Although urocortin is a potent agonist at the classic CRF_1 receptor, the physiological role of urocortin and its involvement in the pathophysiology of psychiatric disorders remains unexplored.

In mammals, the endocrine stress response is mediated through the hypothalamic–pituitary–adrenal (HPA) axis (Fig. 1). During stress, the synthesis of CRF in the PVN increases and CRF is released from terminals in the median eminence into the hypothalamo–hypophysial portal vascular system (Antoni 1986, Plotsky 1991). When the peptide reaches the anterior pituitary gland, it binds to CRF receptors and through a cascade of intracellular steps ultimately increases pro-opiomelanocortin (POMC) gene expression and the release of POMC-derived peptides such as adrenocorticotropin (ACTH) and β-endorphin. ACTH, in turn, induces the synthesis and release of glucocorticoids (principally cortisol in primates and corticosterone in rats) from the adrenal cortex. At least two types of glucocorticoid receptors have been described in brain, i.e. the mineralocorticoid receptor (MR, type I) and the glucocorticoid receptor (GR, type II; for review see e.g. Joëls & De Kloet 1994). Corticosterone binds to both receptors but with about 10 times higher affinity for MRs. The distribution of MRs in brain is mainly restricted to limbic structures, i.e. hippocampus, septum, septohippocampal nucleus and amygdala, and they mediate the control of basal HPA activity. The GRs are found throughout the brain, with high density in the limbic system (hippocampus, septum) and in the parvocellular neurons of the PVN, and are also found in relatively high concentrations in the ascending monoaminergic neurons of the brain stem. During stress when corticosterone levels may increase about 100-fold GRs get occupied by corticosterone and their main function in brain is to suppress stress-induced hyperactivity of the HPA axis at the level of the PVN, anterior pituitary, but also at the hippocampal level (see e.g. De Kloet 1991). Thus, it has been suggested that the adaptive function of the HPA axis is critically dependent on glucocorticoid feedback mechanisms to dampen the stressor-induced activation of the HPA axis and to shut off further glucocorticoid secretion (Jacobson & Sapolsky 1991).

CRF systems in the brain have a role in mediating not only the neuroendocrine, but also the autonomic and behavioral responses to stress (see Fig. 1). For example, CNS administration of CRF to laboratory animals produces physiological and behavioral changes almost identical to those observed in response to stress, including increased heart rate and mean arterial pressure due to alterations in the autonomic nervous system, suppression of

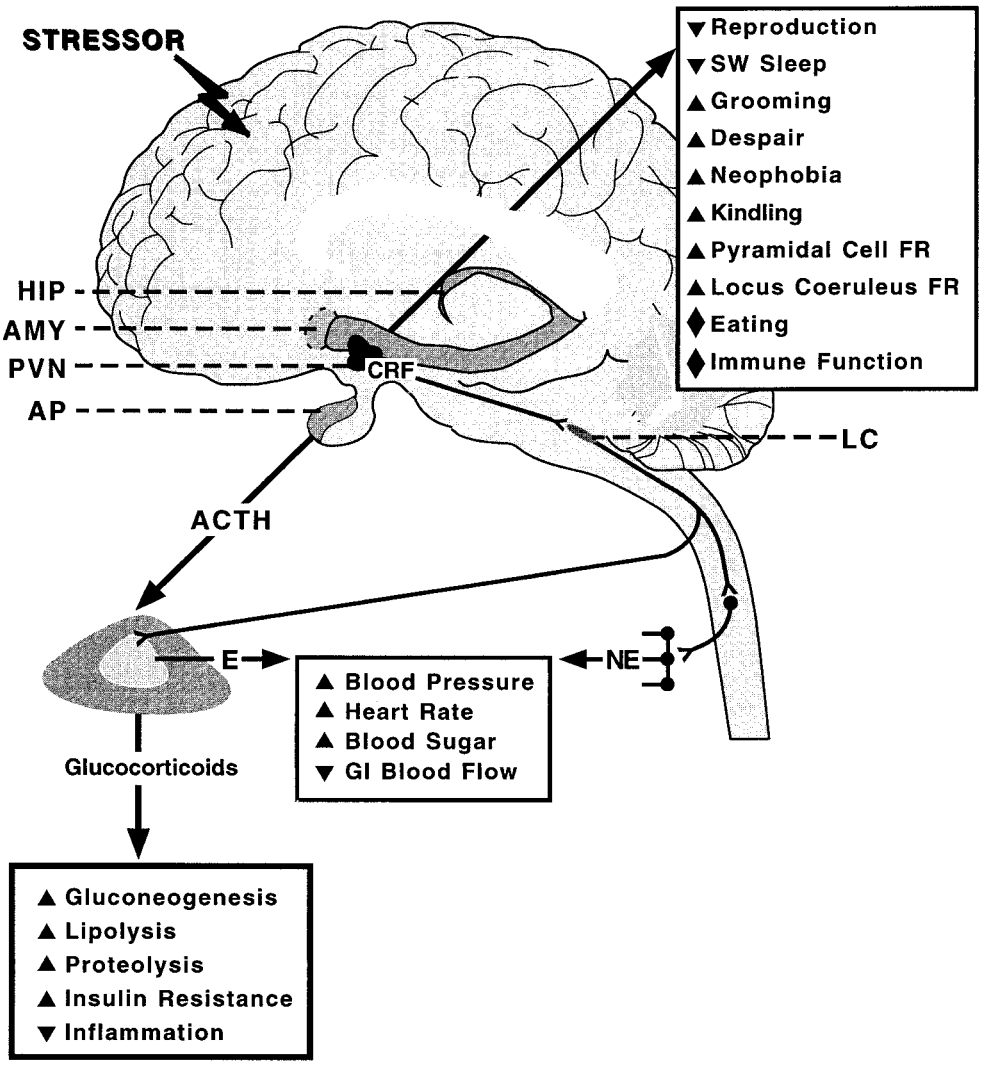

Figure 1 Schematic representation of the endocrine, behavioral, autonomic, and immunologic responses to stress mediated by central CRF neurons. ▲, increase; ▼, decrease; ◆, both increase and decrease; AMY, amygdala; AP, anterior pituitary; E, epinephrine; HIP, hippocampus; LC, locus coeruleus; NE, norepinephrine; PVN, paraventricular nucleus; GI, gastrointestinal; FR, firing rate; SW, slow wave.

exploratory behavior in an unfamiliar environment, induction of grooming behavior, increased conflict behavior, and decreased food intake and sexual behavior (Dunn & Berridge 1990, Owens & Nemeroff 1991, Koob *et al.* 1993). Moreover, centrally administered CRF has been shown to enhance behavioral responses to stressors as evidenced by a reduction in exploratory behavior in a novel, presumably stressful environment, and enhancement of stress-induced freezing behavior (see Koob *et al.* 1993). In non-human primates, central CRF administration increases vocalizations, decreases environmental exploration and increases huddling and lying-down behavior which are symptoms of behavioral despair typically seen after maternal separation in infant monkeys (Kalin 1990). The behavioral effects of centrally administered CRF can be reversed by CRF receptor antagonists and are independent of activation of the HPA axis. Furthermore, CRF receptor antagonist alone attenuates many of the behavioral consequences of stress, underscoring the role of endogenous CRF in mediating many stress-induced behaviors (Heinrichs *et al.* 1995).

Clinical findings in depression

A compelling number of studies have found several measures indicative of a hyperactive HPA axis in depressed patients (for review see Plotsky *et al.* 1995*a*). It

has now been more than 40 years since Board *et al.* (1956) reported that plasma cortisol concentrations are elevated in a majority of patients with major depressive disorder, a finding that has been repeatedly replicated. Moreover, a single dose of the synthetic glucocorticoid dexamethasone (i.e. the dexamethasone suppression test, DST) suppresses plasma ACTH, β-endorphin and cortisol concentrations to a lesser extent and/or for a shorter time in depressed patients compared with healthy non-depressed subjects. Both the hypercortisolemia and dexamethasone nonsuppression normalize upon clinical recovery suggesting that the hyperreactive HPA axis seen in depressed subjects represents a state, rather than a trait, marker.

After intravenous administration of CRF, depressed patients exhibit a blunted ACTH, but normal cortisol, response in comparison to healthy controls (Gold *et al.* 1986, Holsboer *et al.* 1986, Krishnan *et al.* 1993). Moreover, a correlation between dexamethasone nonsuppression of cortisol and a blunted ACTH response to CRF challenge in patients with major depression has been reported (Krishnan *et al.* 1993). After clinical recovery, normalization of the blunted ACTH response to CRF is also observed (Amsterdam *et al.* 1988). Holsboer and collaborators have used a combination of a standard or higher dose of dexamethasone suppression test and a CRF stimulation test in depressed patients. In a series of studies they found that dexamethasone-pretreated patients show enhanced ACTH and cortisol response to CRF compared with control subjects (see Holsboer & Barden 1996). Moreover, this combined test appeared to be a very sensitive diagnostic measure for depression, especially when the patients were clustered into different age groups. Also, healthy non-depressed subjects at high familial risk for affective disorders exhibit disturbed HPA axis activity as induced by the combined DST-CRF test, suggesting that the potential for abnormalities in HPA axis function in depressed patients may be genetically transmitted (Holsboer *et al.* 1995).

One plausible mechanism to explain the blunted ACTH response to CRF challenge observed in depressed patients is down-regulation of pituitary CRF receptors, presumably secondary to increased hypothalamic CRF release. Support for hypersecretion of hypothalamic CRF in depression comes from a series of findings in depressed patients and suicide victims. We have repeatedly observed significantly elevated concentrations of CRF in cerebrospinal fluid (CSF) of drug-free patients with major depression and from suicide victims compared with patients with other psychiatric disorders and healthy controls (Nemeroff *et al.* 1984, Arató *et al.* 1986, 1989, Bánki *et al.* 1987, 1992*a*, France *et al.* 1988, Widerlöv *et al.* 1988). Increased CSF CRF concentrations in depressed subjects have been confirmed by Risch *et al.* (1991). However, other studies have been unable to replicate these observations (Kling *et al.* 1991, 1993, Molchan *et al.* 1993, Pitts *et al.* 1995). Gold and collaborators did not find any difference between CSF CRF concentrations in depressed patients and healthy controls, although depressed patients who were DST nonsuppressors had significantly higher CSF CRF concentrations as compared with depressed DST suppressors (Roy *et al.* 1987). Recently, decreased CSF CRF concentrations have been observed in a group of depressed patients with normal plasma cortisol levels compared with healthy subjects (Geracioti *et al.* 1997). These discrepant findings are almost certainly due to the inclusion of patients with atypical depression or with only mild to moderate depression in these studies. (The reports where CSF CRF concentrations have been measured in depressed subjects are summarized in Fig. 2.) Further support for the postulate that depression is associated with CRF hypersecretion may be derived from postmortem studies which revealed an increase in CRF concentrations and in CRF mRNA expression in the PVN of patients with depression (Raadsheer *et al.* 1994, 1995).

There is evidence that, like measures of HPA axis activity, CSF CRF concentrations normalize when patients recover from depression. Thus, the elevated CSF CRF concentrations of drug-free depressed patients are significantly decreased 24 h after a successful series of electroconvulsive therapy treatments (ECT; Nemeroff *et al.* 1991). In a preliminary report, Kling *et al.* (1994*a*) observed a reduction in diurnal CSF CRF concentrations in depressed patients after successful ECT. In addition, normalization of elevated CRF concentrations in CSF has also been reported after successful treatment of depression with fluoxetine (De Bellis *et al.* 1993). In another study we found a significant reduction of elevated CSF CRF concentrations in fifteen depressed women who remained depression-free for at least 6 months after antidepressant drug treatment (Bánki *et al.* 1992*b*). In contrast, there was a tendency for increased CSF CRF concentrations in the nine patients who relapsed within 6 months. Although CSF CRF concentrations are not correlated with depression severity, these findings suggest that lack of normalization of CRF levels in CSF after antidepressant treatment may predict early relapse. Taken together the above studies indicate that elevated CRF concentrations in CSF appear to be a state, rather than a trait, marker in depression.

Neuropeptides appear to be secreted directly into CSF from brain tissue, and neuropeptides found in CSF are not derived from the systemic circulation (Post *et al.* 1982). Studies using non-human primates suggest that CSF levels of CRF primarily reflect function of extrahypothalamic rather than hypothalamic CRF systems (Kalin 1990). Thus, manipulations that enhance pituitary ACTH release, i.e. physostigmine administration or stress, are not accompanied by an increase in CSF CRF levels. A dissociation between the diurnal variation of CSF CRF and cortisol concentrations has also been described in both humans and primates (Kalin 1990, Kling *et al.* 1994*b*).

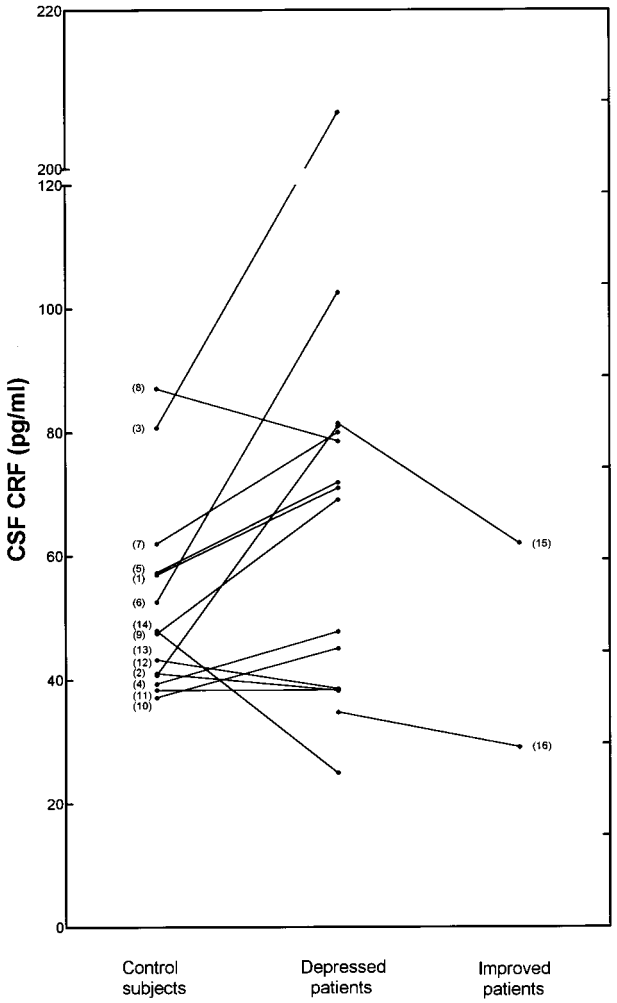

Figure 2 Summary of reports in which CRF concentrations were measured in CSF of depressed patients and suicide victims, or depressed patients after successful antidepressant treatment. Numbers in parentheses represent the following individual references: (1) Nemeroff *et al.* (1984), (2) Bánki *et al.* (1987), (3) Arató *et al.* (1989), (4) Arató *et al.* (1986), (5) Widerlöv *et al.* (1988), (6) France *et al.* (1988), (7) Risch *et al.* (1991), (8) Molchan *et al.* (1993), (9) Bánki *et al.* (1992*a*), (10) Roy *et al.* (1987), (11) Kling *et al.* (1991), (12) Kling *et al.* (1993), (13) Pitts *et al.* (1995), (14) Geracioti *et al.* (1997), (15) Nemeroff *et al.* (1991), (16) De Bellis *et al.* (1993).

Using magnetic resonance imaging (MRI) and computed tomography (CT), enlargement of both the pituitary and the adrenal gland have been observed in depressed patients (Krishnan *et al.* 1991, Axelson *et al.* 1992, Nemeroff *et al.* 1992, Rubin *et al.* 1995). In laboratory animals both hyperplasia and hypertrophy of the anterior pituitary as well as adrenal gland hypertrophy have been observed after enhanced stimulation of the pituitary–adrenal axis (Gertz *et al.* 1987, Sapolsky & Plotsky 1990). Thus, these imaging findings lend further support to the hypothesis of increased hypothalamic CRF secretion in depression.

Finally, we have found a marked decrease in CRF receptor binding sites in the prefrontal cortex of depressed suicide victims, which we hypothesize develops as a compensatory consequence of increased release of CRF in this brain region (Nemeroff *et al.* 1988). Recently we have replicated these findings in a second study.

Preclinical studies of early-life stress

The impact of early-life stress, frequently induced by maternal separation during infancy has been extensively studied in non-human primates (see e.g. Suomi 1991). Thus, rhesus macaques that grew up either alone or with peers only show several signs of behavioral despair, i.e. decreased locomotion, environmental exploration and play, disturbed sleep, decreased, or sometimes increased, food intake (McKinney *et al.* 1984). These behavioral changes resemble many of the cardinal symptoms of human depression. Moreover, they can be alleviated by clinically effective antidepressant treatments such as ECT or chronic treatment with the tricyclic antidepressant (TCA) imipramine. Non-human primates that have been raised without their mothers also respond to acute stress with a greater activation of the HPA axis compared with mother-reared monkeys, as indicated by higher levels of plasma cortisol and ACTH (Suomi 1991). Moreover, a recent study found that repeated social isolation produced sustained hypercortisolism in squirrel monkeys (Levine *et al.* 1997).

Another primate model for adverse early-life experience which may more closely resemble the adverse events hypothesized to predispose to human depression and anxiety disorders (vide supra) has been developed by Rosenblum and collaborators. In this model, bonnet macaque infants are raised under different rearing conditions in which the mothers are confronted with different foraging demands. Mothers that have low foraging demands (LFD) can easily find food, whereas mothers that have consistently high, but predictable, foraging demands (HFD) had to work to find food. A third group of mothers are exposed to variable, unpredictable foraging demands (VFD). The VFD paradigm appears to be the most stressful for the infant and, although the mother is physically present, she is more anxious and more neglectful of her infant. As adults, monkeys raised by VFD mothers exhibit signs of both anxiety and affective disturbances (Rosenblum & Paully 1984). In collaboration with Coplan, Rosenblum and Gorman, we used this paradigm to study the effects of early-life stress on CSF CRF levels in young adult primates. At about 4 months of age the infant monkeys and their mothers were exposed to one of the three foraging demand situations described above for

12 weeks, after which the young animals were subsequently placed in a standard animal colony. CSF samples were obtained from these offspring as young adults. Analogous to what we had previously observed in depressed patients, we found that monkeys reared under stressful (VFD) conditions have higher CSF CRF concentrations when compared with monkeys raised under non-stressful conditions (Coplan *et al.* 1996). More recently we have noted a strong negative correlation between CSF CRF concentrations and the growth hormone response to clonidine, which is blunted in depression (J D Coplan, E L P Smith, R C Trost, B A Scharf, L Bjornson, M J Owens, C B Nemeroff, J M Gorman & L A Rosenblum, unpublished observations). These data suggest that in non-human primates, early-life stress is associated with long-standing CRF neuronal hyperactivity.

In view of the clear association between early-life stress and the later development of affective and anxiety disorders both our laboratory and that of Plotsky, Meaney and colleagues have carried out a series of experiments using the maternal deprivation model of early-life stress in the laboratory rat. In the neonatal rat, the HPA response to certain stressors appears to be blunted during postnatal day 4 through 14 suggesting a stress hyporesponsive period when compared with adult animals (Shapiro 1968, Walker *et al.* 1986, Levine 1994). However, we found that a single 24-h separation of 10-day-old rat pups from their mothers elicited a significant increase in plasma corticosterone levels and a decrease in CRF concentrations in the median eminence (Pihoker *et al.* 1993). In 12- and 18-day-old rat pups, a significant reduction of CRF binding sites in the pituitary was observed after a 24-h maternal separation. This is most likely due to increased hypothalamic CRF release (Nemeroff *et al.* 1993, Pihoker *et al.* 1993). Thus, our results indicate that infant rats can mount a pronounced endocrine response to stress, i.e. maternal separation. Plotsky and collaborators (1995*b*) have previously shown that repeated maternal separation, i.e. three hours daily during postnatal days 2–14, was associated with increased anxiety- and depressive-like behavior of adult rats, as well as a distinct alcohol preference. Most remarkably both increased anxiety and depression as well as alcohol abuse are observed in women who have been victims of childhood abuse (McCauley *et al.* 1997). In subsequent experiments we used repeated maternal separation as a model to study further the effect of early-life stress in the adult rat. Adult animals that had been subjected to daily 6-h maternal separations during postnatal days 2–20 exhibit significant basal and stress-induced increases in plasma ACTH concentrations when compared with non-deprived animals (Ladd *et al.* 1996). Previously, it was reported that maternally deprived rats produce a significantly higher increase in plasma ACTH and corticosterone concentrations in response to restraint stress than non-separated rats (Plotsky & Meaney 1993, Levine 1994). In concert with these findings we also found that these rats exhibited a more than twofold increase in CRF immunoreactivity in the median eminence. Moreover, maternally deprived rats exhibit elevated expression of hypothalamic PVN CRF mRNA. These findings suggest that adult rats previously exposed to early-life stress hypersecrete CRF from the hypothalamus. Consistent with this hypothesis is our observation of a reduction in CRF binding sites in the pituitary as well as an increase in hypophysial portal plasma CRF levels in maternally deprived rats compared with non-deprived rats (Plotsky & Meaney 1993, Ladd *et al.* 1996, Plotsky *et al.* 1998). In addition, more than half of the maternally deprived animals show resistance to suppress corticosterone levels after dexamethasone administration, a finding analogous to the DST results in depressed patients. There exists some evidence that HPA axis hyperactivity may develop from increased exposure of corticosterone during early development. Thus, adult offspring of dams exposed to increased levels of corticosterone during pregnancy either by means of repeated stress or ethanol exposure, show enhanced stress-induced increase in plasma ACTH and corticosterone (Lee *et al.* 1990, Henry *et al.* 1994). Moreover, no alteration in the responsiveness of the HPA axis was observed in offspring of adrenalectomized dams exposed to stress and, conversely, the effects of prenatal stress could be reinstated by corticosterone administration to such dams during stress (Barbazanges *et al.* 1996). However, this was not found by Lee and Rivier (1992). Indeed, significantly higher levels of plasma corticosterone have been observed in 6-day-old maternally deprived pups after they were returned to their mothers, compared with non-deprived pups, most likely as a result of inappropriate behavior of the dams (P M Plotsky, unpublished observations). However, other studies have shown that increased corticosterone during postnatal life produced the opposite effect on adult HPA axis. Thus, both basal and stress-induced cortcosterone and ACTH secretion is decreased in adult rats exposed to increased corticosterone during the first two weeks after birth (Catalani *et al.* 1993), suggesting that other mechanisms are probably involved in the development of HPA axis hyperactivity in maternally deprived animals.

One of our most intriguing observations is the change in extrahypothalamic CRF neuronal systems in adult rats exposed to neonatal maternal deprivation. Thus, a significant increase in CRF binding sites was found in the dorsal raphe nucleus, the major site of origin of the widespread serotonergic innervation of the forebrain (Ladd *et al.* 1996). This finding is of particular interest because abnormalities in serotonergic systems have long been implicated in the pathogenesis of depression, as well as playing a major role in the therapeutic actions of antidepressant drugs (see e.g. Owens & Nemeroff 1994, Maes & Meltzer 1995). In the parabrachial nucleus, an area which receives CRF projections from the central nucleus of the amygdala, an increase in CRF immunoreactvity was also observed. We have

previously shown that local infusion of CRF into the parabrachial nucleus increased both depression- and anxiety-like behaviors suggesting that at least some of the signs of depression and anxiety observed in adult animals subjected to maternal separation during infancy may be mediated through increased CRF activity in the parabrachial nucleus (Weiss *et al.* 1994). In fact, rats exposed to maternal separation show increased expression of CRF mRNA in the central nucleus of amygdala, a brain region involved in the autonomic, endocrine and behavior responses to stress (Menzaghi *et al.* 1993), and increased CRF peptide content in terminal fields in the area of the LC (Plotsky *et al.* 1998). Finally, elevated basal and stress-stimulated CSF CRF concentrations are observed in adult rats that were maternally deprived, and are also consistent with both hyperactivity of extrahypothalamic CRF systems in such animals, as well as with the findings in drug-free depressed patients (vide supra).

CRF and anxiety

Centrally administered CRF produces several signs of increased anxiety and transgenic mice that over-express CRF exhibit increased anxiogenic behavior (Dunn & Berridge 1990, Stenzel-Poore *et al.* 1994). Conversely, central administration of either a CRF antisense oligodeoxynucleotide or a CRF receptor antagonist produce anxiolytic effects in the rat (Dunn & Berridge 1990, Koob *et al.* 1993, Skutella *et al.* 1994). Similar anxiolytic action has recently been reported in transgenic mice lacking CRF_1 receptors (Smith *et al.* 1998, Timpl *et al.* 1998). A recent study by Heinrichs and coworkers (1997) using CRF_1 and CRF_2 receptor antisense oligonucleotides provides evidence that the anxiogenic actions of CRF are mediated by CRF_1 rather than CRF_2 receptors. The anxiogenic effects of CRF have been hypothesized to be mediated through actions of CRF on the LC noradrenergic systems. The activity of the norepinephrine (NE) neuronal system has been observed to be increased during stress and anxiety in several animal species, and states of anxiety and fear appear to be associated with an increase in NE release in humans (see Charney *et al.* 1995). There is anatomical evidence for direct synaptic contact between CRF terminals and dendrites of NE cells in the LC, and both acute and chronic stress increases CRF-like immunoreactivity in the LC (Chappell *et al.* 1986, Van Bockstaele *et al.* 1996). Adult rats exposed to neonatal maternal separation also have markedly elevated LC CRF concentrations (Plotsky *et al.* 1998). In turn, when CRF is locally applied to the LC, increased activity of the NE cells, as well as NE release in terminal fields has been reported (Valentino *et al.* 1983, Smagin *et al.* 1995). Moreover, microinjections of CRF into the LC decrease open-field activity and increase defensive withdrawal, i.e. time spent in a darkened corner of the open-field and an increase in nonambulatory movement (Butler *et al.* 1990, Weiss *et al.* 1994). These behaviors indicate an increase in anxiety after CRF administration into the LC. After repeated stress, the expression of tyrosine hydroxylase (TH), the rate-limiting enzyme in NE synthesis, is elevated and this effect appears to be dependent on endogenous CRF because it can be blocked by the CRF receptor antagonist α-helical $CRF_{9–41}$ (Melia & Duman 1991). Furthermore, in adult rats previously exposed to maternal separation, stress results in increased release of NE in the hypothalamus (Liu *et al.* 1998). In a series of experiments we have found that the clinically effective anxiolytic alprazolam decreases LC CRF concentrations after acute administration, an effect that is maintained during chronic administration (Owens *et al.* 1989, 1991). In view of the hypothesis that anxiety may be associated with increased activity of the LC, our findings suggest that benzodiazepines may exert at least some of their anxiolytic effects through decreasing the CRF stimulatory input to noradrenergic neurons in the LC.

The correlation between childhood abuse or neglect and the development of anxiety disorders (e.g. panic disorder and generalized anxiety disorder) in adulthood, as well as the observed increase in anxiety and hypothalamic and extrahypothalamic CRF neuronal activity in adult animals that have been subjected to maternal deprivation (vide supra) strongly support a link between early-life stress, CRF and the development of anxiety disorders. A blunted ACTH response to CRF challenge has been observed in patients with panic disorder suggesting dysfunction of the HPA axis, whereas CSF CRF levels have not been found to be elevated in this disorder (Roy-Byrne *et al.* 1986, Jolkkonen *et al.* 1993, Fossey *et al.* 1996). On the other hand, elevated CSF CRF concentrations have been reported in patients with obsessive-compulsive disorder (OCD; Altemus *et al.* 1992). Interestingly, successful treatment with clomipramine resulted in a significant decrease in CSF CRF levels in such patients (Altemus *et al.* 1994).

Recently, we reported that Vietnam combat veterans with post-traumatic stress disorder (PTSD), which is characterized by anxiety, flashbacks, and autonomic arousal, show significantly increased concentrations of CRF in CSF (Bremner *et al.* 1997) and they also exhibit a blunted ACTH response to CRF (Smith *et al.* 1989). However, in contrast to depression, patients with PTSD show hypocortisolism and 'supersuppression' to dexamethasone challenge (see e.g. Heim *et al.* 1997, Yehuda 1997). We have also found elevated CSF CRF concentrations in Tourette's syndrome in which patients show enhanced vulnerability to stress and anxiety (Chappell *et al.* 1996), and higher concentrations of CSF CRF during alcohol withdrawal which is characterized by increased anxiety and sympathetic arousal (Hawley *et al.* 1994, Adinoff *et al.* 1996). CSF CRF levels in patients with generalized anxiety disorder are unchanged in comparison

Table 1 Evidence suggesting hyperactivity of central CRF systems in depression

Elevated CSF CRF concentrations in drug-free depressed patients.
Normalization of elevated CSF CRF concentrations with successful antidepressant treatment.
Decreased CRF receptor binding sites in the prefrontal cortex of depressed suicide victims.
Hyperactivity of the HPA axis in depressed patients.
Elevated CRF concentrations and CRF mRNA in the hypothalamic PVN of depressed patients.
Elevated CSF CRF concentrations in adult primates previously exposed to early-life stress, which is associated with an increased risk for depression in humans.
Elevated concentrations of hypothalamic and extrahypothalamic CRF in adult rats previously exposed to early-life stress.

Table 2 Evidence suggesting an involvement of central CRF systems in anxiety disorders

Central administration of CRF to laboratory animals produces increased anxiety.
In laboratory animals stress-induced anxiety can be blocked by CRF receptor antagonists or decreased production of CRF receptors.
CRF increases the activity of the LC noradrenergic system in brain, which has been implicated in the pathophysiology of human anxiety.
Increased CRF concentrations in the LC of adult rats previously exposed to early-life stress, which is associated with an increased risk for anxiety disorders in humans.
Benzodiazepine treatment decreases CRF concentrations in the LC.
CSF CRF concentrations are elevated in certain anxiety disorders (i.e. OCD, PTSD, Tourette's syndrome) and during alcohol withdrawal.

with healthy controls (Bánki *et al.* 1992*a*, Fossey *et al.* 1996). Thus, the clinical data provide some evidence for a role of central CRF neuronal systems in anxiety disorders but not to the extent observed in depression. Moreover, the compelling preclinical evidence for an involvement of CRF, taken together with the seminal role of central noradrenergic systems in stress and anxiety, suggests that CRF-induced alterations of LC functioning may play a role in the pathophysiology of anxiety disorders.

Summary

Evidence from both clinical and preclinical studies strongly supports the view that CRF may be hypersecreted from both hypothalamic and extrahypothalamic neurons in depression. Thus, the well documented hyperactivity of the HPA axis observed in depressed patients may be largely driven by increased secretion of hypothalamic CRF; elevated CSF concentrations of CRF appear to reflect hyperactivity of extrahypothalamic CRF neurons. Similar changes have been found in adult animals that have been subjected to early-life stress, i.e. hyperreactive HPA axis in response to stress, increased concentrations of hypothalamic and extrahypothalamic CRF, and elevated CSF CRF concentrations compared with control animals. Notably, these changes in CRF neuronal activity normalize with successful antidepressant treatment. The anxiogenic effect of CRF may be mediated through its ability to increase the activity of the LC noradrenergic system. Both acute and chronic stress as well as stress in early life increase CRF levels in the LC, whereas anxiolytic drugs decrease the concentration of the peptide in this same area. Clinical studies here revealed that CSF CRF levels are increased in certain anxiety disorders (i.e. OCD, PTSD and Tourette's syndrome) and during alcohol withdrawal, but not in others (i.e. panic disorder and generalized anxiety disorder). Tables 1 and 2 summarize the evidence of involvement of central CRF systems in depression and anxiety disorders. This concatenation of findings suggests that early untoward life events which are associated with the development of depression and anxiety in adulthood, give rise to long-lasting alterations in CRF-containing neurons and may increase an individual's vulnerability for affective and anxiety disorders. These findings also imply that agents that block the actions of CRF, i.e. CRF receptor antagonists, may prove useful in the treatment of mood and anxiety disorders.

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